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ORIGINAL RESEARCH

Baseline Serum Bilirubin and Risk of First Stroke in Hypertensive Patients

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BACKGROUND: Data on the association between serum bilirubin and the risk of stroke are limited and inconclusive. We aimed to evaluate the association between serum bilirubin and the risk of first stroke and to examine any possible effect modifiers in hypertensive patients.

METHODS AND RESULTS: Our study was a post hoc analysis of the CSPPT (China Stroke Primary Prevention Trial). A total of 19 906 hypertensive patients were included in the final analysis. Cox proportional hazards models were used to estimate the hazard ratios (HRs) and 95% CIs for the risk of first stroke associated with serum bilirubin levels. The median follow-up period was 4.5 years. When serum total bilirubin was assessed as tertiles, the adjusted HR of first ischemic stroke for participants in tertile 3 (12.9–34.1 μmol/L) was 0.75 (95% CI, 0.59–0.96), compared with participants in tertile 1 (<9.3 μmol/L). When direct bilirubin was assessed as tertiles, a significantly lower risk of first ischemic stroke was also found in participants in tertile 3 (2.5–24.8 μmol/L) (adjusted HR, 0.77; 95% CI, 0.60–0.98), compared with those in tertile 1 (<1.6 μmol/L). However, there was no significant association between serum total bilirubin (tertile 3 versus 1: adjusted HR, 1.45; 95% CI, 0.89–2.35) or direct bilirubin (tertile 3 versus 1: adjusted HR, 1.27; 95% CI, 0.76–2.11) and first hemorrhagic stroke.

CONCLUSIONS: In this sample of Chinese hypertensive patients, there was a significant inverse association between serum total bilirubin or direct bilirubin and the risk of first ischemic stroke.

Key Words: direct bilirubin ■ first hemorrhagic stroke ■ first ischemic stroke ■ hypertension ■ total bilirubin

Stroke is the leading cause of death in China.[1] Previous studies indicate that traditional risk factors do not account for all strokes.[2,3] Therefore, there is continuous interest in identifying novel modifiable risk factors to further improve stroke primary prevention and reduce the related, enormous disease burden.

Bilirubin is an end metabolic product of heme degradation. More and more studies are demonstrating that even mildly elevated serum bilirubin has potent anti-inflammatory, antioxidant, antiproliferative, and blood lipid-modulating properties.[4–8] Accordingly, recent studies have shown that serum bilirubin is inversely associated with myocardial infarction, coronary heart disease, chronic kidney disease, and all-cause mortality.[9–12] However, although the inverse association between bilirubin and the prevalence of stroke was observed in previous cross-sectional studies,[13,14] data from prospective studies on the association between serum bilirubin levels and incident stroke are limited and inconclusive.[15–18] Notably, few previous studies have comprehensively investigated potential modifiers on the association between bilirubin and first stroke risk.

Hypertension is one of the most important risk factors for stroke and cardiovascular diseases.[19,20] Our

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CLINICAL PERSPECTIVE

What Is New?

- Our study first demonstrates that, even after adjustments for multiple confounding factors, including blood pressure at baseline and during the treatment period, higher baseline serum total bilirubin or direct bilirubin levels were associated with decreased risk of first ischemic stroke in patients with hypertension.

What Are the Clinical Implications?

- Serum bilirubin concentration can be measured easily in the clinical laboratory and applied in medical practice; therefore, if our findings are further confirmed by future studies, routine measurements of bilirubin could help identify those hypertensive patients at high risk of ischemic stroke.

Nonstandard Abbreviations and Acronyms

ALT	alanine aminotransferase
BP	blood pressure
CSPPT	China Stroke Primary Prevention Trial
DBiL	direct bilirubin
HR	hazard ratio
SBP	systolic blood pressure
TBiL	total bilirubin
tHcy	total homocysteine

current study was motivated by the above gaps in knowledge and the opportunity to examine the prospective relationship of serum bilirubin with first stroke and its subtypes among hypertensive adults in the CSPPR (China Stroke Primary Prevention Trial),[21] a randomized, double-blind, clinical study.

METHODS

The parent study (CSPPT: Clinical Trials.gov, NCT00794885) and the current study were approved by the Ethics Committee of the Institute of Biomedicine, Anhui Medical University, Hefei, China (Federal Wide Assurance [FWA] assurance No. FWA00001263). All participants provided written informed consent. The data that support the findings of this study will be available from the corresponding authors on request, after the request is submitted and formally reviewed and approved by the Ethics Committee of the Institute of Biomedicine, Anhui Medical University.

Study Participants and Design

The rationale, study design, and major results for the CSPPT have been described previously.[21–25] Briefly, the CSPPT was a randomized, double-blind, clinical trial conducted from May 19, 2008, to August 24, 2013, with 20 702 hypertensive adults in 32 communities in Jiangsu and Anhui provinces in China. It was designed to evaluate whether a combined therapy of enalapril plus folic acid is more effective than enalapril alone in reducing the risk of first stroke in hypertensive patients. Eligible participants were men and women, aged 45 to 75 years, who had hypertension, defined as seated, resting systolic blood pressure (SBP) of ≥140 mm Hg or diastolic blood pressure (BP) of ≥90 mm Hg at both the screening and recruitment visits or patients who were taking antihypertensive medication. The major exclusion criteria included a history of physician-diagnosed stroke, myocardial infarction, heart failure, coronary revascularization, or congenital heart disease.

The present study was a post hoc analysis of the CSPPT. Among the initial 20 702 participants, those with missing data on the major variables (bilirubin or liver enzymes) (n=144) were excluded. Among the remaining 20 558 participants, the participants with potential Gilbert syndrome (total bilirubin [TBiL] >34.2 μmol/L [>2.0 mg/dL], alanine aminotransferase [ALT] <80 IU/L, aspartate aminotransferase <80 IU/L, γ-glutamyl transpeptidase <80 IU/L, and no self-reported history of hepatobiliary disease; n=96) or potential hepatobiliary disease (TBiL >34.2 μmol/L, ALT ≥80 IU/L, aspartate aminotransferase ≥80 IU/L, serum albumin <3.5 g/dL, or a positive, self-reported history of hepatobiliary disease; n=556) were also excluded to avoid confounding.[15] A total of 19 906 participants were included in the final analysis (Figure S1). Those participants excluded from the analysis did not differ substantially in baseline characteristics from those included in the final analysis (Table S1).

Laboratory Assays

Fasting serum TBiL, direct bilirubin (DBiL), ALT, aspartate aminotransferase, γ-glutamyl transpeptidase, lipids, total homocysteine (tHcy), creatinine, and glucose were measured using automatic clinical analyzers (Beckman Coulter) at the core laboratory of the National Clinical Research Center for Kidney Disease, Guangzhou, China.

Diabetes mellitus was defined as fasting glucose ≥7.0 mmol/L, use of glucose-lowering drugs, or self-reported history of diabetes mellitus.

