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Original Article

# Effect Modification by Total Bilirubin on the Association Between Hypertension and Cerebral Small Vessel Disease

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**Background and Purpose** Bilirubin has potent antioxidant, anti-inflammatory, and neuroprotective effects. Herein, we investigated whether total bilirubin (TBIL) modifies the association between hypertension and cerebral small vessel disease (CSVD).

**Methods** Data were obtained from the PolyvasculaR Evaluation for Cognitive Impairment and vaScular Events study. TBIL and direct bilirubin (DBIL) levels were assayed using fasting venous blood samples. Indirect bilirubin (IBIL) was calculated by subtracting DBIL from TBIL. TBIL was stratified as ≤17 μmol/L and >17 μmol/L based on the biological relevance of Gilbert's syndrome. Hypertension was defined as blood pressure ≥140/90 mm Hg, self-reported hypertension history, or current use of antihypertensive agents. White matter hyperintensity, lacunes, cerebral microbleeds, and enlarged perivascular spaces were evaluated using magnetic resonance imaging and used to rate CSVD burden according to the criteria proposed by Wardlaw et al. and Rothwell et al.

**Results** This study included 3,061 participants, with a mean age of 61.2±6.7 years and 46.5% males. After adjusting for confounders, hypertension was associated with increased odds of presence of CSVD (Wardlaw: odds ratio [OR]=1.86, 95% confidence interval [CI] 1.41–2.44, *P*<0.001; Rothwell: OR=1.84, 95% CI 1.43–2.38, *P*<0.001) and higher modified total CSVD burden (common OR: 1.85, 95% CI 1.45–2.36, *P*<0.001) in participants with TBIL ≤17 μmol/L but not in TBIL >17 μmol/L (*P* for interaction <0.05). Johnson–Neyman analyses showed cut-off concentrations of 22.3–22.4 μmol/L for effect modification by TBIL. IBIL contributed to effect modification, whereas DBIL did not.

**Conclusions** Mildly elevated TBIL may modify the association between hypertension and CSVD.

**Keywords** Cerebral small vessel disease; Hypertension; Bilirubin; Effect modification

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## Introduction

Cerebral small vessel disease (CSVD) is highly prevalent (30.5%) among older adults and is a vital cause of stroke, vascular dementia, intracranial hemorrhage, and Alzheimer's disease.[1,2] Alarmingly, no effective clinical treatments exist for CSVD, as the underlying pathogenesis remains incompletely understood.[3] Prevention and control of CSVD risk factors might be the most feasible strategy to lower the CSVD burden. Among factors contributing to CSVD, including aging, hypertension, diabetes mellitus, dyslipidemia, smoking, and high salt diet, hypertension is a paramount and modifiable risk factor.[4] Patients with hypertension have increased oxidative stress and inflammatory responses, which directly injure the vascular endothelium to allow the perivascular infiltration of toxic materials into neural tissues or blockage of clearance via the glymphatic pathway, ultimately leading to CSVD.[5]

Strategies focusing on blood pressure (BP) control alone appear to be insufficient for reducing the risk of CSVD in patients with hypertension. For example, a cohort study demonstrated a significant reduction in cerebral blood flow in patients with hypertension despite BP control to <140/90 mm Hg after 4 years of follow-up.[6] The reduction in cerebral blood flow was comparable between treated and untreated patients.[6] Randomized controlled studies have reported that treatment with telmisartan, candesartan, or perindopril combined with indapamide lowers systolic blood pressure (SBP) but does not slow the progression of cerebral white matter damage in patients with hypertension.[7] Thus, improved strategies are needed to address these challenges in CSVD prevention.

Bilirubin is a tetrapyrrolic compound originating from heme catabolism and has potent antioxidant, anti-inflammatory, and neuroprotective properties.[8,9] Recently, the levels of bilirubin, as a substance similar to endocrine hormones, have been shown to be inversely associated with cardiovascular disease and vascular risk factors due to its potential metabolic protective effects.[8,10,11] The low incidence of adiposity, non-alcoholic fatty liver disease, metabolic syndrome, and diabetes mellitus in people with Gilbert's syndrome (GS) is partly attributed to the promoting effects of mildly elevated total bilirubin (TBIL) levels on metabolism.[11,12] However, the association of TBIL with CSVD remains inconsistent. Two studies reported that TBIL is inversely associated with cerebral white matter lesions in the general population, whereas another study did not observe an association between TBIL and CSVD.[13-15] Additionally, a recent study found that TBIL may modify the association between diabetes and stroke by suppressing inflammatory factors,[16] suggesting that TBIL may also act as a modifying factor for the association between hypertension and CSVD. Thus, we hypothesized that mildly increased TBIL concentrations may reduce the deleterious effects of hypertension on CSVD owing to the potent antioxidant and anti-inflammatory effects of TBIL.[1,8,9] Validating the hypothesis can provide a new insight for patients with hypertension to prevent CSVD.

Therefore, this cross-sectional study investigated whether TBIL would modify the association between hypertension and CSVD in a community-based population.

## Methods

### Study design and participants

Data were obtained from the baseline survey of the PolyvasculaR Evaluation for Cognitive Impairment and vaScular Events (PRECISE) study (ClinicalTrials.gov Registry No. NCT03178448). The rationale and design of the PRECISE study have been described elsewhere.[17] Briefly, the PRECISE study is an ongoing population-based prospective cohort study that aims to evaluate the prevalence and progression of clinical or subclinical polyvascular lesions in community-dwelling older adults in China using advanced vascular imaging techniques. Using the cluster sample method, PRECISE enrolled 3,067 participants aged 50–75 years from six villages and four communities in Lishui city, Zhejiang province, between May 2017 and September 2019. The exclusion criteria were individuals with contraindications to magnetic resonance imaging (MRI) and computed tomography angiography, life expectancy ≤4 years, and mental diseases. The participants in PRECISE represented a nationwide sample of demographics and medical histories.[17] In this study, we further excluded individuals with missing data on hypertension, CSVD, and TBIL. This study complied with the principles of the Declaration of Helsinki and was approved by the Institutional Review Boards (IRBs) of Beijing Tiantan Hospital (IRB Approval No. KY2017-010-01) and Lishui Central Hospital (IRB Approval No. 2016-42). Informed consent was obtained from all participants before enrollment.

### Data collection

Baseline data were collected through face-to-face interviews by trained research coordinators at Lishui Central Hospital. Standard questionnaires were used to collect data on demographics, smoking and drinking habits, salt intake, medical history (hypertension, diabetes, and dyslipidemia), and medication use (antihypertensive, lipid-lowering, antidiabetic, antiplatelet, and anticoagulant medications). Height, body weight, and BP were measured during physical examinations. BP was measured three times in a seated position after resting for 5 minutes using an automated sphygmomanometer (OMRON Model HEM-7071, Omron Co., Dalian, China). The average of the second and third


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BP measurements was used in this study. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. Fasting venous blood samples were drawn to measure hemoglobin, fasting blood glucose (FBG), glycosylated hemoglobin (HbA1c), total cholesterol (TC), triglyceride, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, serum creatinine, and high-sensitivity C-reactive protein (hsCRP) levels. An oral glucose tolerance test was performed in participants without diabetes to assay two-hour postload glucose levels.

Hypertension was defined as SBP ≥140 mm Hg, or diastolic blood pressure (DBP) ≥90 mm Hg, or self-reported hypertension history, or current use of antihypertensive agents.[18] Diabetes was defined as FBG ≥7.0 mmol/L, two-hour postload glucose ≥11.1 mmol/L, HbA1c ≥6.5%, self-reported diabetes history, or current use of antidiabetic agents.[19] Dyslipidemia was defined as TC ≥240 mg/dL, LDL-C ≥160 mg/dL, HDL-C <40 mg/dL (to convert cholesterol to mmol/L, multiply by 0.0259), or self-reported dyslipidemia previously diagnosed by a physician.[20] Abnormal liver function was defined as elevated ALT (>47 U/L), elevated AST (>37 U/L), or reduced albumin (<3.7 g/dL).[21] Anemia in adults was defined as hemoglobin levels <13 g/dL in males and <12 g/dL in females.[22] Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease-Epidemiology Collaboration equation.[23]

## Measurement of serum bilirubin levels

Fasting venous blood samples were drawn to assay TBIL and direct bilirubin (DBIL) levels using an ARCHITECT c16000 autoanalyzer (Abbott Laboratories, Chicago, IL, USA). Indirect bilirubin (IBIL) was calculated by subtracting DBIL from TBIL. Chronically and mildly elevated bilirubin concentrations are associated with lower risks of cardiovascular diseases, metabolic diseases, certain cancers, autoimmune diseases, and neurodegenerative diseases due to the antioxidant, anti-inflammatory, metabolically protective, and neuroprotective properties of bilirubin.[8,9,11] This phenomenon, known as GS, manifests as mildly increased blood TBIL levels (>17 μmol/L) caused by specific mutations of uridine diphosphate-glucuronosyltransferase 1-1 alongside normal serum activities of liver transaminases, biliary damage markers, and red blood cell counts.[11,24] Based on this biological relevance, we defined high and low TBIL concentrations as >17 μmol/L and ≤17 μmol/L, respectively. Abnormally elevated TBIL was defined as TBIL concentrations >2 times the upper limit of the normal range (41 μmol/L).[25]

## MRI acquisition and assessment

Participants underwent head MRI using a 3.0T MRI scanner (Ingenia 3.0T; Philips, Best, The Netherlands) at Lishui Central Hospital Medical Center. The MRI sequences involved three-dimensional T1-weighted magnetization-prepared rapid acquisition gradient echo, axial T2-weighted fluid-attenuated inversion recovery, and axial susceptibility-weighted imaging. MRI data were stored in the digital imaging and communications in medicine format on discs and analyzed at the Imaging Research Center of Beijing Tiantan Hospital. CSVD imaging markers were identified according to the Standards for Reporting Vascular Changes in Neuroimaging.[26] White matter hyperintensity (WMH) was defined as an increased brightness of the brain white matter on T2 images. Periventricular and deep WMH were evaluated using the Fazekas rating scale.[27] Lacunes were defined as rounded or ovoid lesions of the cerebrospinal fluid signal measuring 3–20 mm in diameter. Cerebral microbleeds (CMBs), including cortical and subcortical CMBs, were defined as round or oval hypointense lesions measuring 2–10 mm on gradient-recalled echo or susceptibility-weighted images. The number of CMBs was recorded to define the CMB burden as grade 0 (absent), grade 1 (1–4 microbleeds), or grade 2 (≥5 microbleeds). Enlarged perivascular space (EPVS) was defined as small punctate (<3 mm) or linear hyperintensities on T2 images. EPVS in the basal ganglia (BG-EPVS) were graded using a semi-quantitative rating scale.[28] Each CSVD imaging marker was independently evaluated by two of four well-trained raters (M Zhou, Y Chen, J Pi, and M Zhao, with one rater assessing two markers) who were blinded to the participants' clinical data. Inconsistent results were further evaluated by another senior neurologist (Y Yang), who was blinded to the initial results. The kappa coefficients of CSVD markers between raters were 0.82 for the Fazekas rating scale of WMH, 0.80 for the presence of lacune and CMBs, and 0.90 for the severity of BG-EPVS. The criteria proposed by Wardlaw et al. was used to calculate the total CSVD burden score.[29] This score is independently associated with common vascular risk factors for CSVD and lacunar stroke subtype, and has demonstrated predictive value for recurrent stroke[29,30]; thus, it can better capture the overall effect of CSVD on the brain than individual CSVD imaging markers.[29] We also applied the criteria defined by Rothwell et al., which modified the total CSVD burden score by redefining microbleed burden and WMH and adjusting the cut-off of basal ganglia perivascular space in the criterion.[30] This modified score may improve the predictive power for intracerebral hemorrhage.[30] We applied these two criteria to enhance the generalizability of our findings to estimate the CSVD burden score. According to the Wardlaw criteria, the total CSVD burden score was rated on an ordinal scale from 0 to 4, with one point allocated for WMH

burden (periventricular WMH Fazekas 3 or deep WMH Fazekas 2–3), presence of lacune, presence of CMBs, and moderate-to-severe BG-EPVS (n>10).[29] According to the Rothwell criteria, the modified total CSVD burden score was rated on an ordinal scale from 0 to 6, with one point allocated for the presence of lacune, CMB burden (n=1–4), severe BG-EPVS (n>20), modified WMH burden (total periventricular+subcortical WMH grade 3–4), two points allocated for CMB burden (n≥5) and modified WMH burden (total periventricular+subcortical WMH grade 5–6).[30] The presence of CSVD was defined as total CSVD or modified total CSVD burden scores ≥1, respectively.

