Renal Sub-study of the
China Stroke Primary Prevention Trial (CSPPT)

Rationale and Study Design

(in supplement of the CSPPT protocol)

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May 2013
1. Rationale

Chronic kidney disease (CKD), characterized by impaired renal function and markers of renal damage (e.g. proteinuria), confers significantly increased risk of end-stage renal disease (ESRD), cardiovascular diseases, and death. It is estimated that more than 119 million Chinese have CKD and are at high risk of developing ESRD. The mechanisms of CKD progression are not fully understood. Angiotensin converting enzyme inhibitors or angiotensin II receptor antagonists are found to slow down the progression of CKD, though the effect is often limited. Despite treatment, the number of patients with ESRD and treated with renal replacement therapy is constantly increasing. Developing new therapeutic interventions for slowing down the progression of CKD is of pivotal importance.

Hyperhomocysteinemia has long been shown to be associated with increased risk of adverse cardiovascular and renal outcomes. However, whether homocysteine-lowering therapy slows down the progression of CKD has not been adequately investigated. There have been many homocysteine-lowering randomized clinical trials to investigate the effects of folic acid and other B vitamins on prevention of cardiovascular events and death, covering a wide arrange of patients from different regions and ethnic groups and with different profiles of disease risk. However, few have evaluated their effects on renal outcomes, especially in populations without folic acid fortification and in patients with mild to moderate CKD. Up to date only two clinical trials (HOST and DIVINe) have investigated the effects of supplementation of folic acid and B-vitamins on renal outcomes and found a null or even a harmful effect. Both trials were conducted in populations with folic acid fortification. The HOST study reported that treatment with
extremely high doses of folic acid and B-vitamins did not improve survival or delay the
time to initiating dialysis in patients with advanced CKD or ESRD. The DIVINe trial
showed that, compared with placebo, treatment with high dose of folic acid and B-
vitamins (including cyanocobalamin) resulted in a greater decrease in GFR and an
increase in vascular events in 238 patients with diabetic nephropathy. It has been
suggested that cyanocobalamin is nephrotoxic and the culprit of the harmful treatment
effect observed.

The China Stroke Primary Prevention Trial (CSPPT) compares 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.
The renal sub-study aims to examine the effects of combination of enalapril and folic acid
with enalapril alone in slowing down the renal function decline in the CSPPT participants,
especially in those with mild to moderated CKD.

2. Study objectives

The primary objective is to evaluate whether combination of enalapril and folic acid
is more effective than enalapril alone in reducing progression of CKD among adults with
hypertension. Progression of CKD was defined as a decrease in eGFR of ≥30% and to a
level of <60 ml/min/1.73m² at the exit visit if the baseline eGFR was ≥60 ml/min/1.73m²,
or a decrease in eGFR of ≥50% at the exit visit if the baseline eGFR was <60
ml/min/1.73m², or ESRD (eGFR<15 ml/min/1.73m² or need for dialysis).

Secondary objectives are to evaluate whether combination of enalapril and folic acid
is more effective than enalapril alone on the following treatment outcomes:
(1) A composite outcome of progression of CKD and all-cause death;

(2) Rapid decline in renal function, defined as an average decline in eGFR of \( \geq 5 \) ml/min/1.73m\(^2\) per year;

\[(3) \text{Annual rate of relative decline in eGFR, estimated as } \left( 1 - t \frac{\text{eGFR at exit}}{\text{eGFR at baseline}} \right) \times 100\% \text{, where } t \text{ is the time length in years from baseline to the exit visit.}\]

3. Study design

CSPPT is a multi-community, randomized, double blinded, and actively controlled trial that evaluated the effects of folic acid on prevention of stroke and other cardiovascular events in hypertensive adults. The study is conducted in 32 communities in Anhui and Jiangsu provinces of China and enrolled a total of 20,702 hypertensive adults without a history of major cardiovascular disease. Participants are randomized to receive treatment with either a combination of enalapril and folic acid or enalapril alone, and followed up every three months. See the protocol of CSPPT for the details of the trial design.

The renal sub-study is initiated three years after completion of the enrollment of CSPPT. Serum creatinine of the participants at baseline and at the end of the study will be measured at a central lab for the assessment of renal function decline. Therefore high completion rate of the exit visit is essential for the success of the renal sub-study. With consideration of the historical followup data and the study power, the renal sub-study will be limited to 20 communities in Jiangsu province, from which a total of 15486 participants have been randomized.
3.1 Participants

The CSPPT participants who meet the following criteria will be included for the renal sub-study: (1) from the communities in Jiangsu province, and (2) baseline eGFR >30 ml/min/1.73m².

3.2 Renal outcomes

The primary outcome is progression of CKD, defined as (a) a decrease in eGFR of ≥30% and to a level of <60 ml/min/1.73m² at the exit visit if the baseline eGFR was ≥60 ml/min/1.73m², or (b) a decrease in eGFR of ≥50% at the exit visit if the baseline eGFR was <60 ml/min/1.73m², or (c) ESRD (eGFR<15 ml/min/1.73m² or need for dialysis).

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/1.73m² per year; and (3) annual rate of relative decline in eGFR, estimated as $1 - \frac{t}{\sqrt{\frac{eGFR \text{ at exit}}{eGFR \text{ at baseline}}}} \times 100\%$, where $t$ is the time length in years from baseline to the exit visit.

3.3 Power consideration

The renal sub-study aims to detect an effect size of ≥25% decrease in the risk of the primary outcome. Figure 1 gives out the statistical power curve along sample size at various 5-year event rates of the primary outcome, presuming a drop out rate of 10% and a type I error rate of 0.05. With a 5-year event rate of 3% and a drop out rate of 90%, the renal sub-study is expected to have a statistical power of 83%.
Figure 1. Curves of statistical power to detect an effect size of $\geq 25\%$ decrease in the risk of the primary outcome, given a dropout rate of 10% and a type I error rate of 0.05.
Renal Sub-study of the
China Stroke Primary Prevention Trial (CSPPT)

Statistical Analysis Plan

Version: 2014.05.18

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1. Introduction

Hyperhomocysteinemia has long been shown to be associated with increased risk of adverse cardiovascular and renal outcomes\textsuperscript{1-4}. However, whether homocysteine-lowering therapy slows down the progression of CKD has not been adequately investigated. There have been many homocysteine-lowering randomized clinical trials to investigate the effects of folic acid and other B vitamins on prevention of cardiovascular events and death, covering a wide range of patients from different regions and ethnic groups and with different profiles of disease risk. However, few have evaluated their effects on renal outcomes\textsuperscript{5,6}, especially in populations without folic acid fortification and in patients with mild to moderate CKD.

The China Stroke Primary Prevention Trial (CSPPT) compares 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. The renal sub-study aims to examine the effects of combination of enalapril and folic acid with enalapril alone in slowing down the renal function decline in the CSPPT participants, especially in those with mild to moderated CKD.

This document describes the information and procedures deemed relevant for the analysis of the renal sub-study. For more background information of CSPPT, see the protocol of CSPPT.

2. Study Objectives and Study Design

2.1 Study Objectives

The primary objective is to evaluate whether combination of enalapril and folic acid is more effective than enalapril alone in reducing progression of CKD among adults with hypertension. Progression of CKD was defined as a decrease in eGFR of $\geq 30\%$ and to a level of $<60$ ml/min/1.73m$^2$ at the exit visit if the baseline eGFR was $\geq 60$ ml/min/1.73m$^2$, or a decrease in eGFR of $\geq 50\%$ at the exit visit if the baseline eGFR was $<60$ ml/min/1.73m$^2$, or ESRD (eGFR<15 ml/min/1.73m$^2$ or need for dialysis).

Secondary objectives are to evaluate whether combination of enalapril and folic acid is more effective than enalapril alone on the following treatment outcomes:

(1) A composite outcome of progression of CKD and all-cause death;

(2) Rapid decline in renal function, defined as an average decline in eGFR of $\geq 5$ ml/min/1.73m$^2$ per year;
(3) Annual rate of relative decline in eGFR, estimated as 
\[
1 - \frac{t}{\sqrt{\frac{\text{eGFR at exit}}{\text{eGFR at baseline}}}} \times 100\%
\]
where \(t\) is the time length in years from baseline to the exit visit.

### 2.2 Study Design

CSPPT is a multi-community, randomized, double-blind, actively-controlled trial (clinicaltrials.gov; identifier: NCT00794885) conducted in 32 communities in Jiangsu and Anhui province.

The CSPPT trial consists of three phases: the screening and recruitment period, followed by a 3-week run-in treatment period, and then a 5-year randomized treatment period. During the screening phase, trained researchers screened local residents to identify eligible hypertensive patients in participating communities. Participants who met all of the inclusion criteria and none of the exclusion conditions were entered into the 3-week run-in treatment phase in which concomitant use of other antihypertensive drugs was permitted. The main purposes of run-in treatment phase were to identify and exclude participants with poor medication compliance and intolerant to enalapril maleate. After the run-in period, qualified participants were randomly assigned to one of the two treatment groups with stratification on MTHFR C677T genotypes (CC, CT, or TT). One treatment arm received a daily oral dose of one enalapril maleate and folic acid tablet containing 10mg enalapril maleate and 0.8mg folic acid. The other arm received a daily oral dose of 10mg enalapril maleate only. The treatment assignments were masked to both the participants and the investigators. The expected treatment length was five years. Combination of other antihypertensive drugs but not B-group vitamins was allowed during the trial. After randomization, participants were scheduled for follow-up every 3 months.

The renal sub-study was initiated three years after completion of the enrollment of CSPPT. Serum creatinine of the participants at baseline and at the end of the study will be measured at a central lab for the assessment of renal function decline. Therefore high completion rate of the exit visit is essential for the success of the renal sub-study. With consideration of the historical followup data, study cost, and the study power, the renal sub-study was confined to the communities in Jiangsu province.

### 2.3 Inclusion Criteria

Inclusion criteria:

1. CSPPT participants randomized at one of the communities in Jiangsu province;
2. Baseline eGFR >30 ml/min.
3. Definitions for Statistical Analysis

3.1 Study Hypothesis

The primary study hypotheses are that enalapril maleate and folic acid tablet is more effective than enalapril maleate alone in reducing the incidence of CKD progression in all patients with essential hypertension, and in those with mild to moderate CKD at baseline.

3.2 Study Time Points and Visits

The trial consists of three phases: the screening and recruitment period, followed by a 3-week run-in treatment period, and then a 5-year randomized treatment period with visits for every 3 months. The visits are to be numbered as following:

V0: screening and recruitment patients, eligible ones entered into run-in treatment phrase;

V1: end of screening and recruitment phase, qualified patients randomized allocated to randomized treatment phase;

V2: the first 3-month visit since V1;

…

V21: 60-month visit since randomized treatment phase;

V22: exit visit, not a routine visit, conducted after the Steering Committee terminated the study, so the time periods varied with participants.

For visits after randomization (V2-V22), a successful visit for a participant is defined as the participant himself/herself showed up in the follow up and vital signs are measured (with at least two measurement of blood pressure).

3.3 Analysis Sets

The following sets will be used for different analyses:

3.3.1 Intent-to-treat set, ITT

ITT includes all subjects met the inclusion criteria (section 2.3). The ITT set will be the set for the primary efficacy analysis.

3.3.2 Per-protocol Set, PPS

PPS is a subset of ITT. PPS consists of all subjects who have no major deviation from the protocol and an overall compliance rate of 70% or higher at the end of the study.

