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Plasmatic trimethylamine N-oxide and its relation to stroke

A systematic review and dose-response meta-analysis

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Abstract

Background: Elevated circulating concentrations of the gut metabolite, trimethylamine N-oxide (TMAO), were found in patients who experienced stroke. However, it has not been reported whether a high level of TMAO is associated with a significantly increased risk of stroke. This study aimed to review the available scientific evidence about the relationship between TMAO levels and the risk of stroke in a dose-response meta-analysis.

Methods: The PubMed, Embase, Cochrane library, and China National Knowledge Infrastructure databases were searched for studies starting from September 1996 to December 2020. Nine studies including 4402 subjects were reviewed in this study.

Results: The results of meta-analysis showed that high levels of circulating TMAO were associated with an increased risk of stroke in patients in the random-effects model (odds ratio [OR], 1.64; 95% confidence interval [CI], 1.12–2.41; $P = 0.047$). The OR for the prevalence of stroke increased by 48% per 5-μmol/L increment (OR, 1.05; 95% CI, 1.16–1.78; $P < 0.001$) and by 132% per 10-μmol/L increment (OR, 2.32; 95% CI, 1.38–3.86; $P < 0.001$) in circulating TMAO concentration according to the dose-response meta-analysis.

Conclusion: There was a significant association between higher plasma TMAO concentrations and the risk of stroke. Further in-depth studies are warranted to validate this interaction and explore potential mechanisms.

Abbreviations: BMI = body mass index, CI = confidence interval, OR = odds ratio, SMD = standardized mean difference, TMAO = trimethylamine N-oxide.

Keywords: dose-response, meta-analysis, risk, stroke, TMAO

1. Introduction

Stroke is a major cause of long-term disability and mortality worldwide. Recently, a large amount of research data showed that ≈8 million people experienced stroke, and there are still 10,000 to 20,000 newly diagnosed and recurrent strokes every year.[1,2] Despite major breakthroughs in the diagnosis, treatment, and prevention of stroke, it is still a common health problem among all ages due to the elevated rate of premature death and high economic burden.[3] Thus, there is an urgent need for prognostic markers that provide novel insights for understanding the pathophysiological mechanisms and suggest research avenues for developing potential strategies for intervention and decreasing the burden of stroke.[4]

Recently, studies derived from murine models and human trials have demonstrated a contribution of the metabolites of the intestinal flora in the modulation of vascular function in obesity and the metabolic syndrome.[5] Trimethylamine N-oxide (TMAO) is a gut microbiota–derived metabolite, primarily produced from nutrient precursors, such as choline, phosphatidylcholine, and L-carnitine, under the action of oxidase.[6] Numerous studies have demonstrated that the blood TMAO concentrations are positively correlated with the short-term and long-term cardiovascular mortality, especially in patients with hypertension, coronary atherosclerosis, and obesity.[7] A prospective population-based study revealed that serum TMAO concentration improves the prediction of cardiovascular or cerebrovascular disease risk.[8] Furthermore, a cohort study reported that the plasma concentration of TMAO is higher in fasting diabetic patients and portended higher major adverse cardiac events and mortality risks than normal controls, independent of traditional risk factors, such as renal function and glycemic control.[9] Notably, it has been reported that TMAO could directly contribute to platelet hyperreactivity and enhanced thrombosis potential, indicating that the circulating levels of TMAO are associated with an increased risk of an acute ischemic event.[10] Furthermore, one study examined the relationship between TMAO concentration and the risk of

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The data in current study are available from the corresponding author on reasonable request.

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incident cardiovascular events in patients with a recent first episode of ischemic stroke.[11,12] In conclusion, these results suggest potentially significant roles for the gut microbiota TMAO in cardio- and cerebrovascular diseases including stroke and transient ischemic attack.

However, no study has specifically evaluated the relationship between the circulating TMAO concentration and stroke till date. Hence, we conducted a dose-response meta-analysis of prospective studies to quantitatively evaluate the relationship between the circulating TMAO concentrations and the risk of stroke.