Outcomes

The study outcomes included first stroke and its subtypes (first ischemic and hemorrhagic stroke), excluding subarachnoid hemorrhage and silent stroke.

Table 1. Characteristics of the Study Participants, According to Baseline TBiL Concentrations

Characteristics	TBiL, μmol/L		
	Tertile 1 (<9.3)	Tertile 2 (9.3–12.9)	Tertile 3 (12.9–34.1)
No. of participants	6621	6634	6651
Age, y	59.5 (7.6)	60.1 (7.5)	60.4 (7.5)
Men, N (%)	1726 (26.1)	2693 (40.6)	3630 (54.6)
BMI, kg/m^2	25.6 (3.8)	25.0 (3.6)	24.3 (3.5)
Enalapril-folic acid, N (%)	3344 (50.5)	3294 (49.7)	3345 (50.3)
Current smoker, N (%)	1092 (16.5)	1567 (23.6)	1977 (29.7)
BP, mm Hg			
Systolic BP at baseline	168.0 (20.8)	167.3 (20.5)	165.5 (19.8)
Diastolic BP at baseline	94.0 (11.8)	94.2 (11.9)	94.0 (12.1)
Time-averaged systolic BP	140.2 (11.7)	139.6 (11.5)	139.7 (11.2)
Time-averaged diastolic BP	83.1 (7.6)	83.0 (7.6)	83.0 (7.8)
Laboratory results			
Fasting glucose, mmol/L	6.0 (1.8)	5.8 (1.6)	5.6 (1.6)
Total cholesterol, mmol/L	5.7 (1.2)	5.6 (1.2)	5.3 (1.2)
Triglycerides, mmol/L	1.8 (1.5)	1.6 (0.9)	1.6 (0.9)
HDL-C, mmol/L	1.3 (0.3)	1.4 (0.3)	1.4 (0.4)
Alanine transaminase, IU/L	12.9 (6.0)	13.8 (6.8)	15.4 (7.9)
Aspartate transaminase, IU/L	23.0 (7.7)	25.1 (8.6)	28.2 (9.6)
γ-Glutamyl transpeptidase, IU/L	23.7 (22.4)	26.1 (22.6)	31.1 (34.5)
Total homocysteine, μmol/L	13.9 (7.7)	14.4 (8.3)	15.1 (8.7)
Total bilirubin, μmol/L	7.6 (1.2)	11.0 (1.0)	17.5 (4.3)
Direct bilirubin, μmol/L	1.3 (0.5)	2.0 (0.5)	3.5 (1.1)
Medication use, N (%)			
Antihypertensive drugs	3339 (50.4)	3043 (45.9)	2817 (42.4)
Lipid-lowering drugs	58 (0.9)	48 (0.7)	55 (0.8)
Glucose-lowering drugs	134 (2.0)	93 (1.4)	77 (1.2)
Antiplatelet drugs	238 (3.6)	199 (3.0)	150 (2.3)
Self-reported history of disease			
Coronary heart disease	131 (2.0)	96 (1.4)	97 (1.5)
Arrhythmia	70 (1.1)	81 (1.2)	63 (0.9)
Diabetes mellitus	287 (4.3)	189 (2.8)	146 (2.2)

Continuous variables are presented as mean (SD), and categorical variables are presented as number (percentage). BP indicates blood pressure; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; and TBiL, total bilirubin.

All outcomes were reviewed and adjudicated by an independent end-point adjudication committee whose members were unaware of study group assignments.

Statistical Analysis

Means (SDs) or proportions were calculated for population characteristics by TBiL tertiles. The differences in population characteristics were compared using ANOVA tests, signed rank tests, or χ^2 tests, accordingly.

Cox proportional hazards models were used to estimate the hazard ratios (HRs) and 95% CIs for the risk of first stroke associated with serum bilirubin levels without and with adjustments for treatment group (enalapril or enalapril+folic acid), age, sex, body mass index, smoking status, SBP and diastolic BP, total cholesterol, fasting glucose, triglycerides, ALT, γ-glutamyl transpeptidase, tHcy, folate levels, antihypertensive drug use, lipid-lowering drug use, glucose-lowering drug use, and antiplatelet drug use at baseline, as well as time-averaged SBP and diastolic BP during the treatment period. In addition, possible modifications of the association between serum bilirubin (per SD increment) and first ischemic stroke were also assessed for the following variables: age (<60 versus ≥60 years), sex, treatment group (enalapril versus enalapril+folic acid), body mass index (<25 versus ≥25 kg/m^2), total cholesterol (<5.2 versus ≥5.2 mmol/L), current smoking (yes versus

Table 2. Relationship of Baseline Serum TBiL Levels With First Stroke and Its Subtypes

Total Bilirubin, μmol/L	Events/N (%)	HR (95% CI)		
		Unadjusted	Adjusted*	*P* Value*
First stroke				
Per SD increment	608	0.92 (0.85–1.00)	0.97 (0.88–1.06)	0.48
Tertiles				
Tertile 1 (<9.3)	225/6621 (3.4)	Reference	Reference	
Tertile 2 (9.3–12.9)	192/6634 (2.9)	0.84 (0.69–1.01)	0.88 (0.72–1.08)	0.22
Tertile 3 (12.9–34.1)	191/6651 (2.9)	0.82 (0.68–0.99)	0.87 (0.71–1.08)	0.20
P value for trend	…	0.04	0.20	…
First ischemic stroke				
Per SD increment	489	0.87 (0.79–0.96)	0.91 (0.82–1.02)	0.10
Tertiles				
Tertile 1 (<9.3)	190/6621 (2.9)	Reference	Reference	
Tertile 2 (9.3–12.9)	159/6634 (2.4)	0.82 (0.66–1.01)	0.85 (0.68–1.06)	0.15
Tertile 3 (12.9–34.1)	140/6651 (2.1)	0.71 (0.57–0.89)	0.75 (0.59–0.96)	0.02
P value for trend	…	<0.01	0.02	…
First hemorrhagic stroke				
Per SD increment	117	1.14 (0.97–1.35)	1.14 (0.93–1.37)	0.16
Tertiles				
Tertile 1 (<9.3)	34/6621 (0.5)	Reference	Reference	
Tertile 2 (9.3–12.9)	33/6634 (0.5)	0.95 (0.59–1.53)	1.03 (0.62–1.70)	0.91
Tertile 3 (12.9–34.1)	50/6651 (0.8)	1.42 (0.92–2.19)	1.45 (0.89–2.35)	0.14
P value for trend	…	0.10	0.12	…

HR indicates hazard ratio; and TBiL, total bilirubin.