PPS is mainly used for sensitive analysis of the primary outcome.
3.4 Subgroup Analyses

We plan to group the participants according to the specified baseline parameters; to perform subgroup analyses for the primary outcome and to assess the modifiers effect of the grouping factor on efficacy. The groupings include:

- Age; <55, 55-65, ≥65 year-old;
- Sex; male, female;
- Folic acid; tertiles;
- Hcy; tertiles;
- MTHFR C677T; CC, CT, TT;
- eGFR 30–60, ≥60 ml/min;
- Body mass index, BMI; tertiles;
- Total cholesterol, TC; tertiles;
- Diabetes yes, no
- Smoke yes, no;

We acknowledge that subgroup and interaction analyses may lack of power due to limited sample size. The subgroup analyses are exploratory.

3.5 Treatment Groups and Treatment Compliance

All patients were randomized into one to the two groups in a 1:1 ratio. One treatment arm received a daily oral dose of one enalapril maleate and folic acid tablet containing 10mg enalapril maleate and 0.8mg folic acid. The other arm received a daily oral dose of 10mg enalapril maleate only. During the study, other kinds of antihypertensive drugs can be added to control blood pressure under the guidance of doctors. The efficacy analysis will be intent-to-treat with the ITT set. PPS will be used for sensitivity analysis.

The study drugs include the masked enalapril maleate and folic acid tablet and enalapril maleate. Every 3 months, participants were followed up, their medication during the last visit period were recorded, and new study drugs were distributed. The treatment compliance in a period is calculated using the formula: number of pills taken/ days in the period x 100%. If the study drug was distributed in the last visit and the number of pills taken since the last visit is missing, the compliance rate of the current period will be filled by the method of “Last Observation Carried Forward, LOCF”. Compliance ≥70% is regard as satisfactory whereas <30% as poor.

3.6 Lost to followup and Handling of Missing Data

The serum creatinine at both baseline and the exit visit (V22) are used to evaluate the renal function decline. Lost to follow-up is defined as alive at V22 with unknown renal outcome. Participants lost to followup will be treated as missing and excluded from the renal outcome analyses.
Participants with missing smoking status and dipstick test at baseline will be assigned as non-smokers and without proteinuria, respectively, in the subsequent analyses. Values for other missing covariates at baseline (such as serum folate, total homocysteine, total cholesterol, fasting glucose, and body mass index) will be imputed from age and gender.

In calculation of treatment compliance, when the number of pills taken in a visit is missing, if study drug is distributed in the last visit and the data at the last visit is available and not filled by LOCF, the number of pills taken will be filled by the method of LOCF. Otherwise, the number is filled with “0”.

### 3.7 Definition for CKD at baseline and other variables

Participants with an eGFR <60 ml/min/1.73m² and/or proteinuria at baseline were classified as having CKD. Diabetes was defined as having a history of diabetes or a fasting glucose ≥7mmol/L 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 cumulative days taking the drug of interest.

### 3.8 Endpoint Outcomes

#### 3.8.1 Primary outcomes

The primary outcome is progression of CKD, defined as a decrease in eGFR of ≥30% and to a level of <60 ml/min/1.73m² at the exit visit if the baseline eGFR was ≥60 ml/min/1.73m², or a decrease in eGFR of ≥50% at the exit visit if the baseline eGFR was <60 ml/min/1.73m², or ESRD (eGFR<15 ml/min/1.73m² or need for dialysis).

#### 3.8.2 Secondary outcomes

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/1.73m² per year; and (3) annual rate of relative decline in eGFR, estimated as \(1 - \frac{t}{\frac{\sqrt{eGFR at exit}}{eGFR at baseline}}\) ×100%, where \(t\) is the time length in years from baseline to the exit visit.

### 3.9 Statistical Significance

We will perform analyses on the primary outcome in total, CKD, and non-CKD populations. To adjust for multiple tests, the distribution of test-wise p-values under the null will be obtained empirical by permutation of the treatment assignment. A cutoff for the test-wise p-value will be calculated from the empirical distribution to maintain a study-wise type I error rate of 0.05.

For the efficacy analysis of the three secondary outcomes, it will not be adjusted for multiple tests and a test-wise p-value of 0.05 will be used as significance boundary.
Similarly, for the exploratory subgroup analyses, correction for multiple tests will not be applied and a test-wise p-value of 0.05 will be used as significance boundary.

4 The Flow of Patients

The flow chart of patients: specify the numbers of patients randomized; the numbers of ITT patients stratified by CKD status in the two treatment arms; the numbers of patients excluded due to missing baseline eGFR or baseline eGFR<30 ml/min, and lost to follow-up.

5 Baseline Characteristics

To present summary statistics of the following baseline characteristics in strata of treatment arms and CKD status. Wilcoxon-Mann-Whitney test and $\chi^2$ test will be used to compare the difference of continuous and categorical variables, respectively, between the two treatment arms.

5.1 Demographic Characteristics

Continuous variables include: age, waist circumference, and body mass index (BMI).

Categorical variables include: sex, age groups, and BMI groups.

5.2 Vital Signs

Continuous variables include: systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure (PP) and pulse.

5.3 Biomarkers

Continuous variables include: serum tHcy, folate, B12, fasting glucose (FPG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG).

Categorical variables include: MTHFR C677T genotype (CC, CT, TT), tertiles of tHcy, folate and B12, FPG groups (<5.6, 5.6-7.0, ≥7.0 mmol/L), and TC groups (<5.2, 5.2-6.2, ≥6.2 mmol/L).

5.4 Behaviors and Habits Associated with CVD Risk

Categorical variables include: current smoking (yes, no), alcohol drinking (yes, no).
5.5 Antihypertensive Drugs at the Screening Visit

Categorical variables include: any antihypertensive drugs, calcium channel blocker (CCB), angiotensin-converting enzyme Inhibitor (ACEI), angiotensin receptor blocker (ARB), diuretic, and beta-receptor blocker (BB).

Table: usage frequency and types of antihypertensive drugs by treatment arms.

5.6 Glucose-lowering Drugs at the Screening Visit

Categorical variables include: any glucose-lowering drugs, or ATC classification and the generic name.

5.7 Lipid-lowering Drugs at the Screening Visit

Categorical variables include: any lipid-lowering drugs, or ATC classification and the generic name.

5.8 Anti-platelet Drugs at the Screening Visit

Categorical variables include: any anti-platelet drugs, aspirin, clopidogrel, or ATC classification and the generic name.

5.9 Disease History

Categorical variables include: system organ class (SOC) and preferred term (PT) according to Medical Dictionary for Regulatory Activities (MedDRA).

6 Medications

6.1 Study Drugs

• Table: the numbers of patients, summary statistics of drug compliance, and the number and percentage of patients with good compliance (≥70%) during each visit (V2-22) by treatment arms in ITT and PPS, respectively.

6.2 Concomitant Medication

• Tables: the numbers of patients with concomitant regular use of CCB, ACEI, ARB, diuretics, and BB by treatment arms in ITT and PPS, respectively.
• Tables: the numbers of patients with concomitant regular use of insulin, oral glucose-lowering drugs, statins, fibrates, nicotinic acids, aspirin and clopidogrel by treatment arms in ITT and PPS, respectively.
7 Vital Signs

- Table: the numbers of patients reached the visit and successful visit, summary statistics (q25, q50, q75, mean) of SBP, and percentage of on target (SBP<140 mmHg) by treatment arms in ITT and PPS, respectively.
- Table: the numbers of patients reached the visit and successful visit, summary statistics (q25, q50, q75, mean) of DBP, and percentage of on target (DBP<140 mmHg) by treatment arms in ITT and PPS, respectively.
- Table: the numbers of patients reached the visit and successful visit, summary statistics (q25, q50, q75, mean) of pulse pressure, and percentage of on target (SBP<140 mmHg and DBP<90 mmHg) by treatment arms in ITT and PPS, respectively.

8 Efficacy Analyses

All efficacy analyses will be intent-to-treat using the ITT set. PPS will be used for sensitivity analysis.

8.1 Primary and Secondary Outcomes

- Table: the numbers of primary and secondary outcome events, including progression of CKD, the composite event, rapid renal function decline by treatment arms and CKD status at baseline in ITT and PPS, respectively.
- Table: the mean and sd of relative decline rate in eGFR decline by treatment arms and CKD status at baseline in ITT and PPS, respectively.

8.2 Primary Efficacy Analyses

8.2.1 Primary Analyses

- Table: Odds ratios (ORs) and 95% confidence intervals (CIs) from generalized linear regression models without covariate adjustment among all participants, those with and without CDK at baseline, respectively.
- Table: ORs and 95% CIs from generalized linear regression models with adjustment for covariates including age, gender, BMI, eGFR, proteinuria, SBP, serum glucose and total cholesterol at baseline, as well as timed-average of SBP, among all participants, those with and without CDK at baseline, respectively.

8.2.2 Sensitivity Analyses

The following sensitivity analyses are planned:

- Efficacy analyses of the primary outcome using the PPS set.
8.2.3 Exploratory Analyses

This section described planned exploratory analyses, including subgroup analyses and analyses of effect modifiers.

Grouping variables for subgroup analyses include baseline age, sex, serum folate, tHcy, TC, MTHFR C677T genotype, BMI, diabetes and smoking at baseline (see 3.4).

Table: ORs and 95% CIs for each subgroup and p-value for the interaction term from generalized linear regression models with adjustment for covariates including age, gender, BMI, eGFR, proteinuria, SBP, serum glucose and total cholesterol at baseline, as well as timed-average of SBP.

8.3 Secondary Analyses

Efficacy analyses for each of the three secondary outcomes will be performed similarly in ITT. We will not perform sensitivity and exploratory analyses for secondary outcomes.

- Table: ORs and 95% CIs from generalized linear regression models for the composite event, with and without adjustment for covariates.
- Table: ORs and 95% CIs from generalized linear regression models for rapid decline in renal function, with and without adjustment for covariates.
- Table: Difference in group means and 95% CIs from generalized linear regression models for the relative rate of renal function decline, with and without adjustment for covariates.

8.4 Other Efficacy Analyses

- Table: Changes of serum folate and Hcy levels from baseline to the exit visit (absolute and relative values) by treatment arms and the corresponding p-value for group difference.

9 Safety Analyses

Renal sub-study will not perform any safety analyses.

10 References


Enalapril Maleate and Folic Acid Tablets for Primary Prevention of Stroke in Patients with Hypertension: A Post-marketing, Double-blind, Randomized Controlled Trial

(China Stroke Primary Prevention Trial, CSPPT)

Clinical Trial Protocol

Study Drug:

Enalapril maleate and folic acid tablets

Comparator:

Enalapril maleate tablets

Principal Investigator:

Yong Huo, M.D, Peking University First Hospital, Beijing, China

Chief Coordinating Organization:

Anhui Biomedical Institute, Anhui Medical University, Hefei, China

Sponsor:

Shenzhen AUSA Pharmed Co. Ltd, Shenzhen, China

Protocol Version 3.0: May 13, 2009

(Previous versions: Version 1.0: April 12, 2008; Version 2.0 August 21, 2008)
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1. CSPPT PROTOCOL SYNOPSIS

<table>
<thead>
<tr>
<th><strong>Official Title</strong></th>
<th>Enalapril Maleate and Folic Acid Tablets for Primary Prevention of Stroke in Patients with Hypertension: A Post-marketing, Double-blind, Randomized Controlled Trial</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary Objective</strong></td>
<td>The purpose of this trial is to confirm that enalapril-folic acid tablet is more effective in preventing stroke among patients with essential hypertension when compared to enalapril.</td>
</tr>
<tr>
<td><strong>Study Design</strong></td>
<td>Randomized, double-blind, multi-community, long-term parallel comparative clinical trial. The trial consists of three stages: screening and recruitment; a 3-week run-in treatment period; and a 5-year randomized treatment period. Screening and recruitment: During the screening stage, each participant completes a physical examination and questionnaires on lifestyle and disease and medication history. Blood pressure will be taken in two separate days. Run-in period: participants are given an enalapril 10 mg tablet for three weeks. Those who show good tolerance and compliance with the treatment are eligible to enter into the next period. Genotyping for methylenetetrahydrofolate reductase (MTHFR) C677T polymorphisms is also performed at this stage.</td>
</tr>
</tbody>
</table>
### Patient Population

Hypertensive patients without history of stroke or myocardial infarction (MI).