2. Methods

2.1. Literature search strategy

This study was designed according to the requirements of Preferred Reporting Items for Systematic Reviews and Meta-Analyses. We searched electronic databases including PubMed, Excerpta Medica data BASE, Cochrane Library, China National Knowledge Infrastructure Database, Chinese Scientific Journals Full Text Database, Wanfang Data Knowledge Service Platform, and the Chinese Biomedical Literature Service System (CBMdisc), for literature starting from September 1996 to December 2020 using the key words "trimethylamine N-oxide," "TMAO," "stroke," as well as "clinical trials." Two authors (P.C. and Z.G.) independently examined the titles and abstracts of citations. Finally, references of included papers, full texts, and bibliographies of all potential articles including relevant reviews and meta-analyses were thoroughly scanned to identify additional eligible studies and ensure that no studies were missed. Our study has got approval from the Ethics Committee of Renmin Hospital of Wuhan University.

2.2. Selection criteria

Studies were included if the reported estimates on the relationship between TMAO levels and stroke were classified or continuous variables. Studies were excluded if any of the following criteria were observed:

duplicate publication data
lack of data on TMAO plasma levels and their correlation with outcomes
review articles, systematic reviews, meta-analyses, commentaries, editorials, or meeting abstracts
nonhuman studies or those not published in English
total sample size was <50.

2.3. Data extraction

Two authors (P.C. and Z.G.) independently screened the titles and abstracts of each study. A third reviewer (D. Li) was invited to examine and resolve the conflicting data. The following information was extracted from each study: the first author, year of publication, total number of patients enrolled in the study, definition of stroke stated in the study, proportion of people experiencing diabetes and hypertension, TMAO levels, method used for assessment of TMAO concentration, and other adjusted estimates for the outcomes of interest. Estimates and their 95% confidence intervals (CIs) for TMAO plasma levels in relation to stroke were extracted as they were presented in the original reports. We also converted the units that were not unified for TMAO levels.

2.4. Qualitative assessment

To evaluate the methodological quality of the included literature, the Newcastle-Ottawa quality assessment scale was used to assess the quality of cohort and case-control studies. Three major aspects were evaluated using this scale, including selection, comparability, and exposure/outcome, with 8 detailed questions (Table S1, Supplemental Digital Content, http://links.lww.com/MD/G892). Studies with a rating of ≥6 stars were deemed to be of high quality. An 11-item checklist recommended by the Agency for Health Care Research and Quality was used to evaluate the methodological quality of cross-sectional studies (Table S2, Supplemental Digital Content, http://links.lww.com/MD/G892).

2.5. Statistical analysis

2.5.1. Main and secondary analyses. In primary studies, the exposure variable (TMAO concentration) was reported in different ways (either as a continuous or as a categorical trait), and the effect measures with their corresponding 95% CIs were reported per different increments in TMAO levels (e.g., per 1 unit, per 10 mol/L, or per 1-SD increment in the continuous trait; or per tertiles, quartiles, or quintiles in TMAO levels; or comparison of individuals having TMAO concentrations higher or lower than the median). In this meta-analysis, to harmonize the presentation of data, we provided effect measures for the top (high TMAO) versus the bottom (low TMAO) tertile of TMAO distribution. Thus, we performed a main analysis to estimate the odds ratio (OR) of stroke associated with high and low concentrations of TMAO. Furthermore, a standardized mean difference (SMD) of TMAO levels in the peripheral blood of stroke and nonstroke populations was estimated after unit conversion to further analyze the relationship between TMAO concentrations and the risk of stroke. Estimates of the effect were calculated with a fixed- or random-effects model and expressed as SMD.

The between-study heterogeneity was assessed using the χ^2 test. If I^2 was <50% ($P > 0.05$), the fixed-effects model was used; if not ($I^2 > 50\%$; $P < 0.05$), the random-effects model was used, and we attempted to discover the cause of the heterogeneity. A subgroup analysis was also performed. A P value <0.05 was used as a cutoff for statistical significance of heterogeneity.