*Adjusted for age, sex, treatment group, body mass index, systolic blood pressure, diastolic blood pressure, smoking status, fasting glucose, total cholesterol, triglycerides, alanine aminotransferase, γ-glutamyl transpeptidase, total homocysteine, folate levels, antihypertensive drug use, lipid-lowering drug use, glucose-lowering drug use, and antiplatelet drug use at baseline, as well as time-averaged systolic blood pressure and diastolic blood pressure during the treatment period.

no), SBP (<160 versus ≥160 mm Hg), diabetes mellitus (absence versus presence), tHcy (median, <12.5 versus ≥12.5 μmol/L), folate (median, <8.1 versus ≥8.1 μmol/L), antihypertensive drug use (yes versus no), lipid-lowering drug use (yes versus no), glucose-lowering drug use (yes versus no), and antiplatelet drug use (yes versus no) at baseline, as well as time-averaged SBP (<140 versus ≥140 mm Hg) during the treatment period. Heterogeneity across subgroups was assessed by Cox proportional hazards models, and interactions between subgroups and serum bilirubin concentrations were examined by likelihood ratio testing.

A 2-tailed $P<0.05$ was considered to be statistically significant in all analyses. R software version 3.4.1 (http://www.R-project.org) was used for all statistical analyses. The "epicalc," "forestplot," and "survival" packages of R were used in our statistical analyses.

RESULTS

Study Participants and Characteristics

General characteristics of study participants are presented by TBiL tertiles in Table 1. The mean and median of serum TBiL and DBiL levels were 12.0 (SD, 4.9) and 10.9 μmol/L and 2.3 (SD, 1.2) and 2.0 μmol/L, respectively. That is to say, most of the participants had normal or mildly elevated TBiL or DBiL levels.

Serum TBiL levels were positively associated with age, male sex, current smoking, ALT, aspartate aminotransferase, γ-glutamyl transpeptidase, tHcy, and high-density lipoprotein cholesterol at baseline; and were inversely associated with body mass index, SBP, total cholesterol, triglycerides, fasting glucose, use of antihypertensive drugs, glucose-lowering drugs, and antiplatelet drugs, and the self-reported history of coronary heart disease and diabetes mellitus at baseline, as well as time-averaged SBP during the treatment period (Table 1).

Relationship of Serum TBiL and DBiL With Study Outcomes

During a median follow-up period of 4.5 years, there were 608 first strokes, including 489 ischemic strokes, 117 hemorrhagic strokes, and 2 uncertain types of stroke.

Overall, there was no significant association between TBiL (per SD increment; adjusted HR, 0.97;

95% CI, 0.88–1.06) or DBiL (adjusted HR, 0.91; 95% CI, 0.82–1.01) levels and first stroke (Tables 2 and 3). However, there was an inverse association between TBiL (Figure 1A; per SD increment; adjusted HR, 0.91; 95% CI, 0.82–1.02) or DBiL (Figure 1B; adjusted HR, 0.85; 95% CI, 0.75–0.95) levels and first ischemic stroke. Consistently, when TBiL was assessed as tertiles, the adjusted HR for first ischemic stroke for participants in tertile 3 (12.9–34.1 μmol/L) was 0.75 (95% CI, 0.59–0.96), compared with participants in tertile 1 (<9.3 μmol/L) (Table 2). When DBiL was assessed as tertiles, a significantly lower risk of first ischemic stroke was observed in participants in tertile 3 (2.5–24.8 μmol/L) (adjusted HR, 0.77; 95% CI, 0.60–0.98), compared with those in tertile 1 (<1.6 μmol/L) (Table 3). However, there was no significant relation of serum TBiL (tertile 3 versus 1; adjusted HR, 1.45; 95% CI, 0.89–2.35) or DBiL (tertile 3 versus 1; adjusted HR, 1.27; 95% CI, 0.76–2.11) with first hemorrhagic stroke. Consistently, there was also no significant association between serum TBiL (tertile 3 versus 1; adjusted HR, 0.87; 95% CI, 0.71–1.08) or DBiL (tertile 3 versus 1; adjusted HR, 0.87; 95% CI, 0.69–1.08) and first stroke (Tables 2 and 3).

Similar results were found in participants with normal levels of TBiL (3–≤17 μmol/L[26]) (Table S2) or normal levels of DBiL (≤6.8 μmol/L[27]) (Table S3). Further adjustment for the baseline serum albumin did not materially change the results (Table S4). Similar findings were also found for further adjustment for the history of diabetes mellitus, arrhythmia, and coronary heart disease at baseline (Table S5).

Furthermore, during the treatment period, participants with lower TBiL levels had a higher frequency use of glucose-lowering drugs and diuretics and a lower frequency use of calcium channel blockers and antiplatelet drugs (Table S6). However, further adjustment for the use of medications (glucose-lowering drugs, lipid-lowering drugs, antiplatelet drugs, calcium channel blockers, and diuretics) during the treatment period did not substantially change the association between serum TBiL or DBiL and first ischemic stroke (Table S7).

Stratified Analysis by Potential Effect Modifiers

We further performed stratified analyses to assess the relationship of baseline serum DBiL (per SD increment) (Figure 2 and Table S8) and TBiL (per SD increment)

Table 3. Relationship of Baseline Serum DBiL Levels With First Stroke and Its Subtypes

Direct Bilirubin, μmol/L	Events/N (%)	HR (95% CI)		
		Unadjusted	Adjusted*	*P* Value*
First stroke				
Per SD increment	608	0.87 (0.80–0.95)	0.91 (0.82–1.01)	0.07
Tertiles				
Tertile 1 (<1.6)	223/6603 (3.4)	Reference	Reference	
Tertile 2 (1.6–2.5)	201/6568 (3.1)	0.89 (0.74–1.08)	0.91 (0.75–1.11)	0.37
Tertile 3 (2.5–24.8)	184/6735 (2.7)	0.78 (0.65–0.95)	0.87 (0.69–1.08)	0.21
P value for trend	…	0.02	0.20	…
First ischemic stroke				
Per SD increment	489	0.80 (0.72–0.89)	0.85 (0.75–0.95)	0.01
Tertiles				
Tertile 1 (<1.6)	187/6603 (2.8)	Reference	Reference	
Tertile 2 (1.6–2.5)	169/6568 (2.6)	0.90 (0.73–1.10)	0.91 (0.73–1.13)	0.40
Tertile 3 (2.5–24.8)	133/6735 (2.0)	0.68 (0.54–0.85)	0.77 (0.60–0.98)	0.04
P value for trend	…	<0.01	0.04	…
First hemorrhagic stroke				
Per SD increment	117	1.13 (0.97–1.31)	1.09 (0.90–1.32)	0.37
Tertiles				
Tertile 1 (<1.6)	35/6603 (0.5)	Reference	Reference	
Tertile 2 (1.6–2.5)	32/6568 (0.5)	0.91 (0.56–1.47)	0.96 (0.58–1.59)	0.87
Tertile 3 (2.5–24.8)	50/6735 (0.7)	1.36 (0.88–2.09)	1.27 (0.76–2.11)	0.36
P value for trend	…	0.15	0.34	…

DBiL indicates direct bilirubin; and HR, hazard ratio.