**Inclusion criteria for Run-in Period:**

1. Seated systolic blood pressure (SBP) $\geq 140$ mmHg or diastolic blood pressure (DBP) $\geq 90$ mmHg at both the screening and recruitment visit; or currently under anti-hypertension treatment;
2. 45 - 75 years old;
3. For pre-menopausal women, agreed to use contraceptives during the trial;
4. Signed the written informed consent.

**Inclusion criteria for Double-blind Treatment Period**

After the run-in period, qualified participants who met all of the inclusion criteria as following and none of the exclusion conditions entered randomized treatment phase.

---

Randomization and treatment: Qualified participants are randomly assigned to one of the two double-blind treatment groups stratified by MTHFR C677T genotypes (CC, CT, or TT). One treatment group receives a daily oral dose of one enalapril-folic acid tablet containing 10 mg enalapril and 0.8 mg folic acid. The other group receives a daily oral dose of 10 mg enalapril.
|   | 1. Satisfactory compliance and tolerant to adverse reaction;  
|   | 2. No cardiovascular events in the run-in treatment period;  
|   | 3. No plans of long-term working or living off town, can be followed up at least every 6 months;  
|   | 4. MTHFR C677T genotype available.  

**Sample Size**

We aim to have at least 80% power to detect a minimal true effect size of HR=0.8 with an alpha level of 0.05 during a five year follow-up. Assuming an annual stroke incidence rate of 0.7%, a sample size of 20337 is required.

**Randomization and Blinding**

Treatment allocation is randomized with a fixed block size of 4 and stratification on MTHFR C677T genotype in a 1:1 ratio. The randomization is performed by phone call to the Study-drug Management Center. The study drugs, enalapril-folic acid tablet and enalapril tablet, are concealed in a single-capsule formulation and identical in appearance, size, color, and taste.

**Treatment**

After the Run-in Period, qualified participants are randomly assigned to one of the two treatment groups:

- One group receives a daily oral dose of one
enalapril-folic acid fixed dose combination tablet containing 10 mg enalapril and 0.8 mg folic acid.

- The other group receives a daily oral dose of 10 mg enalapril.

Expected treatment period is 5 years. Combination of other anti-hypertensive drugs but not B-vitamins is allowed.

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Primary outcome:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First attack of symptomatic stroke</td>
</tr>
</tbody>
</table>

**Secondary outcomes:**

- Composite cardiovascular events;
- All-cause death;
- First attack of ischemic stroke;
- First attack of hemorrhagic stroke;
- Myocardial infarction

**Other outcomes**

- Malignant tumors

<p>| Study Period | A total of 7 years to complete the study: 1 year for enrollment; 5 years of double-blind treatment; and 1 year for data lock, statistical analysis, and preparation of the clinical study report (CSR) and publication. |</p>
<table>
<thead>
<tr>
<th>Safety Measures</th>
<th>Adverse events and serious adverse events recorded.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sites</td>
<td>32 sites in Jiangsu Province and Anhui Province, China</td>
</tr>
<tr>
<td>Statistical Analysis</td>
<td>The primary study hypothesis is that enalapril and folic acid tablet is more effective than enalapril alone in reducing the incidence of stroke in patients with essential hypertension. The primary outcome is time from randomization to the first attack of symptomatic stroke. The primary analysis will be intent-to-treat. The cumulative event rates of the primary outcome in the enalapril-folic acid and the enalapril group, respectively, will be estimated using the Kaplan-Meier method and compared using the log-rank test. The hazard ratio and 95% confidence intervals will be estimated by the Cox proportional hazard regression model. Pre-specified subgroup analyses will also be performed according to the Statistical Analysis Plan. Secondary outcomes will also be analyzed similarly by intent-to-treat. Interim analyses are scheduled for every increment of 200 confirmed stroke events. However, unscheduled interim analysis is also allowed. To maintain the overall type-I error at an alpha level of 0.05, the O’Brien-Fleming alpha-spending function will be used to define the boundaries of statistical significance of the interim and the final</td>
</tr>
</tbody>
</table>
1.1 Study Flow Chart

![Study Flow Chart](image)

Figure 1. Study Flow Chart of the China Stroke Primary Prevention Trial (CSPPT)
1.2. The Follow-up Visits and Items

Table 1. The follow-up visit schedule and scope of data collection

<table>
<thead>
<tr>
<th>Items</th>
<th>Follow-up visits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V0</td>
</tr>
<tr>
<td>Months from V1</td>
<td>-21 days</td>
</tr>
<tr>
<td><strong>General Information</strong></td>
<td></td>
</tr>
<tr>
<td>Informed consent</td>
<td>√</td>
</tr>
<tr>
<td>Demographic, and medical history</td>
<td>√</td>
</tr>
<tr>
<td>Physical exams</td>
<td>√</td>
</tr>
<tr>
<td>BP &amp; pulse</td>
<td>√</td>
</tr>
<tr>
<td>Questionnaires</td>
<td>√</td>
</tr>
<tr>
<td><strong>Experimental drugs and drug combination</strong></td>
<td></td>
</tr>
<tr>
<td>Drug dispense</td>
<td>√</td>
</tr>
<tr>
<td>Compliance</td>
<td>√</td>
</tr>
<tr>
<td>Concomitant medications</td>
<td>√</td>
</tr>
<tr>
<td><strong>Efficacy and safety indicators</strong></td>
<td></td>
</tr>
<tr>
<td>Outcomes</td>
<td>√</td>
</tr>
<tr>
<td>Adverse events</td>
<td>√</td>
</tr>
<tr>
<td><strong>Laboratory</strong></td>
<td></td>
</tr>
<tr>
<td>Biochemical tests*</td>
<td>√</td>
</tr>
<tr>
<td>ECG</td>
<td>√</td>
</tr>
<tr>
<td>tHcy, folic acid*</td>
<td>√</td>
</tr>
<tr>
<td>MTHFR genotype*</td>
<td>√</td>
</tr>
<tr>
<td>Other exams</td>
<td>(✓)</td>
</tr>
</tbody>
</table>

*Tested at central laboratory.

Items for V14, V16, V18, and V20 are the same as for V15.

(✓) For a fraction of the study participants.
2. BACKGROUND AND RATIONALE

Considerable interest and debate remain over whether folic acid supplementation, which corrects folate insufficiency and lowers homocysteine, will help to prevent stroke. In 1969, McCully first suggested that even moderate increases in homocysteine could accelerate atherosclerosis, and then proposed the “homocysteine theory of atherosclerosis” [1, 2]. Subsequently abundant epidemiologic studies, mostly conducted before mandatory folic acid fortification in the US and Canada, shown an independent and graded association between homocysteine levels and cardiovascular risk [3, 4]. And a recent population-based study demonstrated that the decline in stroke mortality accelerated from 1998-2002 across nearly all population strata in the US and Canada; in contrast, the decline in stroke mortality in England and Wales (where fortification is not mandatory) did not change significantly between 1990 and 2002 [5]. However, the relevant randomized trials have not shown a beneficial effect of folic acid supplementation in preventing cardiovascular diseases [6-13]. Notably, while results were negative for other cardiovascular endpoints, the HOPE-2 study showed that fewer patients assigned to active treatment than to placebo had a stroke (HR=0.75; 95%CI: 0.59-0.97) [8]. Consistently, elevated homocysteine was more strongly associated with stroke than ischemic heart disease: for each 5 μmol/L elevation in serum homocysteine, the risk was increased by 59% for stroke versus 32% for ischemic heart disease; and for each 3μmol/L decrease in homocysteine level, the risk reduction was 24% for stroke versus 16% for ischemic heart disease [4]. Another meta-analysis also showed that with a 25% reduction in homocysteine, the stroke risk reduction (19%) was greater than that for ischemic heart disease (11%) [3]. This raises the possibility that folic acid supplementation might be
more effective for stroke prevention than for other cardiovascular outcomes. Furthermore, most relevant trials were conducted in regions with high dietary folate intake or grain fortification with folic acid [6, 12, 13], and therefore may be underpowered to detect a beneficial effect (Table 1). Also, notably, the cohort studies sampled relatively healthy subjects, whereas most clinical trials recruited patients with a history of cardiovascular diseases [6-9, 11-13] (Table 1).

Table 1. Study characteristics and results of eight randomized controlled trials with folic acid using stroke reported as one of the end points from January 1966 to July 2006

<table>
<thead>
<tr>
<th>Data Source</th>
<th>Subjects</th>
<th>Age, yr Mean (SD)</th>
<th>Male,%</th>
<th>Preexistent Diseases</th>
<th>Treatment Duration, mo</th>
<th>Grain Fortification</th>
<th>RR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toole et al⁰</td>
<td>3680</td>
<td>66.3</td>
<td>62.5</td>
<td>Stroke</td>
<td>24</td>
<td>Yes</td>
<td>1.04(0.84~1.29)</td>
</tr>
<tr>
<td>Liem et al¹¹</td>
<td>593</td>
<td>65.2(9.8)</td>
<td>78.0</td>
<td>CHD</td>
<td>42</td>
<td>No</td>
<td>0.65(0.27~1.57)</td>
</tr>
<tr>
<td>Lonn et al⁰³</td>
<td>5522</td>
<td>68.9(6.9)</td>
<td>71.8</td>
<td>CHD</td>
<td>60</td>
<td>Partial</td>
<td>0.76(0.59~0.96)</td>
</tr>
<tr>
<td>Bonaa et al⁰⁷</td>
<td>2815</td>
<td>63.0(11.7)</td>
<td>73.7</td>
<td>CHD</td>
<td>36</td>
<td>No</td>
<td>0.91(0.58~1.45)</td>
</tr>
<tr>
<td>Zoungas et al¹²</td>
<td>315</td>
<td>56(13.5)</td>
<td>32.0</td>
<td>ESRD</td>
<td>43</td>
<td>Yes</td>
<td>0.45(0.20~1.01)</td>
</tr>
<tr>
<td>Wrone et al¹³</td>
<td>510</td>
<td>60.2(15.1)</td>
<td>50.0</td>
<td>ESRD</td>
<td>24</td>
<td>Yes</td>
<td>1.17(0.52~2.61)</td>
</tr>
<tr>
<td>Righetti et al⁰⁹</td>
<td>88</td>
<td>64.3(11.7)</td>
<td>56.0</td>
<td>ESRD</td>
<td>29</td>
<td>No</td>
<td>0.55(0.19~1.62)</td>
</tr>
<tr>
<td>Mark et al¹⁰</td>
<td>3318</td>
<td>55</td>
<td>44</td>
<td>Esophageal dysplasia</td>
<td>72</td>
<td>No</td>
<td>0.63(0.37~1.07)</td>
</tr>
</tbody>
</table>

Our recent meta-analysis included 16,841 subjects from 8 qualified folic acid randomized trials with stroke reported as one of the end points (Table 1) [14]. The analysis was further stratified by factors that may affect the treatment effects. Pooling all of the 8 trials [6-13], folic acid supplementation significantly reduced the risk of stroke by 18% (RR: 0.82; 95% CI: 0.68-1.00; \( P=0.045 \)). In the stratified analyses, a greater beneficial effect was observed among those trials with a treatment duration >36 months (0.71; 0.57-0.87; \( P=0.001 \)) [7, 10-12]; no or partial grain fortification (0.75; 95% CI: 0.62-0.91; \( P=0.003 \))
[7-11]; and no prior history of stroke (0.75; 0.62-0.90; \( P=0.002 \)) [10-13]. In the corresponding comparison groups the estimated RRs were attenuated and insignificant. Our analysis provides coherent evidence that folic acid supplementation could effectively reduce stroke risk in primary prevention and that such effect is likely to be causal. It is recommended that future clinical trials, conducted in regions without grain fortification, with adequate length of follow-up, and among subjects without prior history of stroke, might provide a definitive answer to this important clinical and public health question. The ability to clarify whether there are groups of individuals who may benefit from homocysteine lowering is critical from a research and a population health perspective.