2.5.2. Dose-response meta-analysis. Because most of the primary studies we examined reported different cutoffs of plasma TMAO concentration, we performed a dose-response meta-analysis for primary outcomes using previously reported methods. Studies that reported the OR of stroke and at least 3 TMAO exposure levels were included in the dose-response analysis. We presumed that the groups were equally divided if the number of exposed participants was not reported in each stratification. The missing number of cases in one study was estimated by distributing the total number equally into each quartile. If the median or mean levels of TMAO were not indicated in the study, the approximate medians were estimated using the midpoint of the lower and higher bounds. If the boundaries for the lowest and highest categories were open-ended, then the midpoint of this category was estimated by assuming that the interval was the same as the closest category. We defined the lowest categories of TMAO concentrations as a reference dose for each study. Linear associations were examined using random-effects dose-response meta-analysis. Restricted cubic splines with 3 knots were used to calculate study-specific OR estimates per 1 μmol/L of TMAO increment. All statistical analyses were performed using the STATA software, version 13.0 (StataCorp LP, College Station, TX). A 2-sided P value of <0.05 was considered statistically significant.

3. Results

3.1. Search results

The initial search of literature identified a total of 364 documents through the preliminary examination of the different

databases. Among these, 223 were repetitive and 124 were excluded (confirmed via titles and abstracts) from the analysis because they were review articles, published protocol, lab studies, animal studies, or not of relevance. After analyzing the full-text articles, 8 studies did not meet the inclusion criteria and thus were excluded. Nine studies[13–21] (3 cohort studies and 6 case-control studies) were found eligible according to our criteria for being ultimately included in the meta-analysis. The selection procedure is described in Figure 1.

3.2. Study characteristics and quality assessment

Tables 1 and 2 provide the characteristics of the included studies. These 9 studies[13–21] were published from 2015 to 2020, which reflected the recent interests in the TMAO metabolite. The average circulating TMAO concentrations in the 9 included studies ranged from 1.36 to 6.7 μmol/L. All publications comprehensively reported the baseline information of the enrolled participants. Five studies[13–15,20,21] with a small sample size (<500) were included. All studies assessed the TMAO concentration in fasting blood samples. Overall, 8 studies[13–16,18–21] were conducted in China and only 1[17] in Germany. Study quality was high in most of the studies, with an average Newcastle-Ottawa quality assessment scale score of 7.3 points. One study[21] with a lower quality had a score of 4 stars (Table S1, Supplemental Digital Content, http://links.lww.com/MD/G892). The cross-sectional study scored 7 points using an 11-item checklist recommended by the Agency for Health Care Research and Quality (Table S2, Supplemental Digital Content, http://links.lww.com/MD/G892).

3.3. Circulating TMAO concentrations and the risk of stroke

Five studies,[13–15,18,21] including 2 cohort and 3 case-control studies, with a total of 1117 participants, were considered in the meta-analysis. Overall, the results of the meta-analysis

