

# BMJ Open Association between bilirubin levels and risk of stroke: a systematic review and meta-analysis

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## ABSTRACT

**Objective** To evaluate the association between bilirubin levels and stroke risk.

**Design** Systematic review and meta-analysis, reported in accordance with Meta-analysis Of Observational Studies in Epidemiology guidelines.

**Data sources** The PubMed, Embase, Cochrane Central Register of Controlled Trials and China National Knowledge Infrastructure Databases were searched from inception up to 27 February 2022.

**Eligibility criteria** Cohort studies assessing the dose–response relationship between bilirubin levels and risk of stroke were eligible for inclusion. There were no language restrictions.

**Data extraction and synthesis** All data from eligible studies were collected and assessed by two independent investigators. We generated pooled relative risks (RRs) with 95% CIs. We used a restricted cubic spline model for the dose–response analyses. Subsequent subgroup analyses were conducted according to stroke outcomes, follow-up duration, geographical area and size of the cohort.

**Results** Nine articles including results from 11 cohort studies with 7835 cases of stroke and 263 596 participants met the inclusion criteria. The summarised RR of stroke comparing the highest and lowest bilirubin level was 0.85 (95% CI 0.72 to 0.99). The dose–response analysis indicated that a 15 µmol/L increment of bilirubin level was associated with an 18% lower risk of stroke (RR=0.82, 95% CI 0.67 to 0.99). For ischaemic stroke, the RR was 0.76 (95% CI 0.58 to 0.99). Significant publication bias was not detected.

**Conclusions** Elevated bilirubin levels were associated with a decreased risk of stroke among adults.

**PROSPERO registration number** CRD42017071497.

## INTRODUCTION

Over the past four decades, epidemiological studies have identified numerous risk factors for cardiovascular events, such as stroke.<sup>1 2</sup> However, a better understanding of the multifactorial pathogenesis of atherosclerosis, the underlying entity behind stroke events and the fact that many of these events occur in persons who do not harbour conventional vascular risk factors (eg, smoking, obesity, high blood cholesterol level, high blood pressure, diabetes mellitus and metabolic syndrome) have prompted a search for novel risk factors

## STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ This meta-analysis was based on a systematic search of the literature.
- ⇒ We used the dose–response approach to evaluate the relation between bilirubin levels and risk of stroke.
- ⇒ Only published cohort data were included, which could have increased the risk of publication bias through the exclusion of unpublished studies.
- ⇒ The number of studies included in the subgroup analyses by stroke type was small.

for the prediction of cardiovascular disease (CVD).<sup>3</sup> Nonetheless, the clinical value of most emerging risk factors remains uncertain, largely due to inconsistency of data, lack of prospective studies or insufficient evidence on their predictive ability, which is independent of conventional risk factors.<sup>3</sup>

Bilirubin is a final cytotoxic, lipid-soluble waste product of heme catabolism.<sup>4</sup> It consists of two forms: indirect bilirubin and direct bilirubin (DBIL), the former of which is converted to the latter by the hepatic enzyme uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1) and accounts for up to 70%–80% of the serum circulating total bilirubin.<sup>5–7</sup> Conversely, bilirubin is a potent endogenous antioxidant.<sup>4</sup> Clinical epidemiological studies have examined a decreased stroke risk with elevated bilirubin levels.<sup>8 9</sup>

Many epidemiological studies have investigated the relationship between bilirubin levels and stroke, but they have failed to reach a unanimous conclusion.<sup>10–14</sup> In 2015, Kunutsor *et al* found that bilirubin levels were associated with a decreased risk of stroke (relative risk (RR)=0.93; 95% CI 0.88 to 0.98).<sup>12</sup> These findings were confirmed in three meta-analyses.<sup>15–17</sup>

However, the number of studies in previous meta-analyses was limited. Since 2015, five new studies examining the relationship between bilirubin levels and stroke have been reported.<sup>13 14 18–20</sup> We therefore aimed to



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conduct an up-to-date meta-analysis assessing the consistency and strength of this association in cohort studies.

## METHODS

### Study design

The Meta-analysis Of Observational Studies in Epidemiology reporting guidelines were applied to guide and report this meta-analysis.<sup>21</sup>

The protocol was registered at PROSPERO (CRD42017071497).