Methylenetetrahydrofolate reductase (MTHFR), a major regulatory enzyme for homocysteine metabolism, converts 5, 10-methylenetetrahydrofolate into 5-methyltetrahydrofolate, a co-substrate for homocysteine remethylation to methionine. A single nucleotide polymorphism at nucleotide position 677 (C677T, rs1801133) leads to the substitution of alanine with valine at amino acid 222 of the encoded enzyme. The 677T allele encodes a thermolabile enzyme with reduced activity, which gives rise to increased concentrations of plasma homocysteine and lower levels of serum folate and thereby confers a higher risk for stroke, particularly in those with low folate intake [15].

Furthermore, previous studies have provided strong evidence that hypertension and hyperhomocysteinemia are the two most important modifiable risk factors for stroke [16]. More importantly, these two factors have shown a multiplicative effect on cardiovascular disease risk [17]. Furthermore, Albert et al. [18] reported a significantly interactive effect between angiotensin-converting enzyme inhibitors (ACEI) and folic acid therapy (\( P=0.03 \)) in cardiovascular disease (CVD) risk reduction. Conceivably, individuals with
concomitant hypertension and elevated homocysteine (≥10 μmol/L), which has been named Hyperhomocysteinemia-type hypertension (H-type hypertension) [19], may particularly benefit from homocysteine-lowering therapy along with anti-hypertension therapy, especially with ACEI.

Given the ongoing controversy over the use of homocysteine-lowering therapy to reduce stroke risk and its significance from both a clinical and population health perspective, the China Stroke Primary Prevention Trial (CSPPT) was designed to provide conclusive evidence that the effects of folic acid therapy on stroke primary prevention can be readily observed in populations with low folate intake and other important characteristics such as a high prevalence of elevated homocysteine, hypertension and low cardiovascular disease burden. Furthermore, consideration of the MTHFR C677T genotype will further help to identify individuals who would greatly benefit from folic acid therapy.

CSPPT has the following unique features. First, the CSPPT is by far the largest randomized trial to evaluate the stroke primary prevention effect of a combined anti-hypertension and homocysteine-lowering therapy. Second, CSPPT will be conducted in a population that is expected to greatly benefit from folic acid therapy. Prior to the CSPPT, there had been a particular lack of adequately powered randomized clinical trials specifically targeting stroke primary prevention in Asian countries/regions with a high burden of stroke and without folic acid fortification. For example, stroke has been the leading cause of death, and the age standardized incidence rates of ischemic stroke have increased by 8.7% (CI: 4.3, 8.9, \( p < 0.05 \)) annually from 1984 to 2004 in China [20]. Furthermore, the prevalence of hypertension in the adult Chinese population aged 35 to 74 years was 27.2% from 2000-2001 [21]. Most importantly, about 75% of the
hypertensive adults also had elevated homocysteine [22], which may possibly explain the extremely high incidence of stroke among Chinese hypertensive adults, even after control for blood pressure [23]. Thirdly, the CSPPT focuses on patients without pre-existing stroke or MI. The low vascular disease burden and low percentage use of cardiac and vascular protective drugs (e.g., statin, aspirin, et al) make our results less likely to be confounded by pre-existing clinical conditions and medications that may affect the therapeutic effect. Moreover, the CSPPT will be the first randomized control trial to evaluate the stroke prevention effect of homocysteine-lowering therapy as well as consider individual MTHFR C667T genotypes. Qualified participants are randomly assigned to one of two treatment groups using random permuted blocks stratified by MTHFR C677T genotypes (CC, CT, or TT). This is both innovative and informative for individual risk-benefit assessment and for developing personalized treatment plans.

As such, the CSPPT was launched to evaluate whether a combination therapy (enalapril maleate and folic acid tablets) is more effective than enalapril maleate alone in reducing the incident stroke in patients with essential hypertension but without history of stroke and MI.

3. STUDY OBJECTIVES

3.1 Primary Objective

The primary objective is to evaluate whether an enalapril and folic acid tablet (enalapril 10 mg and folic acid 0.8 mg/day) is more effective than enalapril (10 mg/day) alone in reducing the incidence of stroke in patients with essential hypertension.
3.2 Secondary Objectives

The secondary objectives are to evaluate whether an enalapril and folic acid tablet is more effective than enalapril alone in reducing the incidence of the following endpoint events:

- composite cardiovascular events including death due to cardiovascular causes, stroke and MI;
- all-cause death;
- first ischemic stroke;
- first hemorrhagic stroke; and
- MI.

3.3 Others Objectives

3.3.1 To compare the incidence of malignant tumors between the two treatment groups;

3.3.2 To explore the effect of MTHFR C677T genotypes on modifying the efficacy of an enalapril-folic acid tablet in reducing the risks of cardio-cerebral-vascular events.

4. STUDY DESIGN AND MANAGEMENT OVERVIEW

4.1 Study Design Overview

CSPPT is a randomized, double-blind, actively-controlled trial (clinicaltrials.gov identifier: NCT00794885) to be conducted in 32 community hospitals in Jiangsu Province and Anhui Province, China.
The primary objective is to evaluate whether an enalapril and folic acid tablet (enalapril 10 mg and folic acid 0.8 mg/day) is more effective than enalapril (10mg/day) alone in reducing the incidence of stroke in patients with essential hypertension.

This study was approved by the Independent Ethics Committee (IEC) of the Institute of Biomedicine, Anhui Medical University, Hefei, China (FWA assurance number: FWA00001263).

Anhui Biomedical Institute, Anhui Medical University serves as the chief coordinating organization, whereas the community hospitals are collaborating sites helping to recruit patients and carry out the trial. The Anqing Institute of Biomedical Research and the Lianyungang Cardiovascular Disease Prevention and Control Center are the coordinating centers responsible for coordinating the research activities in Anhui Province and Jiangsu province, respectively.

The trial consists of three stages: screening and recruitment; a 3-week Run-in Period; and a 5-year randomized treatment period (as illustrated in Figure 1).

4.2 Study Milestones

A 7-year study plan has been proposed; key study milestones are outlined below.

**STUDY MILESTONES**

Patient Recruitment 2008.05-2009.05  
Double-blind Follow-Up 2008.06-2014.08
4.3 Randomization of Treatments and Emergency Unblinding Procedure

4.3.1 Method of randomization of treatment allocation

CSPPT has two treatment groups, one receiving treatment with enalapril-folic acid and the other with enalapril. Eligible patients, stratified by the MTHFR C677T genotype, are assigned randomly with a fixed block size of four to one of the two treatment groups in a 1:1 ratio.

Randomization of treatment allocation is accomplished by phone call to the Study-drug Management Center where four randomization tables generated by the Statistical Group are used for the randomization process. Of the four tables, one is a randomization of drug code and treatment allocation, and the other three are MTHFR genotype-specific (CC, CT, and TT, respectively) randomized sequences of allocation assignments with a fixed block size of four. A unique drug-code is assigned to each participant, first by looking up the first available allocation assignment in the genotype-specific table, and then the first available drug-code for the assigned allocation type. The drug-code is used to identify the study participants and the drug received during the trial. All investigators and study participants are blinded to the randomization procedure and the treatment assignments.
4.3.2 Method of blinding

The drugs, enalapril-folic acid tablet and enalapril tablet, will be concealed in a single-capsule formulation and will be identical in appearance, size, color, and taste and bottled in the amount of 100 pills. Bottles of the two types of the concealed drugs will be sent to a centralized Study-drug Management Center. The Study-drug Management Center, who will maintain a copy of the drug-code and treatment allocation table, will label the bottles with corresponding drug-code and distribute the drugs to the study sites.

4.3.3 Emergency unblinding procedure

Investigators will not have access to the randomization code, except in special circumstances, such as the occurrence of a serious adverse event for which knowledge of the study medication would be considered essential for treating the study participant. In such a case, the investigators will make an emergency call to the Study-drug Management Center (24-hour line) for the unblinding service. The investigator will document the date, time of day and reason for the code break, and report all information to the Principal Investigator. The study drug will not be resumed after unblinding.

4.4 Study Organizations

4.4.1 Principal investigator (PI):

Yong Huo, MD, Department of Cardiology, Peking University First Hospital, Beijing, China

4.4.2 Steering committee:

The steering committee will provide scientific and strategic direction for the trial and will have overall responsibility for its design, execution, and publication. The steering
committee will also be responsible for ensuring that study execution and management are of the highest quality. It will approve the protocol and the operational guidelines of the trial prior to its commencement. The steering committee will convene regularly by teleconference or face-to-face meetings to discuss and report on the progress of the trial. The composition of the steering committee and its responsibilities will be described in a charter that will be finalized before the start of the trial.

**Steering committee members:**

Lisheng Liu (Chair), M.D., Division of Hypertension, Fuwai Hospital, Beijing, China.

Yong Huo (Co-Chair), M.D., Department of Cardiology, Peking University First Hospital, Beijing, China.

Kejiang Cao, M.D., Department of Cardiology, Nanjing Medical University, Nanjing, China.

Luyuan Chen, M.D., Department of Cardiology, Guangdong Provincial Hospital Guangzhou, China.

Xiaoshu Cheng, M.D., Department of Cardiology, Second Affiliated Hospital, Nanchang University, Nanchang, China.

Yimin Cui, Ph.D., Department of Pharmacy, Peking University First Hospital, Beijing, China.

Qiang Dong, M.D., Department of Neurology, State Key Laboratory of Medical Neurobiology, Fudan University, Shanghai, China.
Junbo Ge, M.D.: Shanghai Institute of Cardiovascular Diseases, Department of Cardiology, Zhongshan Hospital, Fudan University, Shanghai, China.

Pingjin Gao, M.D.; Shanghai Institute of Cardiovascular Diseases, Department of Cardiology, Zhongshan Hospital, Fudan University, Shanghai, China.

Runlin Gao, M.D., Department of Cardiology, Fuwai Hospital, CAMS & PUMC, Beijing, China.

Dayi Hu, M.D., Department of Cardiology, Peking University People’s Hospital, Beijing, China.

Fanfan Hou, M.D., Ph.D., National Clinical Research Center for Kidney Disease; State Key Laboratory of Organ Failure Research; Nanfang Hospital, Southern Medical University, Guangzhou, China.

Xunming Ji, M.D., Department of Neurology, Xuanwu Hospital, Capital Medical University, Beijing, China.

Jianping Li, M.D., Ph.D., Department of Cardiology, Peking University First Hospital, Beijing, China.

Nanfang Li, M.D., Department of Cardiology, Xinjiang Uygur Autonomous Region People’s Hospital, Urumqi, China.

Xiaoying Li, M.D., Department of Geriatric Cardiology, General Hospital of the People's Liberation Army, Beijing, China.

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Wen Wang, M.D., National Center for Cardiovascular Diseases, Fuwai Hospital 
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Xian Wang, M.D., Ph.D., Department of Physiology and Pathophysiology, School of 
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Chuanshi Xiao, M.D., Department of Cardiology, First Affiliated Hospital, Shanxi 
Medical University, Taiyuan, China.

Xinchun Yang, M.D., Department of Cardiology, Beijing Chaoyang Hospital, 
Capital Medical University, Beijing, China.

Dingliang Zhu, M.D., State Key Laboratory of Medical Genomics, Shanghai Key 
Laboratory of Hypertension, Ruijin Hospital, Jiao Tong University School of 
Medicine, Shanghai, China.