### Statistical analysis

Continuous variables are described as mean±standard deviation or as median (interquartile range) and compared using the Wilcoxon rank-sum test. Categorical variables are described as frequencies (proportions) and compared using chi-square or Fisher's exact tests. The association between hypertension and the presence of CSVD, WMH burden, lacunes, CMBs, and BG-EPVS was evaluated using a binary logistic regression model with odds ratios (ORs) and 95% confidence intervals (CIs) presented. The associations of hypertension with CSVD, modified WMH, and CMB burden were evaluated using an ordinal logistic regression model with common odds ratio (cOR) and 95% CI presented. The product term of the hypertension state and TBIL group was included in the model to assess the effect modification by TBIL. *P* for interaction <0.05 indicated a statistically significant effect modification. The models were adjusted for biological factors (age and sex), vascular risk factors (BMI, current smoking, current drinking, diabetes, and dyslipidemia), medication use (antihypertensive, antiplatelet, and anticoagulant medications), and liver function indicators (ALT and AST). We also adjusted for liver function indicators, as bilirubin may be abnormally elevated in individuals with impaired liver function.[11] Sensitivity analyses were performed by additionally adjusting eGFR, hsCRP, and salt intake (<6 g/d, 6–12 g/d, or >12 g/d) in the models, and were performed in participants with normal TBIL values, normal liver function, without anemia, without use of antihypertensive medication, and without use of anticoagulant medication, respectively. Restricted cubic spline analyses were performed in participants with low and high TBIL concentrations to compare differences in the dose-response relationship between BP and CSVD. The reference was set at an SBP of 120 mm Hg or a DBP of 80 mm Hg. The knots were set at the 5th, 25th, 50th, 75th, and 95th percentiles of BP. Johnson–Neyman analyses were performed to identify effect modifications by TBIL, IBIL, and DBIL, as well as the cut-off concentrations for the presence of effect modification. Subsequently, we performed stratification analyses according to the identified TBIL cut-off concentrations.

All statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) and IBM SPSS Statistics for Windows, version 27.0 (IBM Corp., Armonk, NY, USA). Statistical significance was defined as two-sided *P*<0.05.

## Results

### Baseline participant characteristics

The PRECISE study enrolled 3,067 participants at baseline. After excluding those with missing data on CSVD (n=4) and TBIL (n=2), the final analysis included 3,061 participants (mean age of 61.2±6.7 years, 46.5% males). Among them, 2,219 (72.5%) had low TBIL concentrations (≤17 μmol/L) and 842 (27.5%) had high TBIL concentrations (>17 μmol/L). Participants with high TBIL concentrations had a higher proportion of males and current drinkers, higher DBP levels, and a lower prevalence of dyslipidemia compared with those with low TBIL concentrations (Supplementary Table 1). Participants with and without hypertension showed similar differences in baseline characteristics (Table 1).

### TBIL modified the association between hypertension and CSVD

The distribution of CSVD burden is presented in Supplementary Figure 1. Generally, participants with hypertension had a higher CSVD burden than those without hypertension, regardless of TBIL concentration. After adjusting for potential confounders, hypertension was positively associated with CSVD, whereas TBIL was not. The associations of hypertension with the presence of CSVD and modified total CSVD burden differed significantly according to TBIL concentrations (*P*<0.050). More specifically, hypertension was associated with increased odds of CSVD (Wardlaw: OR=1.86, 95% CI 1.41–2.44, *P*<0.001; Rothwell: OR=1.84, 95% CI 1.43–2.38, *P*<0.001) and higher modified total CSVD burden (cOR: 1.85, 95% CI 1.45–2.36, *P*<0.001) among participants with low TBIL concentrations. In contrast, hypertension was not associated with the presence of CSVD (Wardlaw: OR=1.06, 95% CI 0.68–1.64, *P*=0.802; Rothwell: OR=1.08, 95% CI 0.72–1.64, *P*=0.709) or modified total CSVD burden (cOR: 1.16, 95% CI 0.78–1.72, *P*=0.461) among participants with high TBIL concentrations (Figure 1).

Among CSVD imaging markers, TBIL only modified the association between hypertension and WMH-related burden. Hypertension was associated with increased WMH burden (OR: 2.45, 95% CI 1.76–3.40, *P*<0.001) and modified WMH burden (cOR: 2.04, 95% CI 1.58–2.63, *P*<0.001) in participants with low TBIL concentrations but not in high TBIL concentrations (WMH bur-


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**Table 1.** Baseline characteristics of participants according to hypertension status

| Characteristic | Participants without hypertension | | *P* | Participants with hypertension | | *P* |
|---|---|---|---|---|---|---|
| | TBIL ≤17 μmol/L (n=1,276) | TBIL >17 μmol/L (n=468) | | TBIL ≤17 μmol/L (n=943) | TBIL >17 μmol/L (n=374) | |
| Age (yr) | 59.8±6.4 | 60.3±6.3 | 0.058 | 62.9±6.7 | 63.1±6.8 | 0.538 |
| Male sex | 508 (39.8) | 322 (68.8) | <0.001 | 350 (37.1) | 244 (65.2) | <0.001 |
| Current smoking | 273 (21.4) | 113 (24.2) | 0.220 | 164 (17.4) | 77 (20.6) | 0.176 |
| Current drinking | 198 (15.5) | 127 (27.1) | <0.001 | 130 (13.8) | 118 (31.6) | <0.001 |
| BMI (kg/m$^2$) | 23.1±2.8 | 23.3±2.9 | 0.266 | 24.6±3.2 | 24.4±3.0 | 0.417 |
| SBP (mm Hg) | 120.0±11.0 | 121.3±10.5 | 0.027 | 141.5±15.0 | 140.0±14.4 | 0.087 |
| DBP (mm Hg) | 71.3±7.4 | 73.0±7.3 | <0.001 | 79.4±8.8 | 80.8±9.1 | 0.014 |
| FBG (mmol/L) | 5.7±1.2 | 5.8±1.2 | 0.060 | 6.3±1.8 | 6.3±2.2 | 0.249 |
| HbA1c (%) | 5.9±0.8 | 5.8±0.8 | <0.001 | 6.1±1.0 | 6.0±1.2 | <0.001 |
| TC (mg/dL) | 203.1 (180.1–228.6) | 196.7 (168.9–222.0) | <0.001 | 203.5 (182.2–231.3) | 200.0 (172.6–225.9) | 0.004 |
| TG (mg/dL) | 121.2 (88.9–174.3) | 114.6 (80.5–167.3) | 0.006 | 144.2 (105.3–215.0) | 144.2 (97.3–200.0) | 0.236 |
| HDL-C (mg/dL) | 52.1 (44.0–61.2) | 51.7 (43.8–63.3) | 0.612 | 49.8 (42.9–58.3) | 51.5 (44.0–60.2) | 0.082 |
| LDL-C (mg/dL) | 107.3 (88.6–127.8) | 101.4 (83.6–123.0) | <0.001 | 107.7 (89.2–128.2) | 100.4 (78.0–120.8) | <0.001 |
| TBIL (μmol/L) | 12.0 (9.9–14.2) | 21.2 (18.9–26.2) | <0.001 | 12.0 (9.8–14.2) | 20.5 (18.7–24.3) | <0.001 |
| DBIL (μmol/L) | 4.4 (3.7–5.1) | 7.4 (6.6–8.7) | <0.001 | 4.4 (3.7–5.1) | 7.2 (6.3–8.2) | <0.001 |
| IBIL (μmol/L) | 7.6 (6.0–9.0) | 14.0 (12.4–17.6) | <0.001 | 7.6 (6.0–9.0) | 13.5 (12.2–16.2) | <0.001 |
| Diabetes | 172 (13.5) | 84 (18.0) | 0.020 | 294 (31.2) | 111 (29.7) | 0.595 |
| Dyslipidemia | 488 (38.2) | 162 (34.6) | 0.165 | 466 (49.4) | 161 (43.1) | 0.037 |
| Concomitant medication | | | | | | |
| Antihypertensive | 0 (0.0) | 0 (0.0) | – | 591 (62.7) | 229 (61.2) | 0.626 |
| Lipid-lowing | 15 (1.2) | 12 (2.6) | 0.037 | 64 (6.8) | 29 (7.8) | 0.537 |
| Antidiabetic | 69 (5.4) | 22 (4.7) | 0.557 | 137 (14.5) | 45 (12.0) | 0.237 |
| Antiplatelet | 11 (0.9) | 5 (1.1) | 0.777 | 41 (4.4) | 23 (6.2) | 0.170 |
| Anticoagulant | 0 (0.0) | 0 (0.0) | – | 1 (0.1) | 3 (0.8) | 0.072 |

Values are presented as mean±standard deviation, n (%), or median (interquartile range).
TBIL, total bilirubin; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; HbA1c, glycosylated hemoglobin; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; DBIL, direct bilirubin; IBIL, indirect bilirubin.

den: OR=1.27, 95% CI 0.74–2.17, *P*=0.392; modified WMH burden: cOR=1.14, 95% CI 0.75–1.74, *P*=0.542) (*P* for interaction <0.050). However, lacunes, presence of CMBs, CMB burden, and moderate-to-severe and severe BG-EPVS showed no effect modification by TBIL (*P* for interaction >0.050) (Supplementary Table 2).

## Sensitivity analyses on the effect modification by TBIL

To assess the robustness of the results, we performed sensitivity analyses by additionally adjusting for eGFR, hsCRP, and salt intake in the models and in participants with normal TBIL values, normal liver function, without anemia, without use of antihypertensive medication, and without use of anticoagulant medication. The results were similar to previous findings, except for those in participants without anemia (Supplementary Tables 3-8). Furthermore, comparison of the differences in dose-response relationships between BP and CSVD according to TBIL concentration showed increased odds of CSVD for SBP >130 mm Hg or persistently elevated DBP in participants with low TBIL concentrations (Figure 2A-D). Conversely, SBP and DBP were not associated with CSVD in participants with high TBIL levels (Figure 2E-H). We observed similar differences in CSVD burden between participants with low and high TBIL concentrations, except for the dose-response relationship between DBP and modified total CSVD burden (Supplementary Figure 2).