### Search strategy

Electronic searches were performed in PubMed, EMBASE, the Cochrane Central Register of Controlled Trials and the China National Knowledge Infrastructure to identify eligible studies published from database inception to 27 February 2022. The following search keywords were used: “bilirubin”, “bilirubinaemia”, “hyperbilirubinemia” and “stroke”; “cerebrovascular disorders”, “ischemic stroke”, “intracranial hemorrhage”, “intracranial artery disease”, “cardiovascular disease”, “myocardial ischemia”, “myocardial infarct” and “coronary heart disease”; and “longitudinal studies”, “cohort studies” and “follow-up studies” (online supplemental table 1). We restricted the search to human studies. There were no language restrictions. Citations of relevant publications were also reviewed for study qualification. Moreover, we attempted to contact the original authors to identify additional relevant information through email. In the case of multiple articles using the same or a similar patient cohort, only the most recent or most relevant report was selected for analysis.

### Study selection and data abstraction

Studies were included if they met the following eligibility criteria: (1) collected data obtained within cohort studies, (2) reported multivariate-adjusted RR with 95% CI of first-onset stroke for the highest bilirubin levels versus the lowest bilirubin levels, and (3) follow-up duration of at least 1 year. All data from eligible studies were collected by two independent investigators (SZ and JM). Discrepancies regarding eligibility of publications were resolved by discussion with a third author (ML) and by referring back to the original report.

### Assessment of study quality

The quality of the studies was assessed using the Newcastle–Ottawa Scale (NOS),<sup>22</sup> which contains nine items, categorised into three sections: selection of the study group (four items), quality of the adjustment for confounding factors (two items) and assessment of outcome in the cohorts (three items). A higher score represented better methodological quality. Studies were graded as high quality if they scored  $\geq 7$ . The quality of the articles was independently assessed by two investigators (LQ and ZT), and disagreements were resolved by a third investigator (ML).

### Statistical analysis

RRs were used as the common measure of association between bilirubin levels and stroke, and HRs were considered equivalent to RRs. If the study reported RRs for different sex (men and women) separately, yet without the overall results, then the result for each sex was regarded as a different study. Total bilirubin level was used as the common measure in this meta-analysis. If the study reported only the levels for conjugated or non-conjugated bilirubin, each bilirubin level was regarded as a total bilirubin level. Data analysis used multivariate-adjusted outcome data. These values were converted in every study using their natural logarithms, and the SEs were calculated from the logarithmic numbers and their corresponding 95% CIs. When data on total stroke were unavailable, we used data from ischaemic stroke as an equivalent to total stroke.<sup>23</sup> We combined these estimates using a random-effects model, taking into account both within-study and between-study variabilities.<sup>24</sup> When all included studies use the International System of Unit to report serum bilirubin concentration, we converted mg/dL to  $\mu\text{mol/L}$  by multiplying by 16.81. In the dose–response analysis, the generalised least squares for the trend estimation method described by Greenland and Longnecker and Orsini *et al* was used to calculate study-specific slopes (linear trends) and 95% CI.<sup>25 26</sup> The method requires the distributions of cases and person-years for exposure categories and the median/mean of bilirubin levels for each comparison group. The midpoint of the upper and lower boundaries of each comparison group was assigned to determine the mean bilirubin levels if the median or mean levels were not provided. If the lower or upper boundary for the lowest and highest categories, respectively, was not reported, we assumed that the boundary had the same amplitude as the closest category.<sup>27</sup> Additionally, we first created restricted cubic splines with three knots at fixed percentiles—25%, 50% and 75% of the distribution.<sup>28</sup> A p value for non-linearity was calculated by testing the null hypothesis that the coefficient of the spline transformation is equal to zero. Study heterogeneity was explored using  $\tau^2$ , and the amount of variance in the summary effect due to between-study heterogeneity was defined by  $I^2$ .  $P < 0.10$  was considered statistically significant, as determined by Cochran’s Q statistical test.<sup>29</sup> If there was evidence of heterogeneity, subgroup syntheses and sensitivity analyses were employed to explain what contributed to the heterogeneity. Subsequent subgroup analyses were conducted according to stroke outcomes (ischaemic vs haemorrhagic), follow-up duration ( $\geq 10$  years vs  $< 10$  years), geographical area (Asia vs non-Asia) and size of the cohort ( $\geq 50\,000$  vs  $< 50\,000$ ). To explore possible explanations for heterogeneity and to test the robustness of the association, sensitivity analyses and the aforementioned stratified analysis were conducted. The possibility of publication bias was evaluated using the Begg’s rank correlation test and Egger’s linear regression test at  $p < 0.10$  and visual inspection of the funnel plot.<sup>30–32</sup> In the case of publication bias, the ‘non-parametric trim-and-fill’

method was used to compute risk estimates corrected for this bias.<sup>33</sup> All statistical analyses were conducted using the Review Manager V.5.3 (the Cochrane Collaboration, Oxford, UK) and STATA V.12.0 (StataCorp, College Station, Texas, USA). P values were two sided, and  $p < 0.05$  was considered statistically significant.