Gang Zhao, M.D., Department of Neurology, Xijing Hospital, Forth Military 
Medical University, Xi'an, China.

Lianyou Zhao, M.D., Department of Cardiology, Tangdu Hospital, the Fourth 
Military Medical University, Xi'an, China.

4.4.3 Executive committee:

The responsibilities of the Executive Committee are to supervise the study progress and
provide guidance. They will also work on the execution of the study, assuming the leadership roles in different functions. Important decisions will be made by the committee in face-to-face meetings.

**Executive committee members:**

Yong Huo (Chair and Principal Investigator), M.D., Department of Cardiology, Peking University First Hospital, Beijing, China.

Yefeng Cai, M.D., Department of Neurology, Guangdong Provincial Hospital of Chinese Medicine, Guangzhou, China.

Yimin Cui, Ph.D., Department of Pharmacy, Peking University First Hospital, Beijing, China.

Xiaohua Dai, M.D., Department of Cardiology, First Affiliated Hospital of Anhui University of Traditional Chinese Medicine, Hefei, China.

Fangfang Fan, M.D. Ph.D., Department of Cardiology, Peking University First Hospital, Beijing, China.

Jia Fu, M.D., Department of Neurology, First Affiliated Hospital of Anhui Medical University, Hefei, China.

Xianbin Gao, M.D., Division of Neurology, Donghai County People’s Hospital, Lianyungang, China.

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Jianping Li, M.D., Ph.D., Department of Cardiology, Peking University First Hospital, Beijing, China.

Xianhui Qin, Ph.D., National Clinical Research Center for Kidney Disease; State Key Laboratory of Organ Failure Research; Nanfang Hospital, Southern Medical University, Guangzhou, China; Institute for Biomedicine, Anhui Medical University, Hefei, China.

Guoping Sun, M.D., Department of Oncology, First Affiliated Hospital of Anhui Medical University, Hefei, China.

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Bangning Wang, M.D., Department of Cardiology, First Affiliated Hospital of Anhui Medical University, Hefei, China.

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Huamin Zhang, M.D., Division of Neurology, Ganyu County People’s Hospital, Lianyungang, China.

Chengguo Zhang, M.D., Department of Neurology, First People's Hospital of Foshan, Foshan, China.

Luosha Zhao, M.D., Department of Cardiology, First Affiliated Hospital of Zhengzhou University, Zhengzhou, China.

Yan Zhang, M.D., Ph.D., Department of Cardiology, Peking University First
Hospital, Beijing, China.

4.4.4 Data safety and monitoring board (DSMB):

The responsibility of the DSMB is to provide independent review and evaluation to the safety and efficacy data of the CSPPT to ensure that the study meets the highest standards of ethics and patient safety. DSMB will review the accumulated safety and efficacy data of the trial provided by the investigators, provide comprehensive evaluation of the rationale, safety, and ethical aspects of the trial, and make independent formal suggestions in written on whether to continue, modify, suspend, or end-up the trial to the Steering Committee. The Steering Committee, and the PI will then make the final decision on whether to accept or reject them. All the decisions must be made so as to provide the best protection of the safety and health of study participants in accordance to GCP, the Helsinki Declaration, and the regulations of China SFDA.

DSMB members:

Longde Wang (Chair), M.D. Ph.D., School of Public Health, Peking University Health Science Center, Beijing, China; Chairman of the Chinese Preventive Medicine Association, Beijing, China.

Yundai Chen, M.D., Department of Cardiology, General Hospital of the People’s Liberation Army, Beijing, China.

Aiqun Huang (Secretary), M.D. Ph.D., National Center for Women and Children’s Health, Beijing, China.

Yong Li, M.D., Department of Cardiology, Shanghai Huashan Hospital, Fudan University, Shanghai, China.
4.4.5 Independent statistical group (ISG):

The ISG assumes the following roles:

- Provide a randomization plan and randomization table before initiation of the trial;
- Provide statistical support to the DSMB during the double-blind treatment period of the trial before data-lock;
- Develop the statistical analysis plan before data-lock;
- Execute statistical analysis and draft the statistical analysis report for the trial.

ISG members:

Dafang Chen, Ph.D., Department of Epidemiology & Biostatistics, School of Public Health, Peking University Health Science Center, Beijing, China.
Lee-Jen Wei, Ph.D., Department of Biostatistics, Harvard School of Public Health, Boston, USA.

4.4.6 Event adjudication committee (EAC):

Clinical outcome events (stroke, MI, death and tumor) will be reviewed by independent EAC experts consist of neurologists, cardiologists, oncologists and death statistical expert. An EAC charter including membership, role and responsibilities will be approved before the start of the trial by the Event Adjudication Committee and the Executive Committee.

EAC members:

Yining Huang (Chair), M.D., Department of Neurology, Peking University First Hospital, Beijing, China.

Fang Chen, M.D., Department of Cardiology, Beijing Anzhen Hospital, Capital Medical University, Beijing, China.

Jingwu Dong, M.D., International Disease Classification Collaboration Center, WHO-Peking Union Medical College Hospital, Beijing, China.

Jin Gu; M.D., Ph.D., Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education), Department of Colorectal Surgery, Peking University Cancer Hospital and Institute, Beijing, China.

Jingxuan Guo, M.D., Department of Cardiology, Peking University Third Hospital, Beijing, China.

Lin Shen, M.D., Ph.D., Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education), Department of Gastrointestinal Oncology,
4.5 Site Training, Certification, and Update

The Executive committee has already provided training to all investigators and their assistants on Good Clinical Practice Guidelines, the study protocol and information collection method for outcome events. A formal training program will be completed prior to initiation of patient enrollment. In these training modules, the patient selection criteria and follow-up procedures will be reviewed. Case studies will be used to illustrate potential problems in adhering to the study protocol and blinding requirements.

All investigators and their assistants must complete the following training modules and receive certification:

- Study protocol
- GCP Guidelines
- Information collection method for outcome events, including imaging data (taking and storing electronic pictures)
- Collection, storage and transportation of biological samples
- Other SOPs

A detailed manual of operating procedures will serve as the primary document describing
all study related procedures. It will serve as a guide for training all investigators and their assistants and will be updated periodically. The Executive Committee will formulate answers to the audience in consultation with the Steering Committee.

The Executive Committee will manage and conduct site visits and ensure the integrity and validity of the data recorded on the Study Record Forms.

### 4.6 Follow-up Visits

Subject encounters will include a screening visit (Visit 0, generally occurring over two separate days), randomization visit (Visit 1) after a 3 week Run-in Period, and a follow-up visit every 3 months during the double blind treatment period. Finally, the subject will be visited either in the hospital or at home if there is a possible outcome event.

### 4.7 Outcome Events

According to the objectives of the trial, the outcomes of the trial are divided into primary, secondary, and other outcomes.

**Primary Efficacy Outcome Event**

The primary outcome of this trial is the first attack of symptomatic stroke (fatal and non-fatal).

**Secondary Efficacy Outcome Events**

Secondary outcome events include:

- composite cardiovascular events, including cardiovascular death, cerebrovascular
death, non-fatal stroke (ischemic and hemorrhagic), and myocardial infarction;

- all-cause death, including death due to any known and unknown reason;
- the first attack of ischemic stroke (fatal and non-fatal);
- the first attack of hemorrhagic stroke (fatal and non-fatal); and
- myocardial infarction (fatal and non-fatal).

Other outcomes events

Other outcomes include:

- malignant tumors

The site investigators, with the help of their assistants, will be in charge of collecting information on outcome events according to the protocol and SOP, and submitting it to the independent EAC. The outcomes approved by the Committee will be used in the subsequent analyses.

The diagnostic criteria, procedures of data collection, and diagnostic criteria for the outcome events can be found in the attached protocol: “Event Adjudication SOP”.

5. **SUBJECT SCREEN**

5.1 **Diagnosis of Hypertension:**

According to the 2005 “China Hypertension Treatment and Prevention Guidelines” and the 2003 “WHO/ISH Hypertension Guidelines”, patients with seated systolic blood pressure (SBP) \( \geq 140 \text{ mmHg} \) and/or seated diastolic blood pressure (DBP) \( \geq 90 \text{ mmHg} \) at
both the screening and recruitment visits; or patients with a previous hypertension diagnosis and currently under anti-hypertensive treatment, and with exclusion of secondary hypertension, can be diagnosed with hypertension for this trial.

5.2 Inclusion Criteria for Run-in Period:

1. Seated systolic blood pressure (SBP) ≥140 mmHg or diastolic blood pressure (DBP) ≥90 mmHg at both the screening and recruitment visit; or currently under anti-hypertension treatment;

2. 45 - 75 years old;

3. For pre-menopausal women, agreed to use contraceptives during the trial;

4. Signed the written informed consent.

5.3 Exclusion Criteria:

1. History of stroke;

2. History of myocardial infarction;

3. History of physician-diagnosed heart failure;

4. Post-coronary revascularization;

5. Severe somatic disease who is not able to complete the trial;

6. Secondary hypertension;

7. Congenital or acquired organic heart diseases;

8. Contraindicated or allergic to ACEI;
9. History of severe ACEI adverse effects;

10. Long-term use of folic acid, vitamin B12 or vitamin B6;

11. Pregnant or breastfeeding women;

12. Severe mental disorders;

13. Individuals with abnormal laboratory tests and clinical manifestations who are unsuitable to participate as judged by the investigators;

14. Unwilling to participate in the trial; unwilling to change current antihypertensive treatment.

5.4 Inclusion Criteria for the Double-blind Treatment Period

1. Satisfactory compliance and tolerant to adverse reaction;

2. No CVD events in the run-in treatment period;

3. No plans of long-term working or living off town, can be followed up at least every 6 months;

4. MTHFR C677T genotype available.

5.5 Discontinuation Criteria

1. After entering into the Double-blind Treatment Period, it is discovered that the study participant does not meet the inclusion criteria or shows any item listed in the exclusion criteria which was not discovered during the screening and randomization visits.
2. During the Double-blind Treatment Period, the 12, 13, or 14 item of the exclusion criteria (listed above) occur in a study participant. The participant should then discontinue treatment and detailed records should be noted in the Study Record Form and then transfer to the eCRF.

3. Serious adverse reaction, hypertensive crisis, or severe deterioration of the current disease occurs during the Double-blind Treatment Period, and the Investigator considers that the study participant must stop the trial.

4. Stroke or other cardiovascular event occurs during the Double-blind Treatment Period; the investigator should then decide whether the study participant continues the study treatment based on a risk-benefit balance.

5. Study participant voluntarily withdraws his/her consent.

6. TREATMENTS

6.1 Study Drugs

The objective of this trial is to compare the efficacy of enalapril-folic acid tablet vs. enalapril in reducing stroke and cardiovascular events. A double blind fixed dose long-term treatment observation will be applied in the trial. During the Run-in Period, study participants are given an oral daily dose of 10 mg of enalapril for three weeks. During the Double-blind Treatment Period, study participants are randomly assigned to receive a daily oral dose of 10 mg enalapril or enalapril 10 mg-folic acid 0.8 mg tablet. The expected treatment length is five years.
Investigators will not have access to the randomization (treatment) code, except in special circumstances, such as the occurrence of a serious adverse event for which knowledge of the study medication would be considered essential for treating the study participant (section 4.3).