*Adjusted for age, sex, treatment group, body mass index, systolic blood pressure, diastolic blood pressure, smoking status, fasting glucose, total cholesterol, triglycerides, alanine aminotransferase, γ-glutamyl transpeptidase, total homocysteine, folate levels, antihypertensive drug use, lipid-lowering drug use, glucose-lowering drug use, and antiplatelet drug use at baseline, as well as time-averaged systolic blood pressure and diastolic blood pressure during treatment.

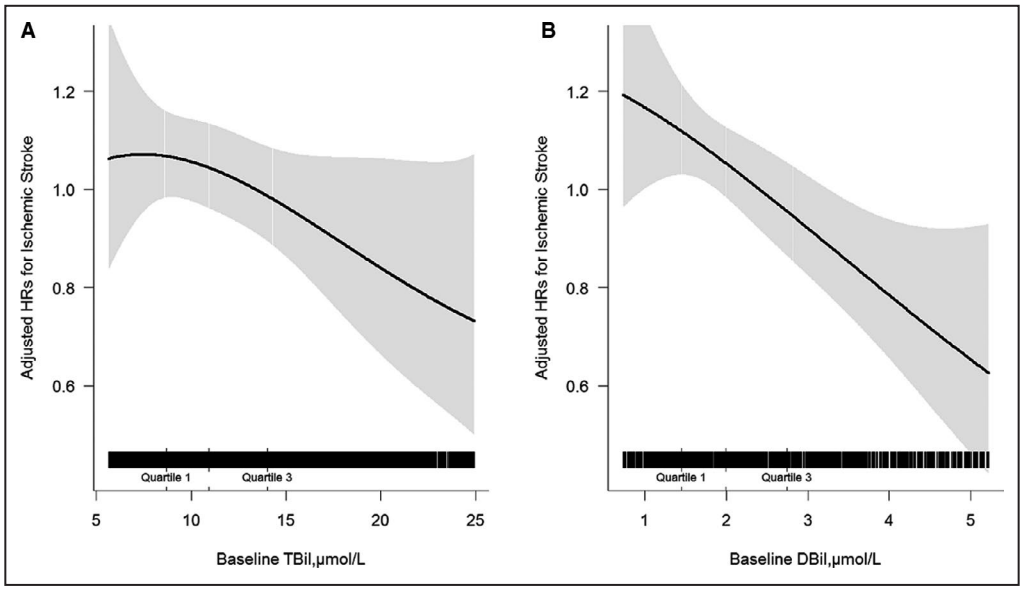

Figure 1. Relationship of total bilirubin (TBiL) (A) and direct bilirubin (DBiL) (B) levels with first ischemic stroke in hypertensive patients.
Adjusted for age, sex, treatment group, body mass index, systolic blood pressure, diastolic blood pressure, smoking status, fasting glucose, total cholesterol, triglycerides, alanine aminotransferase, γ-glutamyl transpeptidase, total homocysteine, folate levels, antihypertensive drug use, lipid-lowering drug use, glucose-lowering drug use, and antiplatelet drug use at baseline, as well as time-averaged systolic blood pressure and diastolic blood pressure during the treatment period. HR indicates hazard ratio.

(Table S9) with the risk of first ischemic stroke in various subgroups.

None of the variables, including age (*P*-interaction=0.24), sex (*P*-interaction=0.32), treatment group (*P*-interaction=0.11), body mass index (*P*-interaction=0.41), current smoking (*P*-interaction=0.51), tHcy (*P*-interaction=0.82), folate (*P*-interaction=0.86), total cholesterol (*P*-interaction=0.33), diabetes mellitus (*P*-interaction=0.42), SBP (*P*-interaction=0.95), antihypertensive drug use (*P*-interaction=0.20), lipid-lowering drug use (*P*-interaction=0.25), glucose-lowering drug use (*P*-interaction=0.70), and antiplatelet drug use (*P*-interaction=0.73) at baseline, as well as time-averaged SBP (*P*-interaction=0.81) during the treatment period, significantly modified the association between DBiL and first ischemic stroke (Figure 2 and Table S8). Similar results were also observed for TBiL levels (Table S9).

DISCUSSION

The current study demonstrates that, even after adjustments for multiple confounding factors, including BP at baseline and during the treatment period, higher baseline serum TBiL or DBiL levels were associated with decreased risk of first ischemic stroke in patients with hypertension.

The association between TBiL and the risk of stroke has been examined in several previous studies. Kimm et al[15] found that higher serum TBiL levels showed a lower HR for ischemic stroke in men but not in women, in a cohort study of health examinees in Korea. However, a previous nested case-control study by Ekblom et al[17] showed that plasma TBiL was lower in stroke cases than in controls, but the difference reached significance only in women. Mahabadi et al[18] reported that there was a significant inverse association between TBiL and stroke risk (only 95 stroke cases) in the general population without known liver disease. The PREVEND (Prevention of Renal and Vascular End-Stage Disease) study[16] demonstrated that there was no significant evidence of an association between TBiL and stroke risk (per SD increment; HR, 0.87; 95% CI, 0.72–1.04) in 7222 participants without a known history of cardiovascular disease. At the same time, using a mendelian randomization design, Lee et al[28] suggested a noncausal association between TBiL and stroke risk. However, except for the inherent limitation of mendelian randomization design, this study did not perform a replication analysis in a powered second group, and could not confirm the exact recurrent stroke. Overall, these studies indicated that the association between blood TBiL and the risk of stroke remains uncertain. Of note, in the previous studies, only ≈10% to 50% of the participants had hypertension. More important, there was no information on BP levels during the follow-up period in all of the previous studies. Therefore, the results could not be generalized to a hypertensive population. Our study provided an opportunity to assess the dose-response relationship between serum bilirubin and first stroke in hypertensive patients receiving