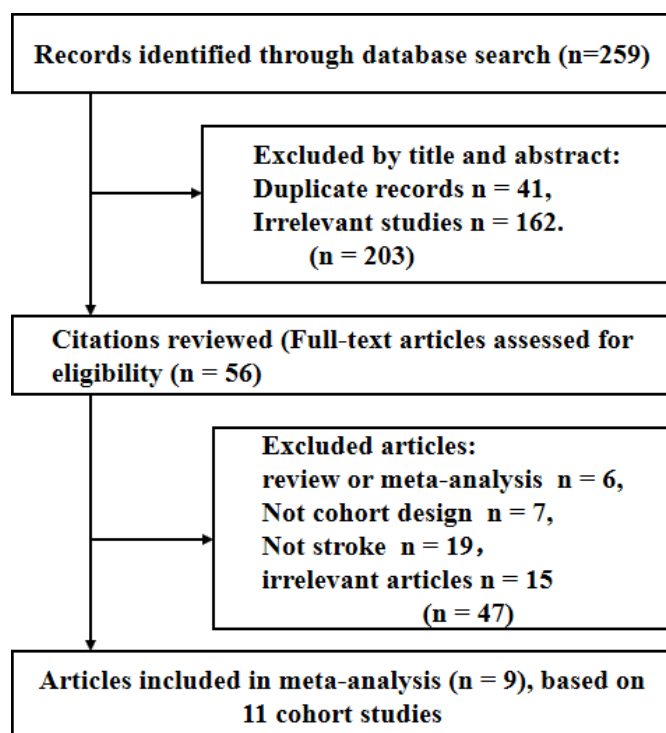
### Patient and public involvement

None.

## RESULTS

### Literature search

Our initial search identified 259 potentially relevant articles. After exclusion of ineligible records, 56 potentially eligible studies were assessed by reviewing the full text. A total of 47 articles were further excluded. The reasons for exclusion of the remaining articles were as follows: the outcome was not stroke or lack of sufficient data for estimation of RRs ( $n=19$ ), review or meta-analysis ( $n=6$ ), lack of cohort design ( $n=7$ ) and irrelevant articles ( $n=15$ ). According to the study selection criteria, the analysis for each sex as an independent comparison was considered, and data were extracted separately. In addition, two articles with the same participants involved the Korean Cancer Prevention Study-II Biobank;<sup>13 20</sup> only the most recent report was selected for analysis. Therefore, the study by Choi *et al*, published in 2020, was included on the basis of the study selection criteria.<sup>20</sup> Finally, a total of 11 independent cohort studies (published in nine articles) were included in the meta-analysis (figure 1).<sup>10–12 14 18–20 34 35</sup>



**Figure 1** Process of literature search and study selection.

### Study characteristics

Eleven independent cohort studies examining the relationship between bilirubin levels and stroke risk (7835 cases among 263 596 individuals) were published between 2009 and 2020 (online supplemental table 2). Among these, two studies were primarily conducted in the USA,<sup>11 14</sup> four in Asian countries (China and Korea)<sup>10 18–20</sup> and three in European countries.<sup>12 34 35</sup> The size of individuals ranged from 316 (in the study conducted by Liu *et al*<sup>19</sup>) to 96 381 (in the study conducted by Marconi *et al*<sup>14</sup>). The median follow-up time varied from 1.89 to 14 years. Of the 11 cohort studies, 8 reported outcomes for ischaemic stroke,<sup>10 14 18–20 34</sup> 7 reported outcomes for total stroke<sup>10–12 18 20 35</sup> and 2 provided data for both stroke subtypes.<sup>10 18</sup> Regarding the assessment of study quality, all articles were considered of high quality according to the NOS (online supplemental table 3).