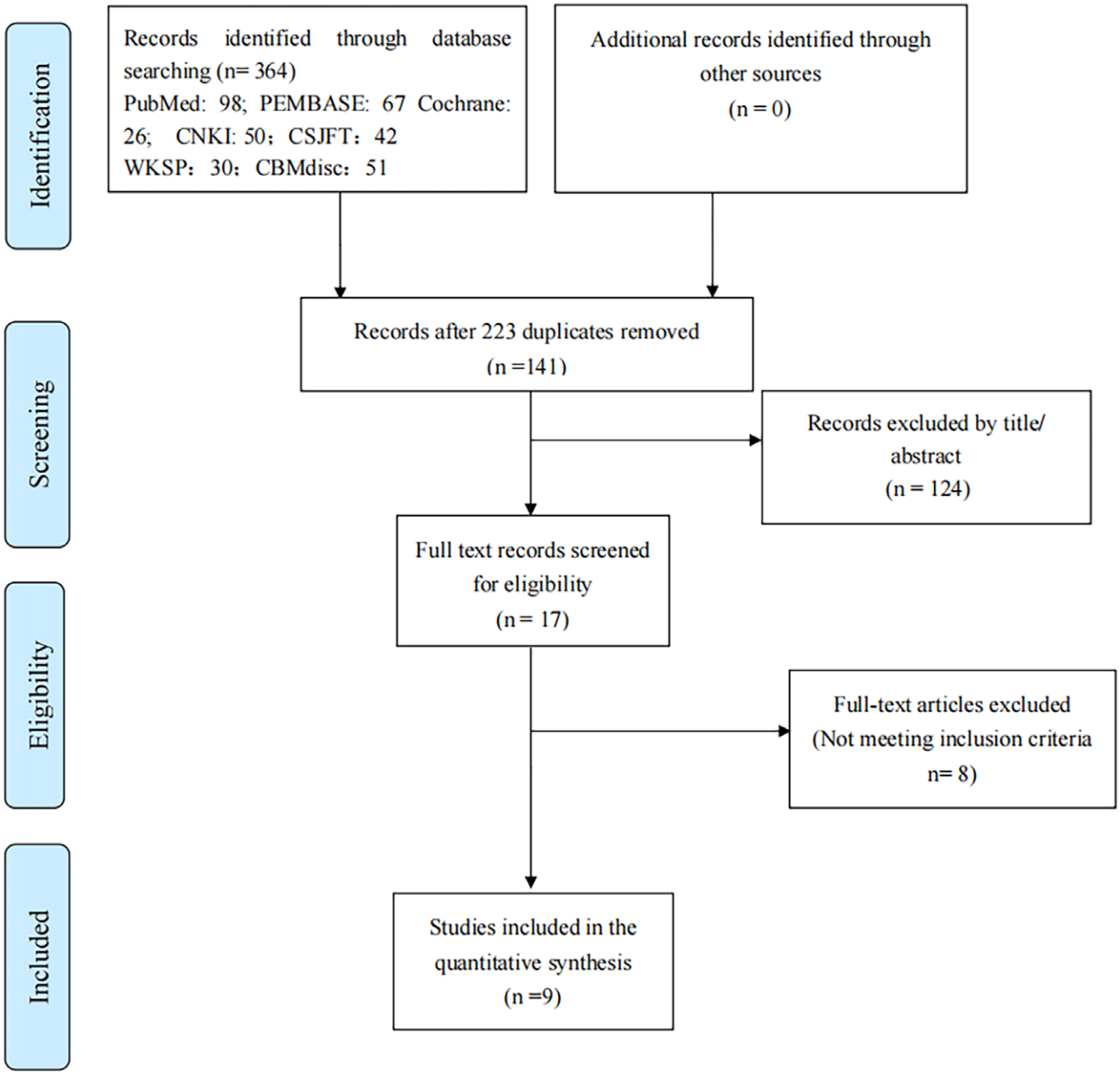

Figure 1. Flowchart of the study selection process. CBMdisc = Chinese Biomedical Literature Service System, CNKI = China National Knowledge Infrastructure, CSJFT = Chinese Scientific Journals Full Text Database, WKSP = Wanfang Data Knowledge Service Platform.

Table 1

Characteristics of included studies.