6.2 Formulations of Study Medications

1. Enalapril Maleate and Folic Acid Tablet of a dosage of 10.8 mg, each tablet contains 10 mg of enalapril maleate and 0.8 mg of folic acid;

2. Enalapril maleate of a dosage of 10 mg, each tablet contains 10 mg enalapril maleate.

3. The tablets are encapsulated for blinding reason during the study, including the Run-in Period.

6.3 Route of Administration

Oral

6.4 Dose Regimen

1. Run-in Period: enalapril maleate 10 mg once a day;

2. Double-blind Period:

   a) Group a: enalapril maleate 10 mg, once a day;

   b) Group b: enalapril maleate and folic acid tablet 10.8 mg, once a day
7 STUDY DRUG HANDLING

7.1 Drug Supply

The Shenzhen AUSA Pharmaceutical Co Ltd will supply the blinded, encapsulated study drugs. All investigational drug supplies in the study will be stored in a secure, safe place, under the responsibility of the Investigator or other authorized individual, and according to the storage conditions described on the labeling.

7.2 Packaging and Labeling

7.2.1 Drug packaging

To administer the investigational products in a double blind way, encapsulated study drugs are used throughout the treatment period. Drugs for the Run-in Period are put into 30ml bottles (21 capsules per bottle); while drugs for the Double-blind Treatment Period are put into 120ml bottles (100 capsules per bottle). Capsules, bottles and caps must pass the drug factory inspections before delivery.

7.2.2 Label design of medicine bottles

The main content printed on the label should include the number of the visit, drug-code, manufacture license, dosage and regime, specification, storage condition, shelf-life, and responsible institution. Below is an example of labeling for the drugs used in the Run-in Period:
Labeling for the drugs used in the Run-in Period

Enalapril-folic acid for prevention of cardiovascular events.

For Clinical Trial Only
Manufacture license: 2008S00281
Specification: 21 Capsules per bottle
Usage and dosage: oral, once daily, one capsule each time
Storage: shading, sealed preservation below 30°C
Use before: March 2010
Responsible institution: Anhui Biomedical Institute, Anhui Medical University

Labeling example for the drugs used in the Double-blind Treatment Period:

Enalapril-folic acid for prevention of cardiovascular events.

For Clinical Trial Only   Drug-Code _____________  V(t)
Manufacture license: 2008S00281
Specification: 100 Capsules per bottle
Usage and dosage: oral, once daily, one capsule each time
Storage: shading, sealed preservation below 30°C
Use before: March 2010
Responsible institution: Anhui Biomedical Institute, Anhui Medical University

‘V’ represents follow-up visit, the following number represents the visit number.

7.2.3 Labeling procedures

1. The labeling and inspection process of drugs used for the Run-in Period will ensure that:

   a. Drugs are packed and sealed in bottles under the GMP conditions.

   b. A label of the Run-in Period will be placed on each bottle. After labeling, the drug administrator shall recount and store the bottles properly.
2. The labeling and inspection process of drugs used for the Double-blind Treatment Period will ensure that:

   a. Drugs are packed and sealed in bottles under the GMP conditions; this will be done by forming a packing group. Investigational drugs and control drugs should be kept in different rooms.

   b. Investigational drug enalapril-folic acid tablets are packed and counted in accordance with the relevant requirements. Labels and code (generated by an independent file) will be placed on the bottles packed with enalapril-folic acid tablets. The number of labels and drug-code should correspond to the number of bottles. The field should be cleaned after labeling and coding; the drug administrator should recount and keep the labeled bottles in proper condition. The entire packaging process should be recorded in detail, including: labeling date, start and finish time, list of labeling personnel, drug counting, bottle and code counting, completion counting, whether or not the field is cleaned, and should be signed by the responsible person.

   c. At a different time or space, the control drug enalapril tablets are packed and counted according to the requirements; labels and codes (generated by an independent file) are placed on the bottles packed with enalapril tablets. Note that the number of labels and codes should correspond to the number of bottles. The field should be cleaned after labeling and coding; the drug administrator will recount and keep the labeled bottles in proper condition. The entire packaging process should be recorded in detail, including: labeling date, start and finish time, list of labeling personnel, drug counting, bottle and code counting,
completion counting, whether or not the field is cleaned, and should be signed by the responsible person.

d. Enalapril-folic acid tablets and Enalapril tablets are placed in different partitions; the bottles will be arranged in accordance with the sequence number of drugs and put into boxes for storage.

7.3 Responsibilities

The investigator, the hospital pharmacist, or other personnel authorized to store and dispense the investigational product will take the responsibility for ensuring that the investigational product used in the clinical trial is securely stored and dispensed as specified in the Package Insert of the drugs and in accordance with the applicable regulatory requirements.

All investigational products shall be dispensed based on the prescription of the investigator (or designated physician, if the investigator is not a licensed physician him/herself), and the investigator should record the pills dispensed and returned back in the Study Record Form.

Under no circumstances will the Investigator supply the investigational product to a third party, allows the investigational product to be used other than as directed by this clinical trial protocol, or dispose of the investigational product in any other manner.

7.4 Concomitant Treatment

Any drugs other than use of folic acid are permitted.

Proper control of blood pressure should be used as a goal for anti-hypertensive medications other than the study drugs.

The dosage of the study drugs are fixed during the trial, with 10 mg of enalapril per day.
If blood pressure is not properly controlled, other anti-hypertensive medications can be added based on the recommendation of the “Chinese Guidelines of Hypertension Management” published in 2005. Controlling of the blood pressure within a normal range is not mandatory. A mild increase in blood pressure is not considered a criterion to withdraw a subject from the trial, unless it brings about a predictable risk for the patient. However, every effort should be made to control blood pressure if it is persistently over 180/110 mmHg.

The first choices of anti-hypertensive drugs to be added are: calcium channel blockers (CCBs) or Diuretics. Alpha-blockers, Beta-blockers, ARBs and other ACEIs are not recommended.

7.5 Treatment Compliance

Compliance will be assessed by counting returned tablets at each visit.

The Investigator (or designated associate) will complete the appropriate page of the Study Record Form or study drug inventory log form.

The date of study drug interruption will also be recorded.

7.6 Treatment Discontinuation

The investigational product should be continued whenever possible. If the investigational product is stopped, it should be determined if the discontinuation can be made temporarily; permanent discontinuation should be a last resort. Any discontinuation should be fully documented. In any case, the patient should remain in the study and be followed-up as long as possible.
In case of unexpected pregnancy, a permanent treatment discontinuation will be determined and it will be treated as a serious adverse event (SAE).

7.7 Retrieval and Destruction of Investigational Product

A detailed log of returned Investigational Product will be established by the Investigator.

7.8 Randomization of Treatments and Emergency Unblinding Procedure

Those study participants who complete the Run-in Period and meet all the inclusion and exclusion criteria for the Double-blind Treatment Period will enter the randomization process. The assignment of treatment group will be made by the use of a stratified (by MTHFR C677T genotype) randomization method.

A two-level blinding procedure will be implemented in this trial. In the first level of blinding, a random sequence table (group A, or B) will be generated with a block size of 4 for each of the three different MTHFR C677T genotypes: CC, CT and TT, respectively. The second level randomization will be used to set the type of treatment for group A and B: enalapril-folic acid and enalapril. An Independent Statistical Group designated by the Principal Investigator will generate the blinding documents using statistical analysis software. The computer blinding source code and the electronic files of the first and second level blinding will be stored in a secure place within sealed envelopes. These cannot be opened before the formal un-blinding of the trial with a formal procedure.

However, the Study-drug Management Center will hold another copy of the blinding document, in order to code the study medications and to dispense the medications based on the treatment-code of each subject. During the recruitment phase of the trial, based on
the chronological order of subjects who meet the inclusion and exclusion criteria, a phone call will be made to the Study-drug Management Center. The Center will then assign the smallest available drug code in the blinding randomization table to the subject, based on his/her MTHFR C677T genotype and the time the call is received.

The Drug-code is to be used to determine the treatment regimen for each subject during the study observation period. The Drug-code confirmation table (including the Study Code, Drug-Code, ID of subject and the address and contact information of the subject) should be securely stored by site investigators. The Drug-Code will be used as the identification code of the subject during the Double Blind Treatment observation period, so that the real identity of the subject can be protected when reporting adverse events and/or other relevant information.

In order to ensure the blinding of the study, the Investigator may not view the blinding information. If, due to a clinical management reason, the real drug used by a study participant must be open to the investigator, the investigator can make an emergency call to the Study-drug Management Center (24 hours * 7 days a week) for an emergency unblinding of the study drug information for that participant (ref. SOP for Study Drug Management of CSPPT). The investigator must record the process and report all information to the PI immediately.

8 SAFETY

8.1 Adverse Events Monitoring

All events will be managed and reported in compliance with all applicable regulations and
will be included in the final Clinical Study Report (CSR).

8.2 Definitions of Adverse Events

An Adverse Event in the trial is any untoward medical occurrence in after the informed consent is signed by the study participant, which does not necessarily have to have a causal relationship with the treatment.

The term Adverse Event covers any symptom, syndrome, or disease occurred or deteriorated during the clinical study observation period that can affect patient’s health. It also includes clinical relevant situations discovered from laboratory tests or other diagnostic processes, such as a need for unexpected diagnostic or treatment measures, or that lead to withdrawal from the study, or that have a clinically significant, abnormal laboratory finding.

The manifestations of adverse events may be: a new disease; deterioration of the disease condition, symptom or signs, or deterioration of concomitant diseases. These may be related to the effect of the comparison drug, or not related to the trial, or are a result of the combination of different factors.

8.3 Serious Adverse Event

A Serious Adverse Event is any untoward medical occurrence at any dose that:

1. Results in death, or is life-threatening; or

2. Results in malformation, carcinoma, birth defect; or

3. Results in persistent or significant disability/incapacity; or
4. Results in persistent organ damages; or

5. Requires inpatient hospitalization or prolongation of existing hospitalization.

Medical and scientific judgment should be exercised by the study physicians. Suspected drug should be listed in the report. Any serious adverse events related to the research process, as well as any unexpected associations of drugs and serious adverse events found in the statistical analysis should be reported.

As the study population of the CSPPT includes hypertensive patients with a mean age of 60 years, they are therefore at high risk of cardio-cerebral vascular diseases, tumors, and death during this trial. Furthermore, since this trial is designed as a large-scale and long-term follow-up study, we predict that a larger proportion of study participants will develop cardio-cerebrovascular diseases, tumors, or death (these are also the outcomes of the study). The clinical outcomes of the present study are thus not treated as serious adverse events but study outcomes and handled accordingly, unless the Study Physician adjudicates a casual relation between the event and the study medication.

8.4 Handling and Reporting of Adverse Events (AE) and Serious Adverse Events (SAE)

In the case of a SAE, if the SAE is also a suspected study endpoint, it will be handled by the physicians in the endpoint working group. Otherwise, it should be handled as described below.

1) If the drug relevance evaluation conforms to "Definitely related", "Probably related" or "Possibly related", the investigators must telephone the Principal Investigator (PI) and the drug-safety officer of the sponsor within 24 hours (no later than the next
working day), and fax the AE report and the follow-up reports (if available) to the PI’s office and the drug-safety officer of the Sponsor within five days. The PI’s office is responsible to report to the ethics committee and the regulatory body based on the requirement of China State Food and Drug Administration.

2) If the drug relevance evaluation conforms to "Possibly unrelated", "unrelated" or "Unable to evaluate/to be evaluated", the investigators are required to complete the SAE report form in a timely manner, and report to the PI and the drug-safety officer of the sponsor regularly. The PI’s office is responsible to report to the ethics committee.

9 STUDY PROCEDURES

The trial consists of three stages: the Screening and recruitment, followed by a 3-week Run-in Period, and then a 5-year Double-blind Treatment Period.

9.1 Screening and Recruitment

During the Screening period, community residents are screened by trained investigators for hypertension and medical history, disease diagnosis, and current treatment, according to the inclusion and exclusion criteria of the Run-in Period. At the beginning of the recruitment visit, before any particular study procedure is performed, each participant is asked to provide written informed consent in compliance with the Declaration of Helsinki and the requirements of the Independent Ethics Committee, after a careful explanation of the purpose and the procedures of the trial. Blood sample will be obtained for MTHFR C677T genotyping and other lab tests.
9.2 Run-in Period

During the Run-in Period, patients who meet all of the inclusion criteria and exclusion criteria will be given an enalapril 10 mg tablet for three weeks. Other antihypertensive drugs can be administered in order to reduce blood pressure into the normal range. MTHFR C677T genotype is determined during this period.