### Bilirubin levels and stroke risk

#### Categorical meta-analysis

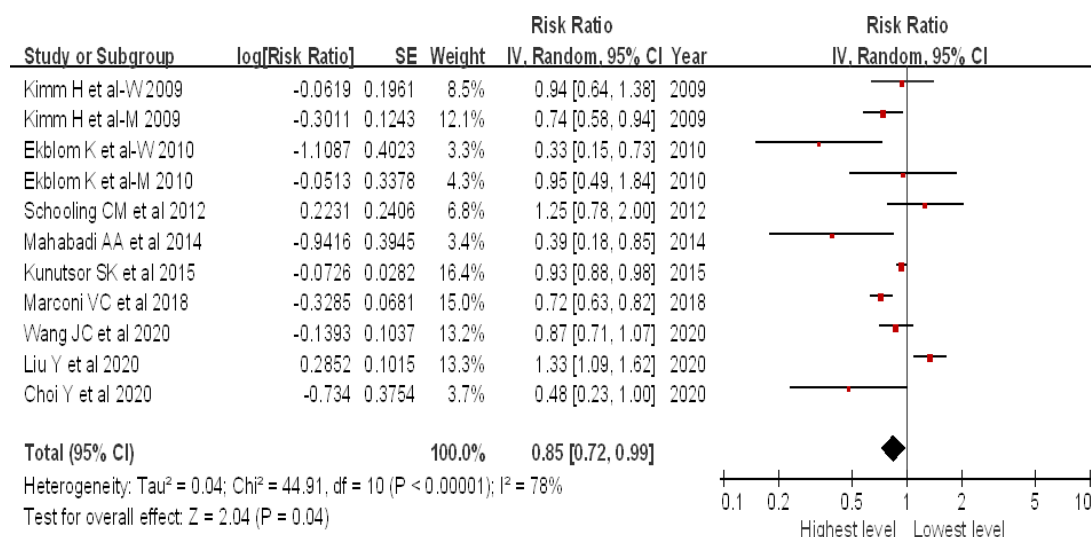
Compared with patients in the lowest bilirubin level, individuals in the highest bilirubin level had a lower stroke risk (RR=0.85; 95% CI 0.72 to 0.99), with substantial heterogeneity among studies ( $I^2=78\%$ ,  $p_{\text{heterogeneity}} < 0.00001$ ) (figure 2). No significant publication bias was observed according to the funnel plot (online supplemental figure 1), Begg's test ( $p=0.119$ ) or Egger's test ( $p=0.346$ ).

#### Dose-response meta-analysis

Three studies could be included in the dose-response meta-analysis.<sup>10 11 14</sup> The remaining studies were excluded, because person-years for exposure categories and the numbers of cases were not available. In the dose-response meta-analysis, bilirubin level was significantly associated with stroke risk, with a significant non-linear trend ( $p_{\text{non-linearity}}=0.04$ ; online supplemental figure 2). The dose-response analysis indicated that a 15  $\mu\text{mol/L}$  increment of bilirubin level was associated with an 18% lower risk of stroke (RR=0.82; 95% CI 0.67 to 0.99).

#### Subgroup and sensitivity analyses

The subgroup analysis of the relationship between bilirubin level and stroke risk is shown in table 1. Authors of non-Asian studies tended to report stronger associations (RR=0.78; 95% CI 0.62 to 1.00),<sup>11 12 14 34 35</sup> as compared with those of the Asian studies (RR=0.89; 95% CI 0.67 to 1.18).<sup>10 18–20</sup> For stroke subtypes, the pooled RR was 0.76 (95% CI 0.58 to 0.99;  $I^2=81$ ;  $p_{\text{heterogeneity}} < 0.00001$ ) for ischaemic stroke,<sup>10 14 18–20 34</sup> which was in contrast to studies that reported on haemorrhagic stroke (RR=1.06; 95% CI 0.77 to 1.47;  $I^2=30$ ;  $p_{\text{heterogeneity}}=0.24$ ).<sup>10 18</sup> After stratification based on follow-up duration, bilirubin level was observed to significantly reduce the risk of stroke (RR=0.82; 95% CI 0.67 to 1.00) in the  $<10$  years group.<sup>12 14 18–20 34 35</sup> The association was attenuated when the analysis was restricted to the size of the cohort (RR=0.74; 95% CI 0.66 to 0.83;  $I^2=0$ ;  $p_{\text{heterogeneity}}=0.44$ ) in the  $\geq 50\,000$  group.<sup>10 14</sup>



**Figure 2** Random-effects analysis of fully adjusted studies for the association between bilirubin level and stroke risk.