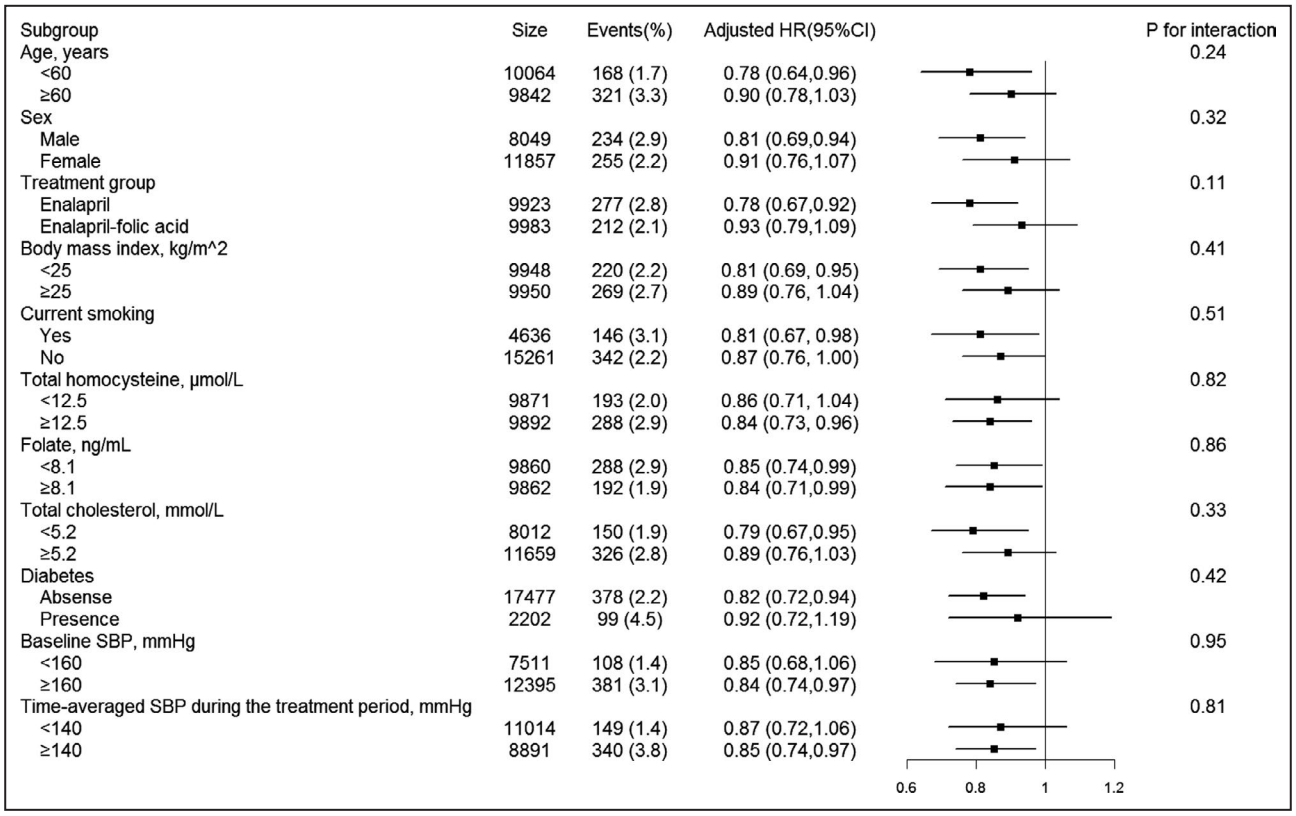

Figure 2. Interactions between serum direct bilirubin (per SD increment) and different variables on first ischemic stroke, examined by likelihood ratio testing.

Adjusted for age, sex, treatment group, body mass index, systolic blood pressure (SBP), diastolic blood pressure, smoking status, fasting glucose, total cholesterol, triglycerides, alanine aminotransferase, γ-glutamyl transpeptidase, total homocysteine, folate levels, antihypertensive drug use, lipid-lowering drug use, glucose-lowering drug use, and antiplatelet drug use at baseline, as well as time-averaged SBP and diastolic blood pressure during the treatment period, if not stratified. Diabetes mellitus was defined as fasting glucose ≥7.0 mmol/L at baseline, use of glucose-lowering drugs, or self-reported history of diabetes mellitus. HR indicates hazard ratio.

standard treatment, with comprehensive adjustments for a series of important confounders and a strict validation of study outcomes.[21]

Our current study found that both higher serum TBiL and DBiL were related to the lower risk of first ischemic stroke in hypertensive patients. Ischemic stroke is a complex process in which atherothrombosis plays an important role.[29] Serum bilirubin is an endogenous antioxidative substance and is positively correlated with total antioxidant capacity.[30] Previous studies reported that the antioxidative activity of bilirubin is independent of its forms (ie, unconjugated and conjugated bilirubins).[31] It has been found that low serum bilirubin was associated with the oxidation of lipids and lipoproteins and the damage of endothelium, consequently leading to the formation of atherosclerotic plaques and arterial thrombosis.[32–34] Accordingly, in a previous double-blind, placebo-controlled, crossover study,[35] treatment with the bilirubin-increasing drug atazanavir induced an increase in average bilirubin levels and improved plasma antioxidant capacity and endothelium-dependent vasodilation in patients with type 2 diabetes mellitus. Moreover, Stein et al[36] reported that higher levels of on-treatment bilirubin (>10.1 versus ≤10.1 μmol/L) were associated with significantly slower progression of carotid artery intima-media thickness. However, the findings and the detailed mechanisms still need to be confirmed in further studies.

The potential limitations of the current analyses should also be considered in interpreting the study results. First, post hoc analyses of randomized trials have inherent limitations, such as the possibility of residual imbalance in some unmeasured factors at baseline. Second, our study was conducted in Chinese hypertensive patients. Although the stratified analysis showed that BP at baseline and during the treatment period did not substantially change the findings, the generalizability of our results to nonhypertensive adults and other populations remains to be determined. Third, our study may be underpowered for evaluating the relation of bilirubin with the risk of hemorrhagic stroke. Fourth, the bilirubin levels of the current study population were mostly in the normal range. And the similar inverse association between serum TBiL or DBiL and first ischemic stroke

was also found in those with normal TBiL or DBiL levels. Of note, our results just suggested the possible beneficial effect of relatively higher TBiL or DBiL levels within normal range on first ischemic stroke. Another limitation of the CSPPT is the lack of the classification of subtypes of ischemic stroke based on mechanisms. Finally, we did not have the direct measurements of indirect bilirubin. Because of these limitations, our results should be regarded as hypothesis generating. All findings need to be further investigated and confirmed in future studies.

In summary, our study suggests that a higher concentration of serum bilirubin is associated with a lower risk of first ischemic stroke. Serum bilirubin concentration can be measured easily in the clinical laboratory and applied in medical practice. If our findings are further confirmed by future studies, routine measurements of bilirubin could help identify those hypertensive patients at high risk of ischemic stroke.

ARTICLE INFORMATION

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Disclosures

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Supplementary Materials

Tables S1–S9
Figure S1

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SUPPLEMENTAL MATERIAL

Table S1. Characteristics of the included and excluded participants in the current study *

Characteristics	Excluded	Included
No. of participants	796	19906
Age, year	59.5 (7.6)	60 (7.5)
Male, No. (%)	448 (56.3)	8049 (40.4)
BMI, kg/m^2	24.7 (3.9)	25.0 (3.7)
Enalapril-folic acid, No. (%)	365 (45.9)	9983 (50.2)
Current smoker, No. (%)	233 (29.3)	4636 (23.3)
Blood pressure, mmHg		
Systolic BP at baseline	166.0 (20.4)	166.9 (20.4)
Diastolic BP at baseline	95.4 (12.1)	94.0 (11.9)
Time-averaged systolic BP	139.9 (11.8)	139.8 (11.4)
Time-averaged diastolic BP	83.8 (8.0)	83.0 (7.6)
Laboratory results		
Fasting glucose, mmol/L	5.7 (1.8)	5.8 (1.7)
Total cholesterol, mmol/L	5.2 (1.3)	5.5 (1.2)
Triglycerides, mmol/L	1.7 (1.4)	1.7 (1.2)
HDL-C, mmol/L	1.3 (0.5)	1.3 (0.4)
Alanine transaminase, IU/L	29.5 (101.6)	14.1 (7.0)
Aspartate transaminase, IU/L	53.2 (177.4)	25.5 (9.0)
Gamma glutamyl transpeptidase, IU/L	56.0 (110.5)	27.0 (27.3)
Total homocysteine, μmol/L	15.0 (10.0)	14.4 (8.3)
Total bilirubin, μmol/L	17.2 (13.3)	12.0 (4.9)
Direct bilirubin, μmol/L	3.8 (5.2)	2.3 (1.2)
Medication use, No. (%)		
Antihypertensive drugs	337 (42.3)	9199 (46.2)
Lipid-lowering drugs	5 (0.6)	161 (0.8)
Glucose-lowering drugs	13 (1.6)	304 (1.5)
Antiplatelet drugs	20 (2.5)	587 (2.9)

*Continuous variables are presented as mean (standard deviation), categorical variables are presented as number (percentage).