## Cut-off value for the presence of effect modification by TBIL

We performed Johnson–Neyman analyses to identify the cut-off concentration of TBIL that potentially modified the association between hypertension and CSVD in the general Chinese population. The effect modification by TBIL was significant for the

presence of CSVD (Wardlaw: *P* for interaction=0.041; Rothwell: *P* for interaction=0.022) but not for CSVD burden (*P* for interaction >0.050) (Supplementary Table 9). The association between hypertension and CSVD presence, as defined by Wardlaw's criteria, gradually weakened as TBIL increased from low concentrations to 22.4 μmol/L and then was not statistically significant

| Outcome | Group | N | n (%) | Adj. OR/cOR (95% CI)§ | *P* | *P* for interaction |
|---|---|---|---|---|---|---|
| Presence of CSVD (Wardlaw)† | All participants* | | | | | |
| | No hypertension | 1,744 | 387 (22.2) | Ref. | – | |
| | Hypertension | 1,317 | 547 (41.5) | 1.58 (1.25–1.99) | <0.001 | |
| | All participants‖ | | | | | |
| | TBIL ≤17 μmol/L | 2,219 | 647 (29.2) | Ref. | – | |
| | TBIL >17 μmol/L | 842 | 287 (34.1) | 1.08 (0.89–1.31) | 0.446 | |
| | Participants with TBIL ≤17 μmol/L* | | | | | |
| | No hypertension | 1,276 | 258 (20.2) | Ref. | – | 0.034 |
| | Hypertension | 943 | 389 (41.3) | 1.86 (1.41–2.44) | <0.001 | |
| | Participants with TBIL >17 μmol/L* | | | | | |
| | No hypertension | 468 | 129 (27.6) | Ref. | – | |
| | Hypertension | 374 | 158 (42.3) | 1.06 (0.68–1.64) | 0.802 | |
| Presence of CSVD (Rothwell)† | All participants* | | | | | |
| | No hypertension | 1,744 | 562 (32.2) | Ref. | – | |
| | Hypertension | 1,317 | 709 (53.8) | 1.59 (1.28–1.98) | <0.001 | |
| | All participants‖ | | | | | |
| | TBIL ≤17 μmol/L | 2,219 | 905 (40.8) | Ref. | – | |
| | TBIL >17 μmol/L | 842 | 366 (43.5) | 1.00 (0.84–1.20) | 0.968 | |
| | Participants with TBIL ≤17 μmol/L* | | | | | |
| | No hypertension | 1,276 | 391 (30.6) | Ref. | – | 0.030 |
| | Hypertension | 943 | 514 (54.5) | 1.84 (1.43–2.38) | <0.001 | |
| | Participants with TBIL >17 μmol/L* | | | | | |
| | No hypertension | 468 | 171 (36.5) | Ref. | – | |
| | Hypertension | 374 | 195 (52.1) | 1.08 (0.72–1.64) | 0.709 | |
| Total CSVD burden (Wardlaw)‡ | All participants* | | | | | |
| | No hypertension | 1,744 | – | Ref. | – | |
| | Hypertension | 1,317 | – | 1.65 (1.32–2.06) | <0.001 | |
| | All participants‖ | | | | | |
| | TBIL ≤17 μmol/L | 2,219 | – | Ref. | – | |
| | TBIL >17 μmol/L | 842 | – | 1.08 (0.90–1.31) | 0.395 | |
| | Participants with TBIL ≤17 μmol/L* | | | | | |
| | No hypertension | 1,276 | – | Ref. | – | 0.082 |
| | Hypertension | 943 | – | 1.88 (1.44–2.46) | <0.001 | |
| | Participants with TBIL >17 μmol/L* | | | | | |
| | No hypertension | 468 | – | Ref. | – | |
| | Hypertension | 374 | – | 1.21 (0.79–1.84) | 0.380 | |
| Modified total CSVD burden (Rothwell)‡ | All participants* | | | | | |
| | No hypertension | 1,744 | – | Ref. | – | |
| | Hypertension | 1,317 | – | 1.62 (1.32–2.00) | <0.001 | |
| | All participants‖ | | | | | |
| | TBIL ≤17 μmol/L | 2,219 | – | Ref. | – | |
| | TBIL >17 μmol/L | 842 | – | 0.99 (0.83–1.18) | 0.914 | |
| | Participants with TBIL ≤17 μmol/L* | | | | | |
| | No hypertension | 1,276 | – | Ref. | – | 0.040 |
| | Hypertension | 943 | – | 1.85 (1.45–2.36) | <0.001 | |
| | Participants with TBIL >17 μmol/L* | | | | | |
| | No hypertension | 468 | – | Ref. | – | |
| | Hypertension | 374 | – | 1.16 (0.78–1.72) | 0.461 | |

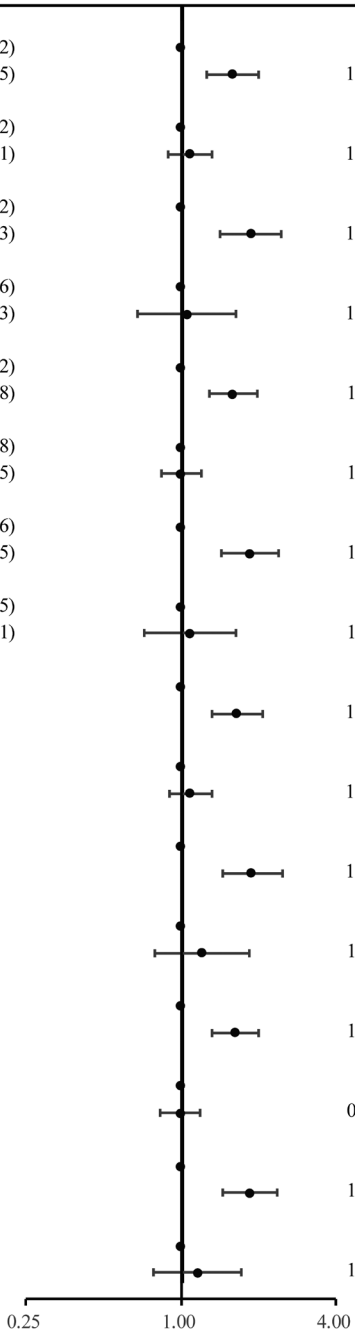


**Figure 1.** Associations between hypertension and CSVD in participants with low and high TBIL concentrations. Adj., adjust; OR, odds ratio; cOR, common odds ratio; CI, confidence interval; CSVD, cerebral small vessel disease; TBIL, total bilirubin; Ref., reference. *Models were adjusted for age, sex, body mass index, current smoking, current drinking, diabetes, dyslipidemia, antihypertensive medication, antiplatelet medication, anticoagulant medication, and alanine aminotransferase and aspartate aminotransferase levels; †Presence of CSVD was defined as total CSVD or modified total CSVD burden scores ≥1; ‡Total and modified total CSVD burdens were classified as grade 0 (total or modified total CSVD burden score 0), grade 1 (total or modified total CSVD burden score 1), grade 2 (total or modified total CSVD burden score 2–3), or grade 3 (total or modified total CSVD burden score ≥4); §The associations of TBIL and hypertension with the presence of CSVD were evaluated using OR, while those with CSVD burden were evaluated using cOR. The product term of the hypertension state and TBIL group was included in the model to assess the effect modification by TBIL; ‖Models were adjusted for the above variables plus hypertension.


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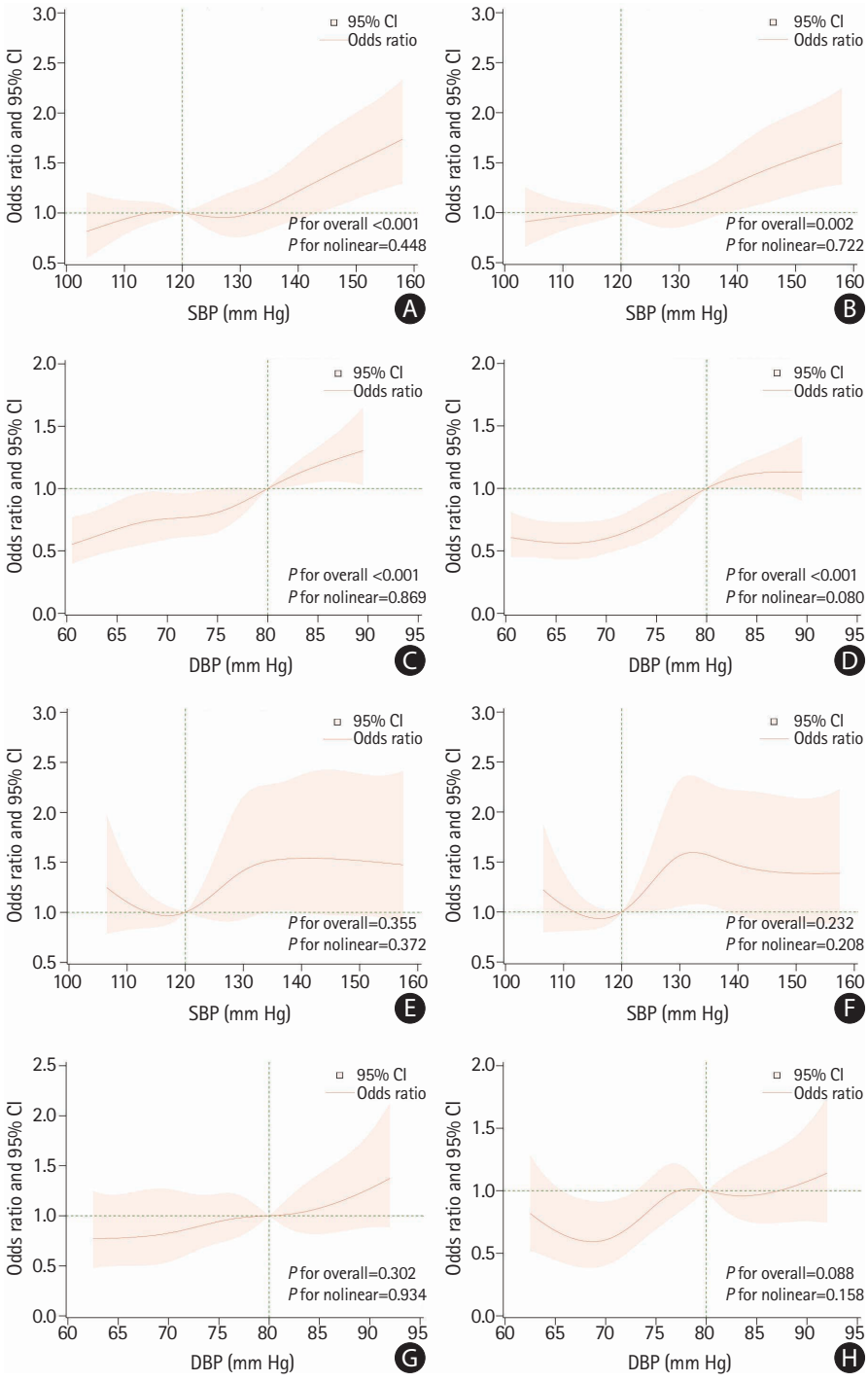


**Figure 2.** Dose-response relationship between blood pressure and the presence of CSVD in participants with low and high TBIL concentrations. (A) Dose-response relationship between SBP and presence of CSVD (Wardlaw) in low TBIL concentrations. (B) Dose-response relationship between SBP and presence of CSVD (Rothwell) in low TBIL concentrations. (C) Dose-response relationship between DBP and presence of CSVD (Wardlaw) in low TBIL concentrations. (D) Dose-response relationship between DBP and presence of CSVD (Rothwell) in low TBIL concentrations. (E) Dose-response relationship between SBP and presence of CSVD (Wardlaw) in high TBIL concentrations. (F) Dose-response relationship between SBP and presence of CSVD (Rothwell) in high TBIL concentrations. (G) Dose-response relationship between DBP and presence of CSVD (Wardlaw) in high TBIL concentrations. (H) Dose-response relationship between DBP and presence of CSVD (Rothwell) in high TBIL concentrations. All models were adjusted for age, sex, body mass index, current smoking, current drinking, diabetes, dyslipidemia, antihypertensive medication, antiplatelet medication, anticoagulant medication, and alanine aminotransferase and aspartate aminotransferase levels. The reference was set at an SBP of 120 mm Hg or a DBP of 80 mm Hg. Knots were set at the 5th, 25th, 50th, 75th, and 95th percentiles of blood pressure. The figures were drawn within the 5th to 95th percentiles of blood pressure to minimize the influence of extreme values on the size of the figures. Presence of CSVD (Wardlaw): total CSVD burden score ≥1 according to the criterion of Wardlaw's group. Presence of CSVD (Rothwell): modified total CSVD burden score ≥1 according to the criterion of Rothwell's group. The sample sizes are 2,219 in A-D and 842 in E-H. CI, confidence interval; SBP, systolic blood pressure; DBP, diastolic blood pressure; CSVD, cerebral small vessel disease; TBIL, total bilirubin.