Sensitivity analysis was conducted to evaluate the robustness of our results. When studies included in the meta-analysis were removed one at a time, the results of the meta-analysis remained largely unchanged, indicating that the results of the present meta-analysis were stable (data not shown). The pooled RRs for stroke ranged from 0.80 (95% CI 0.69 to 0.93; excluding the study by Liu *et al*<sup>19</sup>) to 0.87 (95% CI 0.73 to 1.04; excluding the study by Marconi *et al*<sup>14</sup>).

## DISCUSSION

In this meta-analysis of 11 independent cohort studies involving 7835 cases of stroke and 263 596 participants, we found that bilirubin was associated with a lower risk of stroke. Comparing patients in the highest category of bilirubin level with those in the lowest category, the risk of

stroke was decreased by 15%. In the dose–response meta-analysis, bilirubin level was associated with stroke risk in a non-linear fashion, with a significantly decreased risk at approximately 15  $\mu\text{mol/L}$ . These results were particularly pronounced in non-Asian studies, especially for ischaemic stroke. Furthermore, all studies included in our meta-analysis reported a multivariate-adjusted RR, which probably mitigated the possibility of known confounding factors influencing our results.

## Findings in the context of existing studies

Over the past decades, although previous epidemiological studies have investigated the role of bilirubin in CVDs, the association between bilirubin and the risk of stroke is inconsistent.<sup>10–14</sup> Three studies suggested that bilirubin is associated with a decreased risk of stroke,<sup>12–14</sup> whereas two studies failed to find such an association.<sup>10 11</sup>

**Table 1** Subgroup analyses of bilirubin level and risk of stroke

		Stroke risk			
Subgroup	No of cohorts	RR (95% CI)	I <sup>2</sup> (%)	P <sub>het</sub>	P <sub>eff</sub>
Stroke subtypes					
Ischaemic	8	0.76 (0.58 to 0.99)	81	<0.00001	0.04
Haemorrhagic	3	1.06 (0.77 to 1.47)	30	0.24	0.38
Follow-up duration					
≥10 years	3	0.90 (0.67 to 1.21)	51	0.13	0.50
<10 years	8	0.82 (0.67 to 1.00)	83	<0.00001	0.05
Geographical area					
Asia	5	0.89 (0.67 to 1.18)	79	0.0008	0.42
Non-Asia	6	0.78 (0.62 to 1.00)	80	0.0002	0.05
Size of cohort					
≥50 000	3	0.74 (0.66 to 0.83)	0	0.44	<0.00001
<50 000	8	0.88 (0.70 to 1.09)	76	0.0002	0.23

P<sub>eff</sub>, p value of pooled effect; P<sub>het</sub>, p value of heterogeneity test; RR, relative risk.



An earlier meta-analysis of circulating total bilirubin and the risk of CVD revealed that bilirubin level was inversely associated with cardiovascular risk.<sup>12</sup> In the present meta-analysis, we found that bilirubin was associated with a lower risk of stroke. This result is consistent with the cross-sectional community-based study conducted by Liu *et al*,<sup>36</sup> whereas no association was noted in the Sibutramine Cardiovascular Outcomes trial and nested case-referent setting.<sup>34</sup> Generally, multiple studies have confirmed the strong negative association of bilirubin levels with atherosclerosis, even among those with familial coronary artery disease, peripheral artery disease, carotid plaque and myocardial infarction in the Framingham Offspring Study.<sup>37–39</sup>

Numerous studies have shown an inverse association between bilirubin levels and several oxidative stress-mediated diseases such as diabetes mellitus, metabolic syndrome and CVD.<sup>8,9,40</sup> Individuals with Gilbert syndrome have elevated levels of unconjugated bilirubin because of a defect in UGT1A1 enzyme. Plausible biological mechanisms by which a higher serum bilirubin level contributes to reduced CVD risk include its antioxidant actions, anti-inflammatory effects and antiatherogenic properties.<sup>39,41</sup> As an antioxidant, bilirubin demonstrated an antiatherogenic function through inhibition of low-density lipoprotein oxidation or through inhibition of vascular endothelial activation, which may mediate the antiatherogenic properties of heme oxygenase-1.<sup>42,43</sup> There is even evidence that bilirubin can inhibit neointima formation after arterial injury, block proliferation and migration of human arterial smooth muscle cells, and promote angiogenesis. The antiatherogenic potential of bilirubin was also shown in human studies. Carotid intima-media thickness, a predictor for atherosclerosis, increased in healthy subjects with low bilirubin levels, not only in men but also in women.<sup>44,45</sup> Overall, evidence from these epidemiological and experimental studies lends more credence to our findings about an inverse association between bilirubin level and stroke risk.