The main purpose of the Run-in Period is to evaluate the compliance and adverse reactions of the patients to enalapril treatment, in order to exclude those with poor compliance or enalapril intolerance from entering into the Double-blind Treatment Period.

9.3 Double-blind Treatment Period

Qualified participants are randomly assigned to one of two treatment groups using random permuted blocks stratified by MTHFR C677T genotypes (CC, CT, or TT). One treatment group receives a daily oral dose of one Enalapril-Folic Acid Tablet containing 10 mg enalapril maleate and 0.8 mg folic acid. The other group receives a daily oral dose of 10 mg Enalapril Maleate. The treatment assignments are masked to both the participants and the investigators. The expected treatment length is five years. Concomitant use of other antihypertensive drugs is allowed if needed to achieve better blood pressure control. A formal follow-up visit is conducted every 3 months, and study medications are administered at the visit.

9.4 Follow-up Plan

During the Double-blind Treatment Period, study participants are followed up every 3 months. They are asked to bring all unused drugs and package bottles back to the
investigator for a drug accountability check and record. For details, see the patient flow-chart for the trial below.
10. STATISTICAL CONSIDERATION

The following is an overview of the statistical considerations. Details of the pre-specified statistical analyses can be found in the Statistical Analysis Plan (SAP).

10.1 Statistical Analysis

The primary study hypothesis is that an enalapril- folic acid tablet is more effective than enalapril alone in reducing the incidence of stroke in patients with essential hypertension.
The primary outcome is time from randomization to the first attack of symptomatic stroke. The primary analysis will be intent-to-treat. The cumulative event rates of the primary outcome in the enalapril-folic acid and the enalapril group, respectively, will be estimated using the Kaplan-Meier method and compared using the log-rank test. The hazard ratio (HR) and 95% confidence intervals (CI) will be estimated by the Cox proportional hazard regression model. Pre-specified subgroup analysis will also be performed according to the Statistical Analysis Plan. To accommodate multiple interim analyses (see section 9.3), the statistical significance boundary of the final analysis will be defined by the O’Brien-Fleming alpha-spending function.

The following secondary outcomes will also be analyzed using the strategy similar to that for primary outcome and the intent-to-treat principal:

- composite cardiovascular events including cardiac death, cerebrovascular death, non-fatal stroke and myocardial infarction (MI);
- all cause death;
- ischemic stroke;
- hemorrhagic stroke;
- MI.

Other outcome to be analyzed using the same strategy is malignant tumors.

A two-side test with $p<0.05$ will be considered significant for secondary outcomes.
10.2a Sample Size (version 3.0)

10.2.1a Expected effect size (version 3.0)

Our meta-analysis of the previous reports of randomized controlled trials estimated a stroke hazard ratio (HR) of 0.82 for folic acid supplementation. In subgroups of patients without history of CVDs and from regions without grain fortification of folic acid, the effect size was larger at HR=0.75. The CSPPT participants are without known history of stroke and other CVDs, and from regions without food fortification. As a result, we expect an effect size of HR=0.75 in the CSPPT study. However, to be conservative, we set the minimal effect size to be detected for the primary outcome as HR=0.8.

10.2.2a Stroke incidence rate in the control group (version 3.0)

Stroke incidence rate varies greatly with age, sex, hypertensive status, and geographic regions. It’s hard to predict the stroke incidence rate in the CSPPT participants who are 45-75 years old and hypertensive ad living in Anhui province and Jiangsu province. According to a larger epidemiological study in five cities in China [24], the stroke incidence rate was found to be around 1000 per 100,000 person-years (or 1.0%) in hypertensive patients aged 35 years or older and without stroke history. However, in the accrued preliminary data from CSPPT before May 1, 2009, there were a total of 37 stroke cases from 5868 person-years of follow up without consideration of treatment group, representing an annual incidence rate of 0.63%. The lower incidence rate probably reflects better management of hypertension in the trial. The annual stroke incidence rate in the control group, which was set to 1.0% in the first two versions of the protocol, was revised to 0.7% in the current version.
10.2.3a Sample size calculation (version 3.0)

We aimed to have at least 80% power to detect a minimal true effect size of HR=0.8 with an alpha level of 0.05 during a five year follow-up. Figure 3. gives the curves of the sample sizes required for detecting various true effect size given annual stroke incidence rate of 0.7% and 1.0%, respectively.

Figure 3a. Curves of sample sizes required for detecting true effect sizes
We estimated that, with an annual stroke incidence rate of 0.7% and five years of follow-up, a sample size of 20337 is required for 80% power to detect a true effect size of HR=0.8.

10.2b Sample Size (version 2.0)

10.2.1b Expected effect size (version 2.0)

Our meta-analysis of the previous reports of RCTs estimated a stroke hazard ratio of 0.82 for folic acid supplementation. In subgroups of patients without history of CVDs and from regions without grain fortification of folic acid, the effect size was larger at HR=0.75. The CSPPT participants are without known history of stroke and other CVDs, and from regions with food fortification. As a result, we expect an effect size of HR=0.75 in CSPPT study. However, to be conservative, we revised the minimal effect size to be detected for the primary outcome to HR=0.8.

10.2.2b Stroke incidence rate in the control group (version 2.0)

The stroke incidence rate varies greatly with age, sex, hypertensive status, and regions. It’s hard to predict the stroke incidence rate in the CSPPT participants who are 45-75 years old hypertensive patients from Anhui province and Jiangsu province. According to a larger epidemiological study in five cities in China, the stroke incidence rate is around 1000 per 100,000 person-years (or 1.0%) in hypertensive patients aged 35 years or older and without stroke history. The annual stroke incidence rate in the control group is set to 1.0.

10.2.3b Sample size calculation (version 2.0)

In order to detect a smaller effect size (HR 0.8 instead of 0.75), we will increase the follow up duration from 4 years to 5 years. We aim to have at least 80% power to detect a minimal true effect size of HR=0.8 with an alpha level of 0.05 during a five year follow up. Figure
9.1 gives the curve of the sample sizes required for detecting various true effect sizes given annual stroke incidence rate of 1.0%.

![Graph showing the relationship between true effect size and sample size required.](image)

**Figure 3b. Curves of sample sizes required for detecting true effect sizes**

We estimate that, with an annual stroke incidence rate of 1.0% and five years of follow-up, a sample size of 14333 is required for 80% power to detect a true effect size of HR=0.8.

10.2c  **Sample Size (Version 1.0)**

10.2.1c  **Expected effect size (Version 1.0)**

Our meta-analysis of the previous reports of RCTs estimated a stroke hazard ratio of 0.82 for folic acid supplementation. In subgroups of patients without history of CVDs and from regions without grain fortification of folic acid, the effect size was larger at HR=0.75. The CSPPT participants are without known history of stroke and other CVDs, and from regions with food fortification. As a result, we expect an effect size of HR=0.75 in the CSPPT study.
10.2.2c **Stroke incidence rate in the control group (Version 1.0)**

The stroke incidence rate varies greatly with age, sex, hypertensive status, and regions. It’s hard to predict the stroke incidence rate in the CSPPT participants who are 45-75 years old hypertensive patients from Anhui province and Jiangsu province. According to a larger epidemiological study in five cities in China, the stroke incidence rate is around 1000 per 100,000 person-years (or 1.0%) in hypertensive patients aged 35 years or older and without stroke history. The annual stroke incidence rate in the control group is set to 1.0.

10.2.3c **Sample size calculation (Version 1.0)**

We aim to have at least 80% power to detect a minimal true effect size of HR=0.75 with an alpha level of 0.05 during a four-year follow up. Figure 9.1 gives the curve of the sample sizes required for detecting various true effect sizes given annual stroke incidence rate of 1.0%.
We estimate that, with an annual stroke incidence rate of 1.0% and 4 years of follow-up, a sample size of 11,033 is required for 80% power to detect a true effect size of HR = 0.75.

10.3 Interim Statistical Analysis

During the trial, the Data and Safety Monitoring Board (DSMB) will meet regularly to monitor the progress of the study and to conduct interim analyses. Generally, the interim analyses are scheduled for every increment of 200 confirmed stroke events. However, unscheduled interim analysis by DSMB is also allowed. To maintain the overall type-I error at an alpha level of 0.05, the O’Brien-Fleming alpha-spending function will be used to define the boundaries of statistical significance of the interim and the final analyses.
Results from the interim analyses are only accessible to DSMB members. DSMB may recommend terminating the trial in one of the following scenarios: significant efficacy difference between the two treatment groups; much greater risk to benefit ratio in the enalapril-folic acid group, or a low likelihood of success of the trial within a reasonable period of time (e.g. low drug adherence, low incidence of outcome events, etc.).

10.4 Statistical Analysis Plan (SAP)

Prior to database lock and before code breaking, a final version of the SAP shall be issued and approved by the the study statistician, and the principal Investigator. The SAP will define all “pre-specified, planned analyses” and provide the general specifications for the analysis of the data to be collected and presented in the Clinical Study Report.

11. INFORMED CONSENT, ETHICAL REVIEW AND REGULATORY STANDARDS

11.1 Ethical Review and Regulatory Standards for Clinical Trial

The clinical Trial will be conducted in according to the principles of the Helsinki Assembly, Belmont Report and other applicable ethical amendments recognized by Federal Wide Assurance (FWA), ICH guidelines for Good Clinical Practice, and the ethical amendments laid down by the China State Food and Drug Administration for “Guidelines for New Drug Clinical Trials”. The trial will be in compliance with all international and Chinese laws and regulations and any applicable clinical trial guidelines.
The Investigators and research assistants assuming relevant responsibilities should complete an ethical training course duly assigned.

The clinical trial protocol of the CSPPT will be reviewed and approved by the Independent Ethics Committee (IEC) of the Chief Coordinating Organization. Annual review and approval will also be required during the execution of the trial.

### 11.2 Informed Consent

Investigators and/or their assistants will fully inform study participants about all aspects of the clinical trial at the Screening stage, Run-in stage, and during the 5-year follow-up period, using language and terms that are easily understood, and will assure that all participants are informed to the fullest extent possible about the clinical trial.

Investigators are responsible for obtaining a written consent from each study participant, while providing them with a full understanding of the clinical trial. Prior to a patient's participation in the clinical trial, before any study procedure is performed, a written Informed Consent Form (ICF) should be signed, with the full name of the patient filled in, and personally dated by the patient or by the patient's legally acceptable representative, and by the person who conducted the informed consent discussion. A copy of the signed and dated written ICF will be available to the patient as needed.

### 11.3 Independent Ethical Committee (IEC) Review

Prior to the clinical trial, the Sponsor and PI will submit the Protocol and informed consent to the IEC for review and approval. Other documents to be submitted together include: (1) clinical operation manual, (2) Investigator's Brochure, and (3) CV of the PI.
During the clinical trial, Investigators are required to report to the IEC in the following situations: (1) any event likely to affect the safety of patients or the continued conduct of the clinical trial, in particular any change in safety; (2) any event likely to affect the continued conduct of the clinical trial; (3) any amendment or modification to the clinical trial protocol; (4) to provide an annual brief on the progress of the Trial; (5) to provide a new version of the Investigator's Brochure; and (5) to present a final progress report prior to closing the trial.

12 STUDY MONITORING

12.1 Responsibilities of the Investigator(s)

The Investigator(s) undertake(s) to perform the clinical trial in accordance with the Protocol, ICH guidelines for Good Clinical Practice and the regulatory requirements of the China SFDA.