for TBIL concentrations >22.4 μmol/L (Figure 3A). We observed a similar trend for CSVD presence, as defined by Rothwell's criteria, with a cut-off concentration of 22.3 μmol/L (Figure 3B). After stratification according to these thresholds, hypertension appeared to be differently associated with CSVD presence between participants with TBIL values below and above these

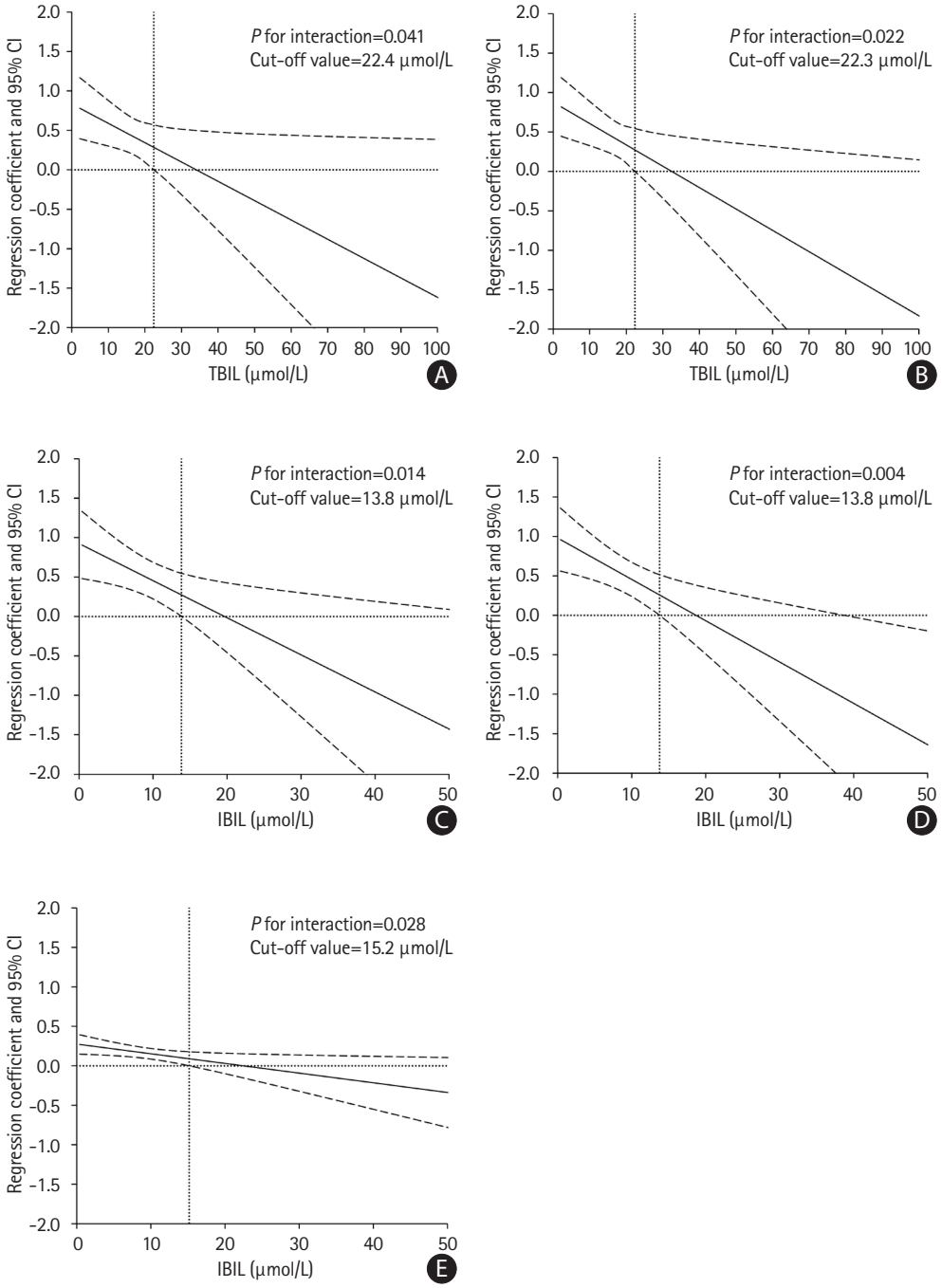


**Figure 3.** Johnson–Neyman analyses on effect modification by TBIL and IBIL. (A) Effect modification on presence of CSVD (Wardlaw) by TBIL. (B) Effect modification on presence of CSVD (Rothwell) by TBIL. (C) Effect modification on presence of CSVD (Wardlaw) by IBIL. (D) Effect modification on presence of CSVD (Rothwell) by IBIL. (E) Effect modification on modified total CSVD burden (Rothwell) by IBIL. The results of CSVD presence were estimated using a binary logistic regression model. The CSVD burden results were estimated using an ordinary least squares regression model. The product terms of the hypertension state and bilirubin concentration were included in the model to assess the effect modification by bilirubin. All results were expressed using a log-odds metric. All models were adjusted for age, sex, body mass index, current smoking, current drinking, diabetes, dyslipidemia, antihypertensive medication, antiplatelet medication, anticoagulant medication, and alanine aminotransferase and aspartate aminotransferase levels. Presence of CSVD (Wardlaw): total CSVD burden score ≥1 according to the criterion of Wardlaw's group. Presence of CSVD (Rothwell): modified total CSVD burden score ≥1 according to the criterion of Rothwell's group. Modified total CSVD burden (Rothwell) was classified as grade 0 (modified total CSVD burden score 0), grade 1 (score 1), grade 2 (score 2–3), and grade 3 (score ≥4). The sample size is 3,061 in A-E. CI, confidence interval; TBIL, total bilirubin; IBIL, indirect bilirubin; CSVD, cerebral small vessel disease.


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thresholds, with marginally significant *P* values for effect modification (Wardlaw: *P* for interaction=0.077; Rothwell: *P* for interaction=0.053) (Supplementary Table 10).

### Effect modification by IBIL and DBIL

To determine the components of TBIL that contribute to the modification of the association between hypertension and CSVD, we performed in-depth analyses of IBIL and DBIL. IBIL modified the association between hypertension and CSVD presence and total burden (*P* for interaction <0.050) (Supplementary Table 9). The corresponding cut-off concentrations for the presence of effect modification by IBIL were 13.8 μmol/L for CSVD presence and 15.2 μmol/L for modified total CSVD burden (Figure 3C-E). However, the effect of DBIL was not significant in the presence of CSVD or CSVD burden (*P* for interaction >0.050) (Supplementary Table 9).

## Discussion

The results of this community-based study demonstrated that hypertension was associated with increased odds of CSVD presence in participants with low TBIL concentrations (≤17 μmol/L) but not high TBIL concentrations (>17 μmol/L). The cut-off concentrations for the presence of effect modification by TBIL were 22.3–22.4 μmol/L in the general Chinese population. IBIL, but not DBIL, contributed to effect modification.

While previous studies have reported potential modifying associations between chronic mild hyperbilirubinemia and cardiovascular diseases, arterial hypertension, diabetes, obesity, metabolic syndrome, certain cancers, autoimmune diseases, ischemic stroke, and neurodegenerative diseases,[9-11] evidence of the potential modifying association between bilirubin and CSVD is limited. Clinical data from 1,121 healthy Japanese adults demonstrated an association between low TBIL concentrations and high prevalence of severe deep white matter lesions.[13] Our study further demonstrated that slightly elevated TBIL may modify the association between hypertension and WMH burden. Another study in 1,128 neurologically healthy adults showed that TBIL was not associated with CSVD,[15] consistent with our direct analysis of the association between TBIL and CSVD. Aging, hypertension, diabetes, dyslipidemia, smoking, and a high-salt diet are the main contributors to CSVD development[4]; an association between TBIL and CSVD was unlikely after including these factors in the models. However, contrary to previous studies,[13,15] the present study provides robust and comprehensive evidence to support the hypothesis that high TBIL levels could modify the association between hypertension and CSVD, showing that mildly elevated TBIL levels may be beneficial for CSVD prevention in patients with hypertension.

Hypertension damages cerebral vessels by suppressing nitric oxide production, inducing reactive oxygen species production, triggering systemic and vascular inflammation, and remodeling the extracellular matrix.[31] Its extended system of conjugated double bonds and reactive hydrogen atom make bilirubin a strong antioxidant molecule to inhibit nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-dependent proliferation of vascular smooth muscle cells and NADPH oxidase-dependent production of superoxide, thereby reducing oxidative stress in vessels.[9] Bilirubin also reduces inflammation by inhibiting complement induction, modulating cytotoxic T-lymphocyte activity, impeding cellular adhesion molecules, and blocking pro-inflammatory cytokine production.[9] These properties may collectively protect cerebral small vessels against endothelial dysfunction and atherosclerosis associated with hypertension, resulting in the effect modification by TBIL observed in the current study.

However, the sensitivity analysis excluding participants with anemia showed negative results. Although the association of anemia with CSVD remains inconclusive, a higher proportion of patients with anemia show white matter lesions, lacunes, and brain atrophy.[32,33] Anemia also contributes to WMH worsening in older adults with hypertension.[34] The underlying mechanism is that low hemoglobin-induced chronic cerebral hypoxia may cause ischemia or infarction in the subcortex and cortex.[32] Anemia also interacts with hypoperfusion consequent to hypertensive small-vessel changes to aggravate cerebral hypoxia.[33] Thus, excluding these high-risk patients from the sensitivity analysis may obscure the effect modification by TBIL. Moreover, the influence of insufficient statistical power in the sensitivity analysis cannot be ruled out, as the *P* values for effect modification were marginally significant.

Among CSVD imaging markers, TBIL potentially modified the association between hypertension and WMH burden. This may be attributed to inflammation and endothelial dysfunction as the primary drivers of WMH rather than other CSVD imaging markers.[35] TBIL can alleviate inflammation and endothelial dysfunction,[9] thereby modifying the association between hypertension and WMH burden. Bilirubin usually exists in the body in unconjugated (IBIL) and conjugated (DBIL) forms.[9] Most unconjugated bilirubin in normal plasma flows is tightly bound to albumin to be transferred to the liver and then converted into conjugated bilirubin.[9] Unbound unconjugated bilirubin can more readily cross the blood-brain barrier and passively diffuse across cells due to its lipid solubility, whereas conjugated bilirubin cannot.[9] These properties may explain why IBIL rather than DBIL contributed to the effect modification by TBIL.

The results of this study have several important clinical im-

plications. First, bilirubin can compensatorily increase under conditions of tissue hypoxia, free radicals, and inflammatory responses.[36] Our findings indicate that mildly elevated TBIL concentrations may be a mechanism of antioxidative stress and anti-inflammation to mitigate the risk of CSVD associated with hypertension. Second, TBIL could be a useful biomarker for risk stratification of CSVD, as patients with hypertension with low TBIL concentrations had a higher risk of CSVD. Such patients should be more alert to cerebral small-vessel lesions and should prioritize BP control. Third, this study proposed potential cut-off concentrations for the presence of effect modification by TBIL in the general Chinese population, which provides a valuable reference for TBIL modulation in clinical practice.