BP, blood pressure; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol.

Table S2. Relationship of baseline serum total bilirubin levels with first stroke and its subtypes with normal total bilirubin levels (3-17 μmol/L).

Total bilirubin, μmol/L	Events/N (%)	HR (95% CI)		
		Unadjusted	Adjusted*	*p* value*
First stroke				
Per SD increment	531	0.96 (0.88,1.05)	0.97 (0.88,1.06)	0.49
Tertiles				
T1 (<8.9)	188/5682(3.3)	REF	REF	
T2 (8.9-11.8)	176/5692(3.1)	0.92 (0.75,1.13)	0.95 (0.77,1.18)	0.64
T3 (≥11.8)	167/5699(2.9)	0.86 (0.70,1.06)	0.86 (0.69,1.08)	0.20
p for trend		0.17	0.20	
First ischemic stroke				
Per SD increment	431	0.91 (0.83,1.01)	0.91 (0.82,1.01)	0.08
Tertiles				
T1 (<8.9)	160/5682(2.8)	REF	REF	
T2 (8.9-11.8)	147/5692(2.6)	0.90 (0.72,1.13)	0.91 (0.73,1.15)	0.43
T3 (≥11.8)	124/5699(2.2)	0.75 (0.60,0.95)	0.73 (0.57,0.94)	0.02
p for trend		0.02	0.02	
First hemorrhagic stroke				
Per SD increment	98	1.19 (0.98,1.45)	1.22 (0.98,1.50)	0.07
Tertiles				
T1 (<8.9)	27/5682(0.5)	REF	REF	
T2 (8.9-11.8)	29/5692(0.5)	1.05 (0.62,1.78)	1.12 (0.64,1.96)	0.69
T3 (≥11.8)	42/5699(0.7)	1.51 (0.93,2.44)	1.63 (0.96,2.79)	0.07
p for trend		0.09	0.06	

*Adjusted for age, sex, treatment group, body mass index, systolic blood pressure, diastolic blood pressure, smoking status, fasting glucose, total cholesterol, triglycerides, alanine aminotransferase, gamma glutamyl transpeptidase, total homocysteine, folate levels, antihypertensive drugs usage, lipid-lowering drugs usage, glucose-lowering drugs usage, and antiplatelet drugs usage at baseline, as well as time-averaged systolic blood pressure and diastolic blood pressure during the treatment period.

Table S3. Relationship of baseline serum direct bilirubin levels with first stroke and its subtypes with normal direct bilirubin levels (≤6.8 μmol/L).

Direct bilirubin, μmol/L	Events/N (%)	HR (95% CI)		
		Unadjusted	Adjusted*	*p* value*
First stroke				
Per SD increment	607	0.88 (0.81,0.96)	0.92 (0.83,1.01)	0.09
Tertiles				
T1 (<1.6)	223/6603(3.4)	REF	REF	
T2 (1.6-2.5)	201/6568(3.1)	0.89 (0.74,1.08)	0.91 (0.75,1.11)	0.36
T3 (≥2.5)	183/6651(2.8)	0.79 (0.65,0.96)	0.87 (0.69,1.09)	0.21
p for trend		0.02	0.21	
First ischemic stroke				
DBiL (μmol/L)				
Per SD increment	488	0.81 (0.74,0.90)	0.85 (0.76,0.96)	0.01
Tertiles				
T1 (<1.6)	187/6603(2.8)	REF	REF	
T2 (1.6-2.5)	169/6568(2.6)	0.90 (0.73,1.10)	0.91 (0.73,1.13)	0.40
T3 (2.5-24.8)	132/6651(2)	0.68 (0.54,0.85)	0.77 (0.60,0.99)	0.04
p for trend		<0.01	0.04	
First hemorrhagic stroke				
DBiL (μmol/L)				
Per SD increment	117	1.17 (0.99,1.38)	1.12 (0.92,1.36)	0.25
Tertiles				
T1 (<1.6)	35/6603(0.5)	REF	REF	
T2 (1.6-2.5)	32/6568(0.5)	0.91 (0.56,1.47)	0.96 (0.58,1.59)	0.86
T3 (2.5-24.8)	50/6651(0.8)	1.37 (0.89,2.12)	1.27 (0.77,2.12)	0.35
p for trend		0.13	0.32	

*Adjusted for age, sex, treatment group, body mass index, systolic blood pressure, diastolic blood pressure, smoking status, fasting glucose, total cholesterol, triglycerides, alanine aminotransferase, gamma glutamyl transpeptidase, total homocysteine, folate levels, antihypertensive drugs usage, lipid-lowering drugs usage, glucose-lowering drugs usage, and antiplatelet drugs usage at baseline, as well as time-averaged systolic blood pressure and diastolic blood pressure during the treatment period.

Table S4. Relationship of baseline serum total bilirubin and direct bilirubin with first ischemic stroke with further adjustment for albumin.

	Events/N (%)	HR (95% CI)		
		Unadjusted	Adjusted*	*p* value*
Total bilirubin (μmol/L)				
Per SD increment	489	0.87 (0.79, 0.96)	0.94 (0.84,1.04)	0.22
Tertiles				
T1 (<9.3)	190/6621 (2.9)	REF	REF	
T2 (9.3-12.9)	159/6634 (2.4)	0.82 (0.66, 1.01)	0.88 (0.70,1.09)	0.24
T3 (12.9-34.1)	140/6651 (2.1)	0.71 (0.57, 0.89)	0.80 (0.62,1.02)	0.07
p for trend		<0.01	0.07	
Direct bilirubin (μmol/L)				
Per SD increment	489	0.80 (0.72, 0.89)	0.86 (0.77, 0.97)	0.01
Tertiles				
T1 (<1.6)	187/6603 (2.8)	REF	REF	
T2 (1.6-2.5)	169/6568 (2.6)	0.90 (0.73, 1.10)	0.94 (0.75,1.17)	0.55
T3 (2.5-24.8)	133/6735 (2.0)	0.68 (0.54, 0.85)	0.80 (0.62,1.03)	0.08
p for trend		<0.01	0.08	

*Adjusted for age, sex, treatment group, body mass index, systolic blood pressure, diastolic blood pressure, smoking status, fasting glucose, total cholesterol, triglycerides, alanine aminotransferase, gamma glutamyl transpeptidase, albumin, total homocysteine, folate levels, antihypertensive drugs usage, lipid-lowering drugs usage, glucose-lowering drugs usage, and antiplatelet drugs usage at baseline, as well as time-averaged systolic blood pressure and diastolic blood pressure during the treatment period.