The Investigator ensures compliance with all procedures required by the clinical trial protocol and with all SOPs provided by the Steering Committee and the Executive Committee (including security rules). The Investigator agrees to provide reliable data and all information required by the clinical trial protocol (which will include the use of the Study Record Form), and will transfer the Study Record Form to eCRF in an accurate and legible manner according to the instructions provided.

The Investigator may appoint other individuals as he/she may deem appropriate as research assistants to assist in the conduct of the clinical trial in accordance with the clinical trial protocol. All research assistants shall be appointed and listed in a timely
written manner. The research assistant will be trained properly before assuming the responsibilities and be supervised by and under the responsibility of the Investigator.

All investigators and research assistants will receive adequate training, using the same kind of recording style and criteria of judgment, before they participate in the trial. The training will be delivered by Executive Committee and Steering Committee members. Training courses topics will include but are not limited to: GCP, protocol and protocol amendments, all relevant SOPs for their positions and functions in the trial, the original Study Record Form, eCRF, informed consent, ethical principles and related diseases and medical knowledge, information collection for diagnosis and differential diagnosis of end point events, etc.

During this trial, the study monitoring and quality control team assigned by the Steering Committee will provide regular visits to the research centers and sites for site monitoring and quality control to ensure that all the components of the study protocol are strictly followed and that the information filled in the Study Record Form is accurate. The main duty of the Monitoring Team is to help the Investigators maintain a high level of ethical, scientific, technical and regulatory quality in all aspects of the clinical trial. The content to be reviewed during the monitoring visits will include but is not limited to the following items: patient informed consent, patient recruitment and follow-up, Serious Adverse Event documentation and reporting, Investigational Product accountability, patient compliance with the Investigational Product regimen, Investigational Drug accountability, the accuracy and legibility of the Study Record Forms, concomitant therapy use, and the overall quality of the data.
12.2 **Source Document Requirements and Study Record Form**

The Study Record Forms will be established for all Subjects. The study monitors will check the information in the SDFs, and verify the data in the eCRFs accordingly. According to the ICH guidelines for Good Clinical Practice, the Monitoring Team must check the Case Report Form entries against the Study Record Form, except for the pre-identified source data directly recorded in the electronic Case Report Form. The ICF will include a statement by which the patient allows the monitoring team, the IEC, and the regulatory authorities to have direct access to source data which support the data on the Study Record Forms and the eCRFs (e.g., patient's medical file, appointment books, original laboratory records, etc.). Such personnel, bound by professional secrecy, must keep confidential all personal identity or personal medical information (according to confidentiality rules).

12.3 **Case Report Forms (CRF)**

The Study Record Form contains core data, other relevant data as well as personal information of the participants. CSPPT will use eCRF to collect the core data of this study with personal information striped. CRF will be filled electronically from the data in the Study Record Forms by authorized personnel at the coordinating centers.

12.4 **Use of Computerized Systems**

A computer-based electronic Case Report Form (eCRF) will be used for data collection. In order to keep adequate source documentation, a Study Record Form that contains all the data and information that will be used to fill-in the eCRF will also be used at the study sites to easily record study specific data and other useful information.
13. PUBLICATION OF RESEARCH FINDINGS

After the study is completed and the database is locked, a writing group, whose members will have unrestricted access to the data, will prepare the study manuscript, which will be subsequently revised by all of the authors.

Patients’ personal information will not be identifiable in the database or in the final report.

14. REFERENCE


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


ATTACHMENT : EVENT ADJUDICATION SOP

Event Adjudication Standard Operation Procedure

<table>
<thead>
<tr>
<th>Version: 3</th>
<th>Revised date: 2011.7.20</th>
<th>Effective date: 2011.7.20</th>
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<tr>
<td>Prepared by: Yefeng Cai</td>
<td>Reviewed by: Yining Huang</td>
<td>Approved by: Yong Huo</td>
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</tbody>
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1. Overview

This SOP specifies the endpoint events of CSPPT and the study-specific diagnostic criteria, and standard procedures for capture, data collection, and adjudication of endpoint events.

There are three types of endpoint events, including the primary endpoint event, the secondary events, and other endpoint event.

1.1 Primary endpoint event

The primary endpoint event is first attack of symptomatic stroke, excluding subarachnoid hemorrhage and silent stroke.

1.2 Secondary endpoint events

Secondary endpoint events include:

- a composite of cardiovascular events consisting of cardiovascular death, nonfatal stroke and nonfatal myocardial infarction (MI);
• the first attack of ischemic stroke (fatal and non-fatal);

• the first attack of hemorrhagic stroke (fatal and non-fatal);

• MI (fatal and non-fatal);

• all-cause death.

1.3 Other endpoint event

The other endpoint event includes malignant tumor.

2. Study-specific Diagnostic Criteria

2.1 First attack of symptomatic stroke

Stroke is defined as rapidly developed clinical signs of focal (or global) disturbance of cerebral function, with symptoms lasting 24 hours or longer (unless interrupted by surgery or death) or a demonstrable lesion on computed tomography (CT) or magnetic resonance imaging (MRI) scan that is consistent with acute stroke, with no apparent causes other than of vascular origin (e.g. of paralysis after epileptic seizure, brain trauma, infection, and brain tumor). Subarachnoid hemorrhage and silent stroke are excluded.

CT or MRI is not absolutely essential for diagnosis of stroke, but is necessary for differentiating the subtypes (ischemic or hemorrhagic) of stroke. Without imaging data, stroke can still be diagnosed in presence of the specific signs and symptoms of focal disturbance of cerebral function, and the subtype shall be “uncertain”.

Whenever possible, patients suspected of stroke should take at least one CT or MRI scan 24 hours to 3 months (best in 3 weeks) after attack. Such data facilitates the diagnosis and classification of stroke.
In this study, patients diagnosed with stroke should meet at least one of the following two conditions:

- Presence of specific signs and symptoms of focal disturbance of cerebral function lasting more than 24 hours, with no apparent causes other than of vascular origin;
- Presence of nonspecific signs and symptoms of disturbance of cerebral function (focal or global) lasting more than 24 hours and consistent imaging changes.

According to the study protocol, patients with history of stroke are excluded. The first attack of symptomatic stroke during the trial shall be regarded as the first attack of the patient unless there is evidence against it. Any stroke after the first attack will be regarded as recurrent stroke, which is not the primary endpoint event of this study.

2.2 Subtypes of stroke

A. Ischemic Stroke: defined as an acute episode of focal cerebral dysfunction caused by infarction of central nervous system. Hemorrhage may be a consequence of ischemic stroke. In this situation, the stroke is an ischemic stroke with hemorrhagic transformation and not a hemorrhagic stroke.

B. Hemorrhagic Stroke: defined as an acute episode of focal or global cerebral dysfunction caused by intraparenchymal, intraventricular or subarachnoid hemorrhage. However, subarachnoid hemorrhage is not an endpoint event of this study.

C. Undetermined Stroke: defined as a stroke with insufficient information to allow categorization of ischemic or hemorrhagic stroke.
2.3 Fatal stroke

Fatal stroke is defined as death within 28 days of a stroke attack that is either due to the stroke or caused by a complication of the stroke.

2.4 Acute myocardial infarction (AMI)

Patients with AMI should meet the following criteria for ischemic symptoms or corresponding ECG changes plus evidence of myocardial damage (c).

The dynamic ECG changes associated with AMI include new pathological Q waves in ≥2 contiguous leads (or new R elevation in V1-V2) without prior left ventricular high voltage or conduction abnormalities; new ST-T changes in ≥2 leads; new left bundle branch block (LBBB); new ST elevation that requires thrombolytic therapy or percutaneous coronary intervention (PCI).

Evidence for myocardial damage include troponin above the lab-specific threshold or other myocardial biomarker (CK, CK-MB) >2ULN (Upper limit of normal).

Priori MI and procedural AMI are not endpoint events of this study.

2.5 Cardiovascular death

Cardiovascular death includes sudden cardiac death, death due to AMI, heart failure, stroke, or cardiovascular invasive procedures, death due to cardiovascular hemorrhage, and death due to other known vascular causes. In particular, unwitnessed death in a subject seen alive and clinically stable ≤ 24 hours prior to being found dead without any evidence supporting a specific non-cardiovascular cause of death shall be considered as sudden cardiac death.
2.6 Composite cardiovascular events

Composite cardiovascular events consist of cardiovascular death, MI, and stroke.

2.7 All-cause death

All-cause death includes death due to any reasons. Evidence for death includes death certificates from hospitals or reports of home visit from investigators.

2.8 Malignant tumor

Malignant tumor can be diagnosed based on either positive pathologic findings or specific clinical manifestations.

Acceptable evidence for pathologic findings includes original or photocopied pathological reports; and original or photocopied medical records from hospitals in which pathological results are cited.

When pathological data are not available, cases will be reviewed independently by two oncologists. Malignant tumor is diagnosed only when both make the same clinical diagnosis based on clinical manifestations and examinations.

3. Capture of Endpoint Events

Suspected endpoint events are identified through the following four mechanisms:

a. Reports from patients and their family members;

b. Monthly telephone follow-up;

c. Regular quarterly follow-up or home visit;

d. Reports from tiered local health network.
Upon receiving a report of suspected endpoint event, investigators should start processing the case as soon as possible.

4. Endpoint Working Group

Endpoint working group are a group of site investigators responsible for collecting relevant medical information and making preliminary assessment of the suspected endpoint events. It consists of at least two neurologists, one cardiologist, one oncologist, and 1-2 clinical research coordinators (CRCs). All suspected stroke cases should be evaluated by two neurologists. All deaths should be verified by home visit, and relevant medical records and death certificate be collected if available.

Every case reported as an endpoint event by the physicians of the working group (both neurologists in case of stroke) will be reviewed an independent Endpoint Adjudication Committee.

5. Endpoint Adjudication Committee

Endpoint Adjudication Committee (EAC) meets regularly and is responsible for reviewing all the endpoint cases reported by the site investigators from the endpoint working group. The review by EAC, blind to treatment allocation and the resultant diagnosis, will be the basis of all efficacy analyses of this study.

6. Event adjudication procedure

Source data for all suspected stroke cases including medical records (written clinical summary, physical examination findings, neurological consult report) and imaging data (CT scan or MRI (original or copy)) as well as event report forms were submitted to the
Event Adjudication Committee (EAC). For each suspected case, source data were reviewed by one of the three neurologists on the EAC to determine if the case met the stroke definition criteria established for the CSPPT study; and if so, the neurologist signed a final Endpoint Adjudication Form to certify an adjudicated stroke case. When a case was questionable, all three neurologists reviewed the source data and could request additional source data to resolve any disagreement; and for such a case, a final Endpoint Adjudication Form was signed by the EAC Chairman (one of the neurologists).

7. Archiving of Endpoint Data

Source data of all suspected endpoint cases including medical records and imaging data collected as well as event report forms should be archived by the site investigators or the chief coordinating organization. Evaluation forms from EAC, together with an electronic copy of all medical documentations of the cases reviewed by EAC will be archived by the data management center of the study.

8. Revision Histories

Version 3

- Added clinical diagnosis of malignant tumor

Version 2

- Refined definition of cardiovascular death
Summary of amendments of protocol

Major Amendments from Version 1.0 to Version 2.0:

1. Sample size increased from 12000 to 15000, due to lower stroke incidence;

2. Modify the Run-in Period from 2 to 3 weeks to 3 weeks, due to MTHFR genotyping test;

3. Delete the exclusion criterion of 3rd degree hypertension.

Major Amendments from Version 2.0 to Version 3.0

1. Sample size further increased from 15000 to 20000, due to lower stroke incidence;

2. Prolongation of the Double-blind Treatment Period from 4 years to 5 years, due to lower stroke incidence.