The major strength of this study was the population selection using a cluster sampling method to ensure demographic properties similar to those of the national sample survey.[17] This made the study results more easily applicable to the general population. Furthermore, using advanced high-resolution imaging techniques, this study comprehensively assessed CSVD imaging markers and calculated the CSVD burden.

Despite these strengths, this study also has some limitations. First, the cross-sectional design prevented us from inferring temporal and causal relationships among bilirubin, hypertension, and CSVD. Large-scale prospective studies are needed to confirm the potential modifying association between TBIL and CSVD. Second, the participants were selected from a single community; thus, the potential influence of selection bias could not be ruled out. Third, this study identified the effect modification by TBIL using the threshold of 17 μmol/L based on the biological relevance of GS.[11,24] This rationale may be insufficient. Further studies are needed to establish a threshold for effect modification by TBIL in different populations. Finally, the PRECISE cohort was based on a single Chinese community population, which limits the generalizability of our findings. Thus, replication of these findings in other ethnic groups and geographic settings is needed.

## Conclusions

Slightly elevated TBIL levels modified the association between hypertension and CSVD in community-based populations. Hypertension is positively associated with CSVD in low but not high TBIL concentrations. This modification effect may be attributed to IBIL rather than DBIL. These findings implicate that bilirubin might protect the cerebral small vessels against damage from hypertension. However, further studies are needed to confirm these findings and investigate the underlying mechanisms.

## Supplementary materials

Supplementary materials related to this article can be found online at https://doi.org/10.5853/jos.2025.01935.

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## Conflicts of interest

The authors have no financial conflicts of interest.

## Author contribution

Conceptualization: Zhang Xia. Data curation: Yingying Yang, Shan Li. Formal analysis: Zhang Xia, Xuan Wang. Funding acquisition: Yilong Wang, Yuesong Pan. Investigation: Xueli Cai, Shan Li. Methodology: Zhang Xia, Yingying Yang, Yuesong Pan. Project administration: Tiemin Wei, Yongjun Wang, Yilong Wang, Yuesong Pan. Resources: Xueli Cai, Tiemin Wei, Yongjun Wang. Software: Yingying Yang, Mengxing Wang, Xuan Wang. Supervision: Xueli Cai, Yuesong Pan. Validation: Xuan Wang. Visualization: Zhang Xia. Writing—original draft: Zhang Xia. Writing—review & editing: Yuesong Pan. Approval of final manuscript: all authors.

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**Supplementary Table 1.** Baseline characteristics of the participants

| Characteristic | All participants (n=3,061) | Participants with TBIL ≤17 μmol/L (n=2,219) | Participants with TBIL >17 μmol/L (n=842) | *P* |
|---|---|---|---|---|
| Age (yr) | 61.2±6.7 | 61.1±6.7 | 61.5±6.6 | 0.060 |
| Male sex | 1,424 (46.5) | 858 (38.7) | 566 (67.2) | <0.001 |
| Current smoking | 627 (20.5) | 437 (19.7) | 190 (22.6) | 0.079 |
| Current drinking | 573 (18.7) | 328 (14.8) | 245 (29.1) | <0.001 |
| BMI (kg/m$^2$) | 23.8±3.0 | 23.8±3.1 | 23.8±3.0 | 0.527 |
| SBP (mm Hg) | 129.3±16.3 | 129.1±16.7 | 129.6±15.5 | 0.344 |
| DBP (mm Hg) | 75.2±9.0 | 74.7±9.0 | 76.5±9.0 | <0.001 |
| FBG (mmol/L) | 6.0±1.6 | 5.9±1.5 | 6.0±1.8 | 0.440 |
| HbA1c (%) | 5.9±0.9 | 6.0±0.9 | 5.9±1.0 | <0.001 |
| TC (mg/dL) | 201.9 (178.0–227.8) | 203.5 (181.1–229.0) | 198.1 (170.7–223.2) | <0.001 |
| TG (mg/dL) | 129.2 (92.0–189.4) | 130.1 (93.8–192.0) | 126.5 (87.6–185.0) | 0.010 |
| HDL-C (mg/dL) | 51.0 (43.6–60.6) | 51.0 (43.6–59.8) | 51.7 (44.0–61.8) | 0.159 |
| LDL-C (mg/dL) | 105.8 (86.9–126.6) | 107.3 (88.8–128.2) | 100.8 (80.7–122.4) | <0.001 |
| TBIL (μmol/L) | 13.6 (10.7–17.7) | 12.0 (9.9–14.2) | 20.9 (18.8–25.2) | <0.001 |
| DBIL (μmol/L) | 4.9 (4.0–6.2) | 4.4 (3.7–5.1) | 7.3 (6.5–8.5) | <0.001 |
| IBIL (μmol/L) | 8.6 (6.7–11.4) | 7.6 (6.0–9.0) | 13.8 (12.3–16.8) | <0.001 |
| Hypertension | 1,317 (43.0) | 943 (42.5) | 374 (44.4) | 0.338 |
| Diabetes | 661 (21.6) | 466 (21.0) | 195 (23.2) | 0.195 |
| Dyslipidemia | 1,277 (41.7) | 954 (43.0) | 323 (38.4) | 0.020 |
| Concomitant medication | | | | |
| Antihypertensive | 820 (26.8) | 591 (26.6) | 229 (27.2) | 0.753 |
| Lipid-lowing | 120 (3.9) | 79 (3.6) | 41 (4.9) | 0.096 |
| Antidiabetic | 273 (8.9) | 206 (9.3) | 67 (8.0) | 0.250 |
| Antiplatelet | 80 (2.6) | 52 (2.3) | 28 (3.3) | 0.128 |
| Anticoagulant | 4 (0.1) | 1 (0.05) | 3 (0.4) | 0.066 |

Values are presented as mean±standard deviation, n (%), or median (interquartile range).
TBIL, total bilirubin; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; HbA1c, glycosylated hemoglobin; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; DBIL, direct bilirubin; IBIL, indirect bilirubin.

**Supplementary Table 2.** Association between hypertension and CSVD imaging markers in participants with low and high TBIL concentrations

| CSVD imaging markers | Group | N | n (%) | Adj. OR/cOR (95% CI)* | *P* | *P* for interaction |
|---|---|---|---|---|---|---|
| WMH burden[†] | Participants with TBIL ≤17 μmol/L | | | | | |
| | No hypertension | 1,276 | 119 (9.3) | Ref. | | 0.047 |
| | Hypertension | 943 | 247 (26.2) | 2.45 (1.76–3.40) | <0.001 | |
| | Participants with TBIL >17 μmol/L | | | | | |
| | No hypertension | 468 | 57 (12.2) | Ref. | | |
| | Hypertension | 374 | 87 (23.3) | 1.27 (0.74–2.17) | 0.392 | |
| Modified WMH burden[‡] | Participants with TBIL ≤17 μmol/L | | | | | |
| | No hypertension | 1,276 | | Ref. | | 0.032 |
| | Hypertension | 943 | | 2.04 (1.58–2.63) | <0.001 | |
| | Participants with TBIL >17 μmol/L | | | | | |
| | No hypertension | 468 | | Ref. | | |
| | Hypertension | 374 | | 1.14 (0.75–1.74) | 0.542 | |
| Lacunes | Participants with TBIL ≤17 μmol/L | | | | | |
| | No hypertension | 1,276 | 32 (2.5) | Ref. | | 0.960 |
| | Hypertension | 943 | 80 (8.5) | 2.27 (1.29–4.00) | 0.005 | |
| | Participants with TBIL >17 μmol/L | | | | | |
| | No hypertension | 468 | 16 (3.4) | Ref. | | |
| | Hypertension | 374 | 42 (11.2) | 2.06 (0.91–4.64) | 0.081 | |
| Presence of CMBs[§] | Participants with TBIL ≤17 μmol/L | | | | | |
| | No hypertension | 1,276 | 95 (7.5) | Ref. | | 0.643 |
| | Hypertension | 943 | 119 (12.6) | 1.16 (0.75–1.79) | 0.506 | |
| | Participants with TBIL >17 μmol/L | | | | | |
| | No hypertension | 468 | 44 (9.4) | Ref. | | |
| | Hypertension | 374 | 55 (14.7) | 1.02 (0.55–1.92) | 0.947 | |
| CMB burden[‖] | Participants with TBIL ≤17 μmol/L | | | | | |
| | No hypertension | 1,276 | | Ref. | | 0.730 |
| | Hypertension | 943 | | 1.16 (0.75–1.79) | 0.513 | |
| | Participants with TBIL >17 μmol/L | | | | | |
| | No hypertension | 468 | | Ref. | | |
| | Hypertension | 374 | | 1.08 (0.58–2.01) | 0.808 | |
| BG-EPVS (moderate-to-severe)[¶] | Participants with TBIL ≤17 μmol/L | | | | | |
| | No hypertension | 1,276 | 72 (5.6) | Ref. | | 0.505 |
| | Hypertension | 943 | 127 (13.5) | 1.42 (0.91–2.21) | 0.123 | |
| | Participants with TBIL >17 μmol/L | | | | | |
| | No hypertension | 468 | 39 (8.3) | Ref. | | |
| | Hypertension | 374 | 62 (16.6) | 1.21 (0.63–2.30) | 0.572 | |
| BG-EPVS (severe)** | Participants with TBIL ≤17 μmol/L | | | | | |
| | No hypertension | 1,276 | 7 (0.6) | Ref. | | 0.811 |
| | Hypertension | 943 | 13 (1.4) | | 0.903 | |
| | Participants with TBIL >17 μmol/L | | | | | |
| | No hypertension | 468 | 7 (1.5) | Ref. | | |
| | Hypertension | 374 | 11 (2.9) | 0.56 (0.10–2.98) | 0.495 | |

All models were adjusted for age, sex, body mass index, current smoking, current drinking, diabetes, dyslipidemia, antihypertensive medication, antiplatelet medication, anticoagulant medication, and alanine aminotransferase and aspartate aminotransferase levels.
CSVD, cerebral small vessel disease; TBIL, total bilirubin; Adj., adjust; OR, odds ratio; cOR, common odds ratio; CI, confidence interval; WMH, white matter hyperintensity; CMBs, cerebral microbleeds; BG-EPVS, basal ganglia enlarged perivascular space; Ref., reference.
*The associations of hypertension with WMH burden, lacune, presence of CMBs, and BG-EPVS were evaluated with OR, while with modified WMH burden and CMB burden were evaluated with cOR. The product term of hypertension state and TBIL group was included in the model to assess the effect modification by TBIL; [†]WMH burden was defined as either (early) confluent deep WMHs (Fazekas score 2 or 3) or irregular periventricular WMHs extending into the deep white matter (Fazekas score 3); [‡]Modified WMH burden was classified into grade 0 (total periventricular+subcortical WMHs score 1–2), grade 1 (total periventricular+subcortical WMHs score 3–4), and grade 2 (total periventricular+subcortical WMHs score 5–6); [§]Presence of CMBs was defined as presence of CMBs; [‖]CMB burden was classified as grade 0 (absent), grade 1 (1–4 microbleeds), and grade 2 (≥5 microbleeds); [¶]BG-EPVS (moderate-to-severe) indicated >10 perivascular space in basal ganglia; **BG-EPVS (severe) indicated >20 perivascular space in basal ganglia.