Table S5. Relationship of baseline serum total bilirubin and direct bilirubin with first ischemic stroke with further adjustment for history of diabetes mellitus, arrhythmia and coronary heart disease at baseline.

	Events/N (%)	HR (95% CI)		
		Unadjusted	Adjusted*	*p* value*
Total bilirubin (μmol/L)				
Per SD increment	489	0.87 (0.79, 0.96)	0.92 (0.82,1.02)	0.10
Tertiles				
T1 (<9.3)	190/6621 (2.9)	REF	REF	
T2 (9.3-12.9)	159/6634 (2.4)	0.82 (0.66, 1.01)	0.85 (0.68,1.06)	0.15
T3 (12.9-34.1)	140/6651 (2.1)	0.71 (0.57, 0.89)	0.76 (0.60,0.97)	0.02
p for trend		<0.01	0.02	
Direct bilirubin (μmol/L)				
Per SD increment	489	0.80 (0.72, 0.89)	0.85 (0.76, 0.96)	0.01
Tertiles				
T1 (<1.6)	187/6603 (2.8)	REF	REF	
T2 (1.6-2.5)	169/6568 (2.6)	0.90 (0.73, 1.10)	0.91 (0.73,1.14)	0.41
T3 (2.5-24.8)	133/6735 (2.0)	0.68 (0.54, 0.85)	0.77 (0.60,0.99)	0.04
p for trend		< 0.01	0.04	

*Adjusted for age, sex, treatment group, body mass index, systolic blood pressure, diastolic blood pressure, smoking status, fasting glucose, total cholesterol, triglycerides, alanine aminotransferase, gamma glutamyl transpeptidase, total homocysteine folate levels, antihypertensive drugs usage, lipid-lowering drugs usage, glucose-lowering drugs usage, and antiplatelet drugs usage, and history of diabetes mellitus, arrhythmia and coronary heart disease at baseline, as well as time-averaged systolic blood pressure and diastolic blood pressure during the treatment period.

Table S6. Concomitant medications usage during the treatment period by tertiles of serum total bilirubin levels[*]

Medication, No. (%)	Total bilirubin, μmol/L			*p* value
	Tertile 1 (<9.3)	Tertile 2 (9.3-12.9)	Tertile 3 (12.9-34.1)	
Anti-hypertension drugs				
Calcium channel blockers	5184 (78.3)	5201 (78.4)	5311 (79.9)	0.05
Diuretics	3668 (55.4)	3218 (48.5)	2785 (41.9)	<0.01
Glucose-lowering drugs	122 (1.8)	90 (1.4)	95 (1.4)	0.05
Lipid-lowering drugs	9 (0.1)	13 (0.2)	11 (0.2)	0.70
Antiplatelet drugs	34 (0.5)	58 (0.9)	50 (0.8)	0.04

[*]Regular concomitant medication use was defined as 180 or more cumulative days of taking the drug of interest.

Table S7. Relationship of baseline serum total bilirubin and direct bilirubin levels with first ischemic stroke with further adjustment for concomitant medication usage during the treatment period.

Total bilirubin, μmol/L	Events/N (%)	HR (95% CI)		
		Unadjusted	Adjusted*	*p* value*
Total bilirubin (μmol/L)				
Per SD increment	489	0.87 (0.79, 0.96)	0.90 (0.81, 1.01)	0.06
Tertiles				
T1 (<9.3)	190/6621 (2.9)	REF	REF	
T2 (9.3-12.8)	159/6634 (2.4)	0.82 (0.66, 1.01)	0.84 (0.67, 1.05)	0.12
T3 (12.8-34.1)	140/6651 (2.1)	0.71 (0.57, 0.89)	0.73 (0.58, 0.93)	0.01
p for trend		<0.01	0.01	
Direct bilirubin (μmol/L)				
Per SD increment	489	0.80 (0.72, 0.89)	0.84 (0.74, 0.94)	<0.01
Tertiles				
T1 (<1.6)	187/6603 (2.8)	REF	REF	
T2 (1.6-2.5)	169/6568 (2.6)	0.90 (0.73, 1.10)	0.88 (0.71, 1.10)	0.26
T3 (2.5-24.8)	133/6735 (2.0)	0.68 (0.54, 0.85)	0.74 (0.58, 0.96)	0.02
p for trend		< 0.01	0.02	

*Adjusted for age, sex, treatment group, body mass index, systolic blood pressure, diastolic blood pressure, smoking status, fasting glucose, total cholesterol, triglycerides, alanine aminotransferase, gamma glutamyl transpeptidase, total homocysteine, folate levels, antihypertensive drugs usage, lipid-lowering drugs usage, glucose-lowering drugs usage, and antiplatelet drugs usage at baseline; as well as time-averaged systolic blood pressure and diastolic blood pressure, glucose lowering drugs usage, lipid lowering drugs usage, antiplatelet drugs usage, calcium channel blockers usage and diuretics usage during the treatment period.

Table S8. Association between serum direct bilirubin (per-SD increment) and risk of first ischemic stroke in various subgroups*

Subgroup	Size	Events (%)	Adjusted HR (95%CI)	*p* for interaction
Antihypertensive drugs usage				0.20
Yes	9199	267 (2.9)	0.91 (0.78,1.06)	
No	10707	222 (2.1)	0.79 (0.67,0.93)	
Lipid-lowering drugs usage				0.25
Yes	161	3 (1.9)	1.62 (0.60,4.40)	
No	19745	486 (2.5)	0.84 (0.75,0.95)	
Glucose-lowering drugs usage				0.70
Yes	304	10 (3.3)	0.99 (0.45,2.18)	
No	19602	479 (2.4)	0.85 (0.75,0.95)	
Antiplatelet drugs uasag				0.73
Yes	587	16 (2.7)	0.96 (0.49,1.89)	
No	19319	473 (2.4)	0.85 (0.75,0.95)	

*Adjusted for age, sex, treatment group, body mass index, systolic blood pressure, diastolic blood pressure, smoking status, fasting glucose, total cholesterol, triglycerides, alanine aminotransferase, gamma glutamyl transpeptidase, total homocysteine, folate levels, antihypertensive drugs usage, lipid-lowering drugs usage, glucose-lowering drugs usage, and antiplatelet drugs usage at baseline, as well as time-averaged systolic blood pressure and diastolic blood pressure during the treatment period.