### Strengths and limitations

A strength of this meta-analysis was the included cohort studies, which should have greatly reduced the potential of selection bias. The large study population (7835 cases of stroke and 263 596 participants) enabled us to conduct a wide range of informative subgroup analyses to confirm the inverse association between bilirubin and stroke risk.<sup>10</sup> In addition, the combined use of categorical and dose-response meta-analyses further provided a comprehensive description of the shape of the association. Furthermore, all articles were published after 2009, which provided us with important timely data linking bilirubin level with stroke risk.

However, a few limitations of our meta-analysis should be considered. First, we could not exclude potential biases due to the different methods used to assess bilirubin and the different ranges between the lowest and highest categories. Second, of the 11 cohort studies included in the

meta-analysis, none accounted for change in serum bilirubin level over time, and the bilirubin level was assessed at baseline only, which would have increased the chance of random measurement error. However, we believe the possibility of confounders from biliary disease might be minimal because subjects with abnormal liver enzymes and histories of hepatobiliary diseases were excluded. Third, only published cohort data were included in our meta-analysis, which could have increased the risk of publication bias through the exclusion of unpublished studies. However, after carefully examining all relevant articles, including several meta-analyses and systematic reviews, we were not aware of any relevant unpublished studies on this topic. Fourth, the number of studies included in the subgroup of stroke type was small, especially cardioembolic stroke. Finally, although the included studies attempted to control for various known risk factors such as age, smoking and education, it is still possible that residual confounding factors could have contributed to the association reported herein due to unknown confounders or imprecise adjustment.

### Clinical implications

Adjustment for stroke subtypes did affect the inverse association between bilirubin level and stroke risk, which indicated that the detrimental association with increased bilirubin level was partially explained by the stroke classification. Our analysis confirmed the previous findings that elevated bilirubin levels were associated with a reduction in the risk of stroke. Moreover, there was statistically significant evidence that the serum total bilirubin level was independently associated with cardioembolic stroke, which can be used as a measure to diagnose the stroke subtype.<sup>46</sup> Moreover, another study proved that serum bilirubin levels were positively associated with the severity of acute ischaemic stroke with higher adjusted OR of severity in the top quartile of total bilirubin and DBIL.<sup>47</sup> Conversely, higher bilirubin levels can reduce the formation of carotid atherosclerotic plaque and the occurrence of acute ischaemic stroke, further underscoring the likelihood that atherosclerosis might be the pathological link between bilirubin and stroke.

Furthermore, in this meta-analysis, heterogeneity between studies was found, which was not considerably altered in the sensitivity analysis. Heterogeneity might have come from several sources, among which follow-up duration and study location were the greatest. Each study had a different sample size in the present meta-analysis, causing divergent power; thus, heterogeneity could vary immensely. Relatively small sample sizes, incomplete matching and insufficient representative samples generated from a single centre constitute the limitations that might have caused heterogeneity. Additionally, the heterogeneity might have been partly due to the inconsistency between categories in the original articles, particularly differences between cut-off points for the highest or lowest category. Certainly, the observed heterogeneity could be attributable to differences in the cohort size

and the geographical area among the individuals. As mentioned earlier, the presence of heterogeneity calls for caution in interpreting the findings on this meta-analysis.

## CONCLUSIONS

Our meta-analysis, based on 11 cohort studies, indicated that elevated bilirubin levels were associated with a decreased risk of stroke among adults. Further studies to clarify cut-off levels for this association and intervention trials that investigate the potential of bilirubin as a therapeutic target for stroke are warranted.

**Contributors** ML conceived and designed the study. SZ and JM searched the databases and checked these according to the eligible criteria and exclusion criteria. ML helped develop search strategies. SZ and JM extracted the quantitative data. LQ and ZT analysed the data. GW and LQ wrote the draft of the paper. All authors read and agree with the results and conclusions of this article. ML is responsible for the overall content as the guarantor.

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**Competing interests** None declared.

**Patient and public involvement** Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

**Patient consent for publication** Not required.

**Ethics approval** Ethical approval and informed consent were not necessary because all the data used for the analyses were extracted from available publications.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data availability statement** All data relevant to the study are included in the article or uploaded as supplemental information.

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