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**Supplementary Table 3.** Sensitivity analysis on the effect modification by TBIL with additional adjustment of eGFR, hsCRP, and salt intake in models

| Outcome | Group | N | n (%) | Adj. OR/cOR (95% CI)* | *P* | *P* for interaction |
|---|---|---|---|---|---|---|
| Presence of CSVD (Wardlaw)† | Participants with TBIL ≤17 μmol/L | | | | | |
| | No hypertension | 1,276 | 258 (20.2) | Ref. | | 0.034 |
| | Hypertension | 943 | 389 (41.3) | 1.87 (1.42–2.48) | <0.001 | |
| | Participants with TBIL >17 μmol/L | | | | | |
| | No hypertension | 468 | 129 (27.6) | Ref. | | |
| | Hypertension | 374 | 158 (42.3) | 1.05 (0.67–1.63) | 0.837 | |
| Presence of CSVD (Rothwell)† | Participants with TBIL ≤17 μmol/L | | | | | |
| | No hypertension | 1,276 | 391 (30.6) | Ref. | | 0.026 |
| | Hypertension | 943 | 514 (54.5) | 1.86 (1.43–2.40) | <0.001 | |
| | Participants with TBIL >17 μmol/L | | | | | |
| | No hypertension | 468 | 171 (36.5) | Ref. | | |
| | Hypertension | 374 | 195 (52.1) | 1.05 (0.69–1.60) | 0.816 | |
| Total CSVD burden (Wardlaw)‡ | Participants with TBIL ≤17 μmol/L | | | | | |
| | No hypertension | 1,276 | | Ref. | | 0.082 |
| | Hypertension | 943 | | 1.89 (1.44–2.48) | <0.001 | |
| | Participants with TBIL >17 μmol/L | | | | | |
| | No hypertension | 468 | | Ref. | | |
| | Hypertension | 374 | | 1.20 (0.78–1.84) | 0.400 | |
| Modified total CSVD burden (Rothwell)‡ | Participants with TBIL ≤17 μmol/L | | | | | |
| | No hypertension | 1,276 | | Ref. | | 0.048 |
| | Hypertension | 943 | | 1.86 (1.45–2.38) | <0.001 | |
| | Participants with TBIL >17 μmol/L | | | | | |
| | No hypertension | 468 | | Ref. | | |
| | Hypertension | 374 | | 1.16 (0.78–1.73) | 0.466 | |

All models were adjusted for age, sex, body mass index, current smoking, current drinking, diabetes, dyslipidemia, antihypertensive medication, antiplatelet medication, anticoagulant medication, alanine aminotransferase, aspartate aminotransferase, eGFR, hsCRP, and habit of salt intake.
TBIL, total bilirubin; eGFR, estimated glomerular filtration rate; hsCRP, high-sensitivity C-reactive protein; Adj., adjust; OR, odds ratio; cOR, common odds ratio; CI, confidence interval; CSVD, cerebral small vessel disease; Ref., reference.
*The association of hypertension with the presence of CSVD was evaluated using OR, while with CSVD burden was evaluated using cOR. The product term of the hypertension state and TBIL group was included in the model to assess the effect modification by TBIL; †Presence of CSVD was defined as total CSVD or modified total CSVD burden scores ≥1; ‡Total and modified total CSVD burdens were classified as grade 0 (total or modified total CSVD burden score 0), grade 1 (total or modified total CSVD burden score 1), grade 2 (total or modified total CSVD burden score 2–3), or grade 3 (total or modified total CSVD burden score ≥4).

**Supplementary Table 4.** Sensitivity analysis on the effect modification by TBIL in participants with normal TBIL values

| Outcome | Group | N | n (%) | Adj. OR/cOR (95% CI)* | *P* | *P* for interaction |
|---|---|---|---|---|---|---|
| Presence of CSVD (Wardlaw)† | Participants with TBIL ≤17 μmol/L | | | | | |
| | No hypertension | 1,276 | 258 (20.2) | Ref. | | 0.047 |
| | Hypertension | 943 | 389 (41.3) | 1.86 (1.41–2.44) | <0.001 | |
| | Participants with TBIL >17 μmol/L | | | | | |
| | No hypertension | 460 | 125 (27.2) | Ref. | | |
| | Hypertension | 370 | 157 (42.4) | 1.08 (0.69–1.67) | 0.747 | |
| Presence of CSVD (Rothwell)† | Participants with TBIL ≤17 μmol/L | | | | | |
| | No hypertension | 1,276 | 391 (30.6) | Ref. | | 0.043 |
| | Hypertension | 943 | 514 (54.5) | 1.84 (1.43–2.38) | <0.001 | |
| | Participants with TBIL >17 μmol/L | | | | | |
| | No hypertension | 460 | 165 (35.9) | Ref. | | |
| | Hypertension | 370 | 193 (52.2) | 1.11 (0.73–1.68) | 0.617 | |
| Total CSVD burden (Wardlaw)‡ | Participants with TBIL ≤17 μmol/L | | | | | |
| | No hypertension | 1,276 | | Ref. | | 0.106 |
| | Hypertension | 943 | | 1.88 (1.44–2.46) | <0.001 | |
| | Participants with TBIL >17 μmol/L | | | | | |
| | No hypertension | 460 | | Ref. | | |
| | Hypertension | 370 | | 1.21 (0.80–1.85) | 0.369 | |
| Modified total CSVD burden (Rothwell)‡ | Participants with TBIL ≤17 μmol/L | | | | | |
| | No hypertension | 1,276 | | Ref. | | 0.057 |
| | Hypertension | 943 | | 1.85 (1.45–2.36) | <0.001 | |
| | Participants with TBIL >17 μmol/L | | | | | |
| | No hypertension | 460 | | Ref. | | |
| | Hypertension | 370 | | 1.19 (0.80–1.76) | 0.393 | |

Participants with abnormal elevated TBIL were excluded. Abnormal elevated TBIL was defined as the TBIL concentrations of more than 2 times the upper limit of the normal range (41 μmol/L). All models were adjusted for age, sex, body mass index, current smoking, current drinking, diabetes, dyslipidemia, antihypertensive medication, antiplatelet medication, anticoagulant medication, and alanine aminotransferase and aspartate aminotransferase levels.
TBIL, total bilirubin; Adj., adjust; OR, odds ratio; cOR, common odds ratio; CI, confidence interval; CSVD, cerebral small vessel disease; Ref., reference.
*The association of hypertension with the presence of CSVD was evaluated using OR, while with CSVD burden was evaluated using cOR. The product term of the hypertension state and TBIL group was included in the model to assess the effect modification by TBIL; †Presence of CSVD was defined as total CSVD or modified total CSVD burden scores ≥1; ‡Total and modified total CSVD burdens were classified as grade 0 (total or modified total CSVD burden score 0), grade 1 (total or modified total CSVD burden score 1), grade 2 (total or modified total CSVD burden score 2–3), or grade 3 (total or modified total CSVD burden score ≥4).


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**Supplementary Table 5.** Sensitivity analysis on the effect modification by TBIL in participants with normal liver function

| Outcome | Group | N | n (%) | Adj. OR/cOR (95% CI)* | *P* | *P* for interaction |
|---|---|---|---|---|---|---|
| Presence of CSVD (Wardlaw)† | Participants with TBIL ≤17 μmol/L | | | | | |
| | No hypertension | 1,201 | 242 (20.2) | Ref. | | 0.068 |
| | Hypertension | 866 | 353 (40.8) | 1.71 (1.28–2.29) | <0.001 | |
| | Participants with TBIL >17 μmol/L | | | | | |
| | No hypertension | 424 | 115 (27.1) | Ref. | | |
| | Hypertension | 321 | 134 (41.7) | 1.02 (0.64–1.64) | 0.927 | |
| Presence of CSVD (Rothwell)† | Participants with TBIL ≤17 μmol/L | | | | | |
| | No hypertension | 1,201 | 366 (30.5) | Ref. | | 0.022 |
| | Hypertension | 866 | 470 (54.3) | 1.74 (1.33–2.27) | <0.001 | |
| | Participants with TBIL >17 μmol/L | | | | | |
| | No hypertension | 424 | 154 (36.3) | Ref. | | |
| | Hypertension | 321 | 163 (50.8) | 1.05 (0.67–1.63) | 0.846 | |
| Total CSVD burden (Wardlaw)‡ | Participants with TBIL ≤17 μmol/L | | | | | |
| | No hypertension | 1,201 | | Ref. | | 0.186 |
| | Hypertension | 866 | | 1.73 (1.30–2.29) | <0.001 | |
| | Participants with TBIL >17 μmol/L | | | | | |
| | No hypertension | 424 | | Ref. | | |
| | Hypertension | 321 | | 1.21 (0.77–1.90) | 0.421 | |
| Modified total CSVD burden (Rothwell)‡ | Participants with TBIL ≤17 μmol/L | | | | | |
| | No hypertension | 1,201 | | Ref. | | 0.057 |
| | Hypertension | 866 | | 1.75 (1.36–2.26) | <0.001 | |
| | Participants with TBIL >17 μmol/L | | | | | |
| | No hypertension | 424 | | Ref. | | |
| | Hypertension | 321 | | 1.13 (0.74–1.72) | 0.582 | |

Participants with abnormal liver function were excluded. Abnormal liver function was defined as elevated alanine aminotransferase (>47 U/L) or elevated aspartate aminotransferase (>37 U/L) or reduced albumin (<3.7 g/dL). All models were adjusted for age, sex, body mass index, current smoking, current drinking, diabetes, dyslipidemia, antihypertensive medication, antiplatelet medication, and anticoagulant medication.

TBIL, total bilirubin; Adj., adjust; OR, odds ratio; cOR, common odds ratio; CI, confidence interval; CSVD, cerebral small vessel disease; Ref., reference.

*The association of hypertension with the presence of CSVD was evaluated using OR, while with CSVD burden was evaluated using cOR. The product term of the hypertension state and TBIL group was included in the model to assess the effect modification by TBIL; †Presence of CSVD was defined as total CSVD or modified total CSVD burden scores ≥1; ‡Total and modified total CSVD burdens were classified as grade 0 (total or modified total CSVD burden score 0), grade 1 (total or modified total CSVD burden score 1), grade 2 (total or modified total CSVD burden score 2–3), or grade 3 (total or modified total CSVD burden score ≥4).

**Supplementary Table 6.** Sensitivity analysis on the effect modification by TBIL in participants without anemia

| Outcome | Group | N | n (%) | Adj. OR/cOR (95% CI)* | *P* | *P* for interaction |
|---|---|---|---|---|---|---|
| Presence of CSVD (Wardlaw)† | Participants with TBIL ≤17 μmol/L | | | | | |
| | No hypertension | 1,172 | 234 (20.0) | Ref. | | 0.064 |
| | Hypertension | 894 | 360 (40.3) | 1.84 (1.38–2.44) | <0.001 | |
| | Participants with TBIL >17 μmol/L | | | | | |
| | No hypertension | 443 | 121 (27.3) | Ref. | | |
| | Hypertension | 357 | 151 (42.3) | 1.08 (0.69–1.70) | 0.737 | |
| Presence of CSVD (Rothwell)† | Participants with TBIL ≤17 μmol/L | | | | | |
| | No hypertension | 1,172 | 352 (30.0) | Ref. | | 0.083 |
| | Hypertension | 894 | 478 (53.5) | 1.81 (1.39–2.36) | <0.001 | |
| | Participants with TBIL >17 μmol/L | | | | | |
| | No hypertension | 443 | 160 (36.1) | Ref. | | |
| | Hypertension | 357 | 189 (52.9) | 1.09 (0.71–1.67) | 0.701 | |
| Total CSVD burden (Wardlaw)‡ | Participants with TBIL ≤17 μmol/L | | | | | |
| | No hypertension | 1,172 | | Ref. | | 0.136 |
| | Hypertension | 894 | | 1.82 (1.38–2.41) | <0.001 | |
| | Participants with TBIL >17 μmol/L | | | | | |
| | No hypertension | 443 | | Ref. | | |
| | Hypertension | 357 | | 1.20 (0.78–1.86) | 0.403 | |
| Modified total CSVD burden (Rothwell)‡ | Participants with TBIL ≤17 μmol/L | | | | | |
| | No hypertension | 1,172 | | Ref. | | 0.096 |
| | Hypertension | 894 | | 1.81 (1.40–2.32) | <0.001 | |
| | Participants with TBIL >17 μmol/L | | | | | |
| | No hypertension | 443 | | Ref. | | |
| | Hypertension | 357 | | 1.17 (0.78–1.75) | 0.448 | |

Participants with anemia were excluded. Anemia in adults was defined as hemoglobin levels of <13 g/dL in males and <12 g/dL in females. All models were adjusted for age, sex, body mass index, current smoking, current drinking, diabetes, dyslipidemia, antihypertensive medication, antiplatelet medication, anticoagulant medication, and alanine aminotransferase and aspartate aminotransferase levels.