Diabetes was defined as fasting glucose ≥7.0 mmol/L at baseline or, use of glucose-lowering drugs or, self-reported history of diabetes disease.

Table S9. Association between serum total bilirubin (per-SD increment) and risk of first ischemic stroke in various subgroups*

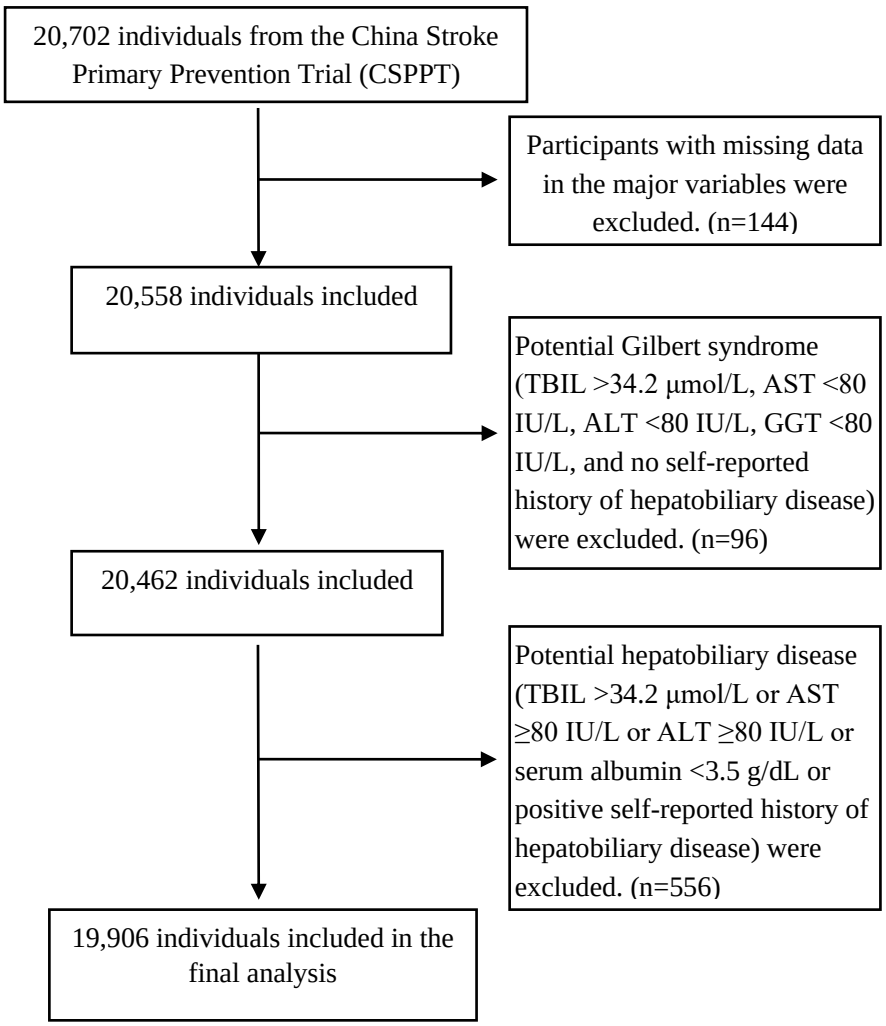
Subgroup	Size	Events (%)	Adjusted HR (95%CI)	*p* for interaction
Age, years				0.27
<60	10064	168 (1.7)	0.85 (0.71, 1.02)	
≥60	9842	321 (3.3)	0.96 (0.85, 1.09)	
Sex				0.59
Male	8049	234 (2.9)	0.89 (0.77, 1.03)	
Female	11857	255 (2.2)	0.94 (0.81, 1.10)	
Treatment group				0.13
Enalapril	9923	277 (2.8)	0.85 (0.74, 0.98)	
Enalapril-folic acid	9983	212 (2.1)	0.99 (0.86, 1.15)	
Body mass index, kg/m^2				0.89
<25	9948	220 (2.2)	0.92 (0.79, 1.07)	
≥25	9950	269 (2.7)	0.91 (0.78, 1.05)	
Current smoking				0.97
Yes	4636	146 (3.1)	0.92 (0.77, 1.09)	
No	15261	342 (2.2)	0.91 (0.80, 1.04)	
Total homocysteine, μmol/L				0.94
<12.5	9871	193 (2.0)	0.91(0.76, 1.08)	
≥12.5	9892	288 (2.9)	0.91 (0.80, 1.04)	
Folate, ng/mL				0.96
<8.1	9860	288 (2.9)	0.91 (0.80,1.05)	
≥8.1	9862	192 (1.9)	0.91 (0.78,1.06)	
Total cholesterol, μmol/L				0.62
<5.2	8012	150 (1.9)	0.88 (0.75, 1.05)	
≥5.2	11659	326 (2.8)	0.93 (0.82, 1.06)	
Diabetes				0.34
Absense	17477	378 (2.2)	0.89 (0.79, 1.00)	
Presence	2202	99 (4.5)	1.00 (0.80, 1.25)	
Baseline SBP, mmHg				0.56
<160	7511	108 (1.4)	0.96 (0.79, 1.17)	
≥160	12395	381 (3.1)	0.90 (0.79, 1.01)	
SBP during the treatment period, mmHg				0.59
<140	11014	149 (1.4)	0.96 (0.80, 1.14)	
≥140	8891	340 (3.8)	0.90 (0.79, 1.03)	
Antihypertensive drugs uasge				0.46
Yes	9199	267 (2.9)	0.95 (0.82, 1.09)	
No	10707	222 (2.1)	0.88 (0.76, 1.02)	

Lipid-lowering drugs usage				0.31
Yes	161	3 (1.9)	1.46 (0.66, 3.21)	
No	19745	486 (2.5)	0.91 (0.82, 1.01)	
Glucose-lowering drugs usage				0.67
Yes	304	10 (3.3)	1.07 (0.51, 2.25)	
No	19602	479 (2.4)	0.91 (0.82, 1.01)	
Antiplatelet drugs usage				0.90
Yes	587	16 (2.7)	0.88 (0.47, 1.64)	
No	19319	473 (2.4)	0.91 (0.82, 1.02)	

*Adjusted for age, sex, treatment group, body mass index, systolic blood pressure, diastolic blood pressure, smoking status, fasting glucose, total cholesterol, triglycerides, alanine aminotransferase, gamma glutamyl transpeptidase, total homocysteine, folate levels, antihypertensive drugs usage, lipid-lowering drugs usage, glucose-lowering drugs usage, and antiplatelet drugs usage at baseline, as well as time-averaged systolic blood pressure and diastolic blood pressure during the treatment period.

Diabetes was defined as fasting glucose ≥7.0 mmol/L at baseline or, use of glucose-lowering drugs or, self-reported history of diabetes disease.

Figure S1. Flow chart of the participants.



TBiL, total bilirubin; DBiL, direct bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyl transpeptidase.