TBIL, total bilirubin; Adj., adjust; OR, odds ratio; cOR, common odds ratio; CI, confidence interval; CSVD, cerebral small vessel disease; Ref., reference.

*The association of hypertension with the presence of CSVD was evaluated using OR, while with CSVD burden was evaluated using cOR. The product term of the hypertension state and TBIL group was included in the model to assess the effect modification by TBIL; †Presence of CSVD was defined as total CSVD or modified total CSVD burden scores ≥1; ‡Total and modified total CSVD burdens were classified as grade 0 (total or modified total CSVD burden score 0), grade 1 (total or modified total CSVD burden score 1), grade 2 (total or modified total CSVD burden score 2–3), or grade 3 (total or modified total CSVD burden score ≥4).


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**Supplementary Table 7.** Sensitivity analysis on the effect modification by TBIL in participants without use of antihypertensive medication

| Outcome | Group | N | n (%) | Adj. OR/cOR (95% CI)* | *P* | *P* for interaction |
|---|---|---|---|---|---|---|
| Presence of CSVD (Wardlaw)† | Participants with TBIL ≤17 μmol/L | | | | | |
| | No hypertension | 1,276 | 258 (20.2) | Ref. | | 0.033 |
| | Hypertension | 352 | 126 (35.8) | 1.81 (1.37–2.39) | <0.001 | |
| | Participants with TBIL >17 μmol/L | | | | | |
| | No hypertension | 468 | 129 (27.6) | Ref. | | |
| | Hypertension | 145 | 50 (34.5) | 1.07 (0.69–1.67) | 0.767 | |
| Presence of CSVD (Rothwell)† | Participants with TBIL ≤17 μmol/L | | | | | |
| | No hypertension | 1,276 | 391 (30.6) | Ref. | | 0.037 |
| | Hypertension | 352 | 171 (48.6) | 1.79 (1.39–2.32) | <0.001 | |
| | Participants with TBIL >17 μmol/L | | | | | |
| | No hypertension | 468 | 171 (36.5) | Ref. | | |
| | Hypertension | 145 | 64 (44.1) | 1.12 (0.74–1.70) | 0.597 | |
| Total CSVD burden (Wardlaw)‡ | Participants with TBIL ≤17 μmol/L | | | | | |
| | No hypertension | 1,276 | | Ref. | | 0.059 |
| | Hypertension | 352 | | 1.83 (1.39–2.41) | <0.001 | |
| | Participants with TBIL >17 μmol/L | | | | | |
| | No hypertension | 468 | | Ref. | | |
| | Hypertension | 145 | | 1.20 (0.79–1.85) | 0.395 | |
| Modified total CSVD burden (Rothwell)‡ | Participants with TBIL ≤17 μmol/L | | | | | |
| | No hypertension | 1,276 | | Ref. | | 0.046 |
| | Hypertension | 352 | | 1.82 (1.42–2.33) | <0.001 | |
| | Participants with TBIL >17 μmol/L | | | | | |
| | No hypertension | 468 | | Ref. | | |
| | Hypertension | 145 | | 1.18 (0.79–1.75) | 0.424 | |

Participants undergoing antihypertensive medication treatment were excluded. All models were adjusted for age, sex, body mass index, current smoking, current drinking, diabetes, dyslipidemia, antiplatelet medication, anticoagulant medication, alanine aminotransferase, and aspartate aminotransferase.
TBIL, total bilirubin; Adj., adjust; OR, odds ratio; cOR, common odds ratio; CI, confidence interval; CSVD, cerebral small vessel disease; Ref., reference.
*The association of hypertension with the presence of CSVD was evaluated using OR, while with CSVD burden was evaluated using cOR. The product term of the hypertension state and TBIL group was included in the model to assess the effect modification by TBIL; †Presence of CSVD was defined as total CSVD or modified total CSVD burden scores ≥1; ‡Total and modified total CSVD burdens were classified as grade 0 (total or modified total CSVD burden score 0), grade 1 (total or modified total CSVD burden score 1), grade 2 (total or modified total CSVD burden score 2–3), or grade 3 (total or modified total CSVD burden score ≥4).

**Supplementary Table 8.** Sensitivity analysis on the effect modification by TBIL in participants without use of anticoagulant medication

| Outcome | Group | N | n (%) | Adj. OR/cOR (95% CI)* | *P* | *P* for interaction |
|---|---|---|---|---|---|---|
| Presence of CSVD (Wardlaw)† | Participants with TBIL ≤17 μmol/L | | | | | |
| | No hypertension | 1,276 | 258 (20.2) | Ref. | | 0.036 |
| | Hypertension | 942 | 388 (41.2) | 1.86 (1.41–2.44) | <0.001 | |
| | Participants with TBIL >17 μmol/L | | | | | |
| | No hypertension | 468 | 129 (27.6) | Ref. | | |
| | Hypertension | 371 | 156 (42.1) | 1.05 (0.68–1.62) | 0.839 | |
| Presence of CSVD (Rothwell)† | Participants with TBIL ≤17 μmol/L | | | | | |
| | No hypertension | 1,276 | 391 (30.6) | Ref. | | 0.031 |
| | Hypertension | 942 | 513 (54.5) | 1.84 (1.43–2.38) | <0.001 | |
| | Participants with TBIL >17 μmol/L | | | | | |
| | No hypertension | 468 | 171 (36.5) | Ref. | | |
| | Hypertension | 371 | 193 (52.0) | 1.07 (0.71–1.62) | 0.742 | |
| Total CSVD burden (Wardlaw)‡ | Participants with TBIL ≤17 μmol/L | | | | | |
| | No hypertension | 1,276 | | Ref. | | 0.084 |
| | Hypertension | 942 | | 1.88 (1.44–2.46) | <0.001 | |
| | Participants with TBIL >17 μmol/L | | | | | |
| | No hypertension | 468 | | Ref. | | |
| | Hypertension | 371 | | 1.18 (0.77–1.79) | 0.455 | |
| Modified total CSVD burden (Rothwell)‡ | Participants with TBIL ≤17 μmol/L | | | | | |
| | No hypertension | 1,276 | | Ref. | | 0.042 |
| | Hypertension | 942 | | 1.85 (1.45–2.36) | <0.001 | |
| | Participants with TBIL >17 μmol/L | | | | | |
| | No hypertension | 468 | | Ref. | | |
| | Hypertension | 371 | | 1.13 (0.76–1.68) | 0.542 | |

All models were adjusted for age, sex, body mass index, current smoking, current drinking, diabetes, dyslipidemia, antihypertensive medication, antiplatelet medication, alanine aminotransferase, and aspartate aminotransferase.
TBIL, total bilirubin; Adj., adjust; OR, odds ratio; cOR, common odds ratio; CI, confidence interval; CSVD, cerebral small vessel disease; Ref., reference.
*The association of hypertension with the presence of CSVD was evaluated using OR, while with CSVD burden was evaluated using cOR. The product term of the hypertension state and TBIL group was included in the model to assess the effect modification by TBIL; †Presence of CSVD was defined as total CSVD or modified total CSVD burden scores ≥1; ‡Total and modified total CSVD burdens were classified as grade 0 (total or modified total CSVD burden score 0), grade 1 (total or modified total CSVD burden score 1), grade 2 (total or modified total CSVD burden score 2–3), or grade 3 (total or modified total CSVD burden score ≥4).


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**Supplementary Table 9.** Effect modification by different types of bilirubin and corresponding cut-off concentrations for presence of effect modification

| Modifying factor | Outcome | Factor | n | *P* for interaction | Cut-off concentrations (μmol/L) |
|---|---|---|---|---|---|
| TBIL | Presence of CSVD (Wardlaw)* | Hypertension | 3,061 | 0.041 | 22.4 |
| | Presence of CSVD (Rothwell)* | Hypertension | 3,061 | 0.022 | 22.3 |
| | Total CSVD burden (Wardlaw)† | Hypertension | 3,061 | 0.278 | |
| | Modified total CSVD burden (Rothwell)† | Hypertension | 3,061 | 0.056 | |
| IBIL | Presence of CSVD (Wardlaw)* | Hypertension | 3,061 | 0.014 | 13.8 |
| | Presence of CSVD (Rothwell)* | Hypertension | 3,061 | 0.004 | 13.8 |
| | Total CSVD burden (Wardlaw)† | Hypertension | 3,061 | 0.182 | |
| | Modified total CSVD burden (Rothwell)† | Hypertension | 3,061 | 0.028 | 15.2 |
| DBIL | Presence of CSVD (Wardlaw)* | Hypertension | 3,061 | 0.081 | |
| | Presence of CSVD (Rothwell)* | Hypertension | 3,061 | 0.101 | |
| | Total CSVD burden (Wardlaw)† | Hypertension | 3,061 | 0.503 | |
| | Modified total CSVD burden (Rothwell)† | Hypertension | 3,061 | 0.223 | |

Results of presence of CSVD were estimated by binary logistic regression model. Results of CSVD burden were estimated by ordinary least squares regression model. The product term of hypertension state and bilirubin concentrations was included in the model to assess the effect modification by bilirubin. Cut-off concentrations were available when there was statistically significant effect modification (*P* for interaction <0.05). All models were adjusted for age, sex, body mass index, current smoking, current drinking, diabetes, dyslipidemia, antihypertensive medication, antiplatelet medication, anticoagulant medication, and alanine aminotransferase and aspartate aminotransferase levels.

TBIL, total bilirubin; IBIL, indirect bilirubin; DBIL, direct bilirubin; CSVD, cerebral small vessel disease.

*Presence of CSVD was defined as total CSVD or modified total CSVD burden scores ≥1; †Total and modified total CSVD burdens were classified as grade 0 (total or modified total CSVD burden score 0), grade 1 (total or modified total CSVD burden score 1), grade 2 (total or modified total CSVD burden score 2–3), or grade 3 (total or modified total CSVD burden score ≥4).

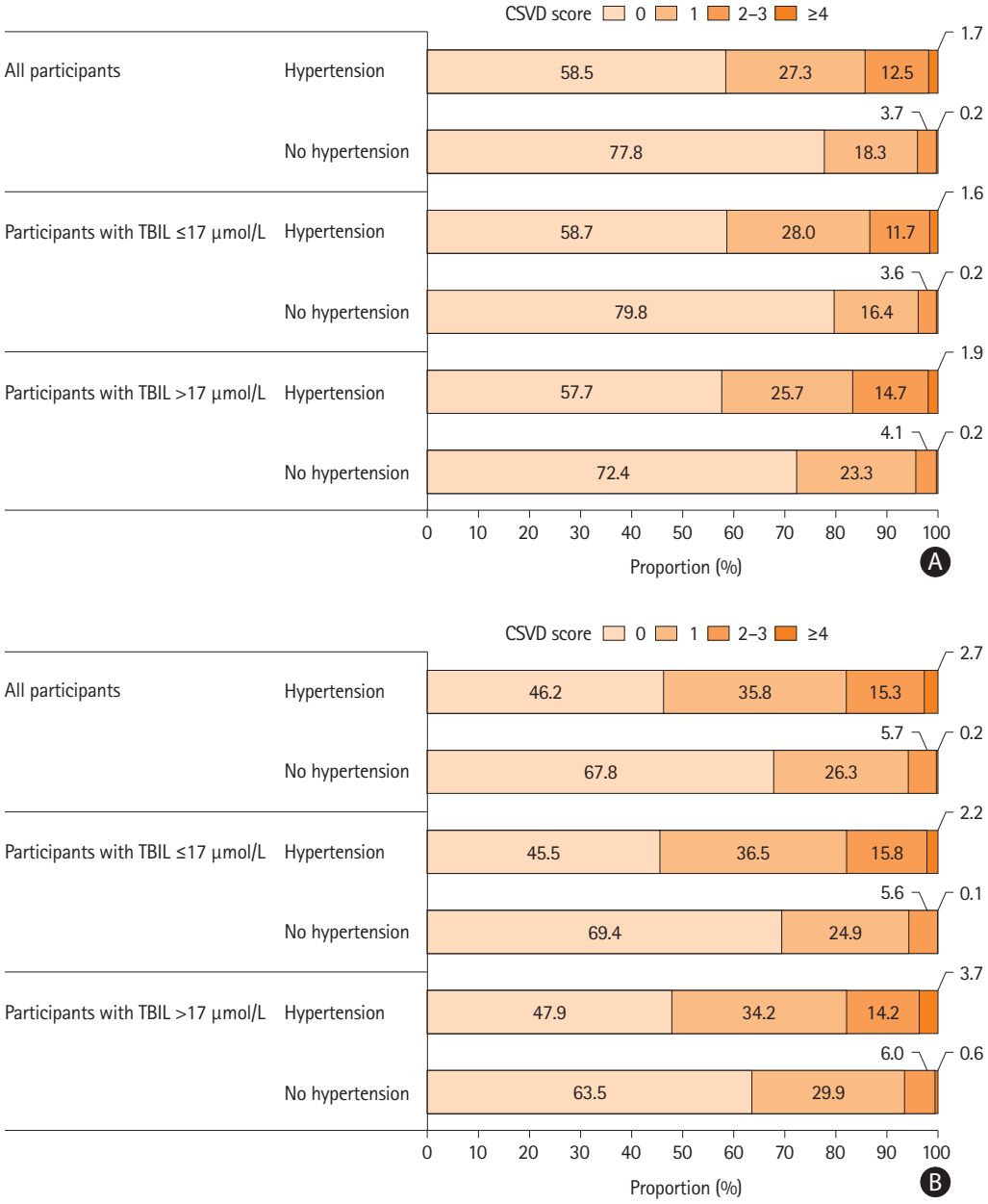
**Supplementary Table 10.** Effect modification by TBIL in participants stratified by identified cut-off concentrations from the Johnson–Neyman analyses

| Outcome | Group | N | n (%) | Adj. OR (95% CI) | *P* | *P* for interaction |
|---|---|---|---|---|---|---|
| Presence of CSVD (Wardlaw)* | Participants with TBIL ≤22.4 μmol/L | | | | | |
| | No hypertension | 1,542 | 324 (21.0) | Ref. | | 0.077 |
| | Hypertension | 1,188 | 492 (41.4) | 1.65 (1.29–2.11) | <0.001 | |
| | Participants with TBIL >22.4 μmol/L | | | | | |
| | No hypertension | 202 | 63 (31.2) | Ref. | | |
| | Hypertension | 129 | 55 (42.6) | 1.21 (0.60–2.46) | 0.596 | |
| Presence of CSVD (Rothwell)* | Participants with TBIL ≤22.3 μmol/L | | | | | |
| | No hypertension | 1,538 | 480 (31.2) | Ref. | | 0.053 |
| | Hypertension | 1,188 | 643 (54.1) | 1.72 (1.36–2.16) | <0.001 | |
| | Participants with TBIL >22.3 μmol/L | | | | | |
| | No hypertension | 206 | 82 (39.8) | Ref. | | |
| | Hypertension | 129 | 66 (51.2) | 0.86 (0.43–1.70) | 0.659 | |

All models were adjusted for age, sex, body mass index, current smoking, current drinking, diabetes, dyslipidemia, antihypertensive medication, antiplatelet medication, anticoagulant medication, and alanine aminotransferase and aspartate aminotransferase levels. The product term of hypertension state and TBIL group was included in the model to assess the effect modification by TBIL.

TBIL, total bilirubin; Adj., adjust; OR, odds ratio; CI, confidence interval; CSVD, cerebral small vessel disease; Ref., reference.

*Presence of CSVD was defined as total CSVD or modified total CSVD burden scores ≥1.




**Supplementary Figure 1.** Distribution of CSVD burden defined by the criterion of Wardlaw's group (A) and Rothwell's group (B). (A) CSVD burden (Wardlaw) was rated on an ordinal scale from 0 to 4, with one point allocated for WMH burden (periventricular WMH Fazekas 3 or deep WMH Fazekas 2–3), presence of lacune, presence of CMBs, and moderate-to-severe BG-EPVS (n>10). (B) CSVD burden (Rothwell) was rated on an ordinal scale from 0 to 6, with one point allocated for the presence of lacune, CMB burden (n=1–4), severe BG-EPVS (n>20), modified WMH burden (total periventricular+subcortical WMH grade 3–4), two points for CMB burden (n≥5) and modified WMH burden (total periventricular+subcortical WMH grade 5–6). CSVD, cerebral small vessel disease; TBIL, total bilirubin; WMH, white matter hyperintensity; CMBs, cerebral microbleeds; BG-EPVS, basal ganglia enlarged perivascular space.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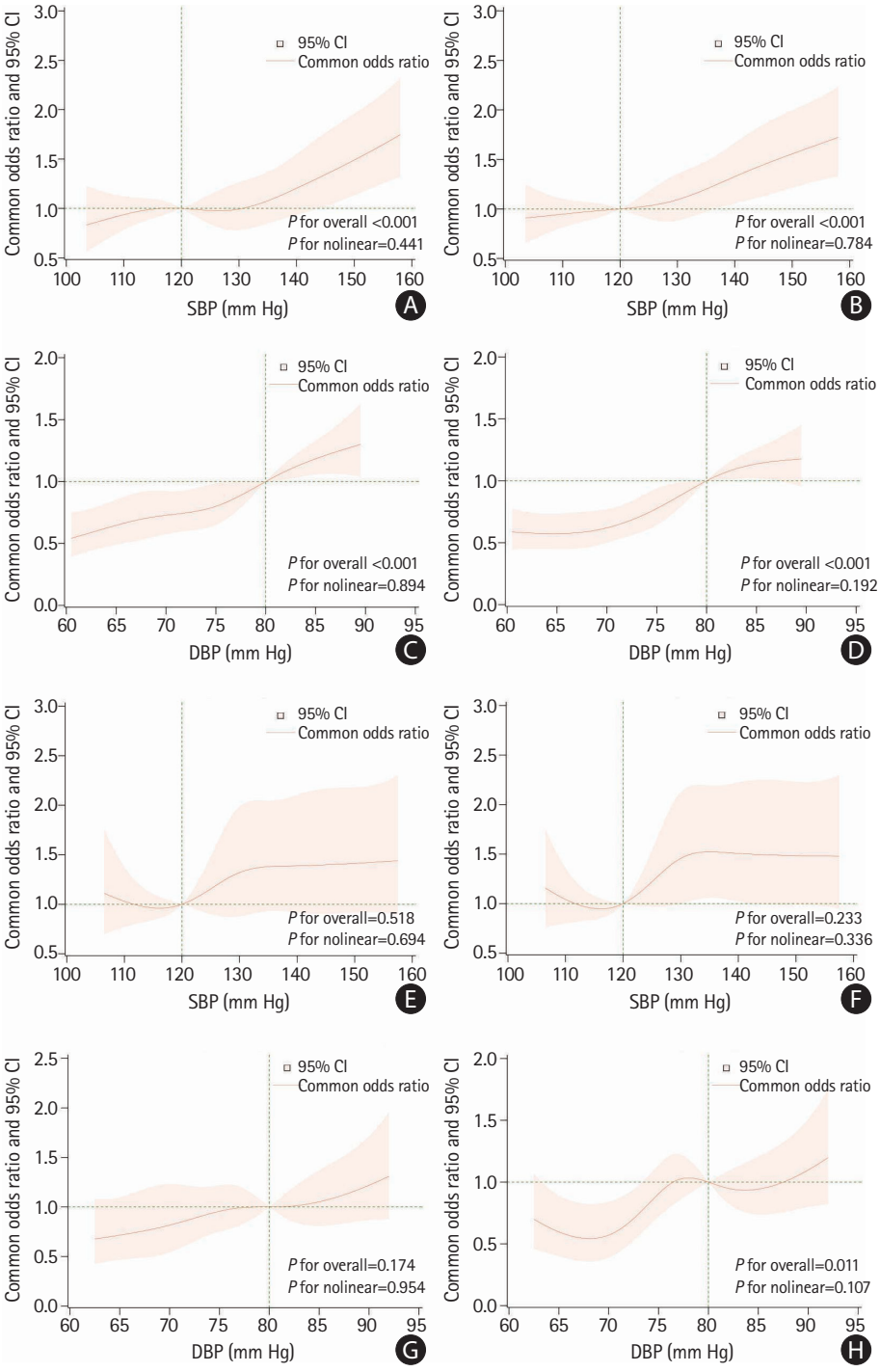


**Supplementary Figure 2.** Dose-response relationship between blood pressure and the CSVD burden in participants with low and high TBIL concentrations. (A) Dose-response relationship between SBP and total CSVD burden (Wardlaw) in low TBIL concentrations. (B) Dose-response relationship between SBP and modified total CSVD burden (Rothwell) in low TBIL concentrations. (C) Dose-response relationship between DBP and total CSVD burden (Wardlaw) in low TBIL concentrations. (D) Dose-response relationship between DBP and modified total CSVD burden (Rothwell) in low TBIL concentrations. (E) Dose-response relationship between SBP and total CSVD burden (Wardlaw) in high TBIL concentrations. (F) Dose-response relationship between SBP and modified total CSVD burden (Rothwell) in high TBIL concentrations. (G) Dose-response relationship between DBP and total CSVD burden (Wardlaw) in high TBIL concentrations. (H) Dose-response relationship between DBP and modified total CSVD burden (Rothwell) in high TBIL concentrations. All models were adjusted for age, sex, body mass index, current smoking, current drinking, diabetes, dyslipidemia, antihypertensive medication, antiplatelet medication, anticoagulant medication, and alanine aminotransferase and aspartate aminotransferase levels. The reference was set at an SBP of 120 mm Hg or a DBP of 80 mm Hg. Knots were set at the 5th, 25th, 50th, 75th, and 95th percentiles of blood pressure. The figures were drawn within the 5th to 95th percentiles of blood pressure to minimize the influence of extreme values on the size of the figures. Total and modified total CSVD burdens were classified as grade 0 (total or modified total CSVD burden score 0), grade 1 (total or modified total CSVD burden score 1), grade 2 (total or modified total CSVD burden score 2–3), or grade 3 (total or modified total CSVD burden score ≥4). CI, confidence interval; SBP, systolic blood pressure; DBP, diastolic blood pressure; CSVD, cerebral small vessel disease; TBIL, total bilirubin.