Reference	Year	Country	Research type	Participants, n	Stroke definition	Blood sample	TMAO measure method	Study period
Hou et al[13]	2020	China	Cohort study	362	NIHSS	Fasting plasma	LC-MS/MS	2018–2019
Wu et al[14]	2020	China	Case-control	337:50	NIHSS	Fasting plasma	LC-MS/MS	2016–2018
Zhu et al[15]	2019	China	Case-control	86:170	NIHSS	Fasting plasma	LC-MS/MS	January 2017 to December 2017
Yin et al[17]	2015	China	Case-control	322:231	NIHSS	Fasting plasma	LC-MS/MS	2014–2015
Haghikia et al[18]	2019	Germany	Cohort study	686	NIHSS	Fasting plasma	LC-MS/MS	NA
Rexidamu et al[19]	2019	China	Case-control	255:255	NIHSS	Fasting plasma	LC-MS/MS	2016–2018
Nie et al[20]	2018	China	Case-control	622:622	NIHSS	Fasting plasma	LC-MS/MS	2008–2013
Liang et al[21]	2018	China	Case-control	111:68	NIHSS	Fasting plasma	LC-MS/MS	2015–2017
Zhai et al[16]	2019	China	Cohort study	225	NIHSS	Fasting plasma	LC-MS/MS	July 2018 to December 2018

LC-MS/MS = liquid chromatograph mass spectrometer/mass spectrometer, NA = not available, NIHSS = National Institute of Health Stroke Scale, TMAO = trimethylamine N-oxide.

showed that the OR of stroke between high and low circulating TMAO concentrations was 1.64 (95% CI, 1.12–2.41) using a random-effects model (I^2 = 58.5%; P = 0.047; Fig. 2). The results indicated that a high circulating TMAO concentration was associated with a higher risk of stroke. Furthermore, different subgroup analyses were performed to evaluate the potential effects of the study population, sample type, and sample size. A high level of TMAO was associated with increased OR of stroke in cohort studies (OR, 1.10; 95% CI, 0.75–1.61; I^2 = 0%; Fig. 2), and a greater association between them was also found in case-control studies (OR, 2.14; 95% CI, 1.29–3.55; I^2 = 56.2%).

Our meta-analysis of continuous variables of the relationship between TMAO concentration and stroke risk included 3212 participants from 6 studies.[14–16,18–20] We calculated the SMD of TMAO levels in stroke and nonstroke populations after unit conversion. TMAO levels in the plasma of stroke patients were much higher than those of people without stroke in the fixed-effects model (SMD, 1.03; 95% CI, 0.95–01.11; I^2 = 0%; Fig. 3A). Subgroup analyses demonstrated that the different male rate also affected the heterogeneity (male% <50%: SMD, 1.05; 95% CI, 0.95–1.15; I^2 = 0%; male% >50%: SMD, 1.01; 95% CI, 0.89–1.12; I^2 = 54.9%; Fig. 3A). Further additional subgroup analyses were performed according to the number of cases, rate of male patients, rate of diabetic patients, rate of hypertensive patients, body mass index (BMI), rate of current smokers in the study, number of patients in each study >300 (SMD, 1.48; 0.99–1.17; I^2 = 40.8%), and number of patients in each study <300 (SMD, 2.34; 0.61–9.00; I^2 = 58.5%; Fig. 3B); diabetes (%) <25% (SMD, 1.08; 95% CI, 0.99–1.17; I^2 = 0) and diabetes (%) >25% (SMD, 0.91; 95% CI, 0.76–1.06; I^2 = 0; Fig. 3C); hypertension (%) <55% (SMD, 1.40; 95% CI, 0.75–2.61; I^2 = 65.4) and hypertension (%) >55% (SMD, 1.91; 95% CI, 1.04–3.52; I^2 = 58.5; Fig. 3D); non-BMI (SMD, 1.67; 95% CI, 0.76–3.68; I^2 = 76.2) and BMI (SMD, 1.71; 95% CI, 1.27–2.32; I^2 = 58.5; Fig. 3E); current smokers (%) >50% (SMD, 2.12; 95% CI, 0.47–9.62; I^2 = 87.9) and current smokers (%) <50% (SMD, 1.62; 95% CI, 1.23–2.13; I^2 = 0; Fig. 3F).

3.4. Dose-response analysis

Six studies with a total of 3212 participants were further used to perform a dose-response analysis and investigate the dose-response relationship between the TMAO concentrations and the OR of stroke. There was no significant evidence of a nonlinear association between plasma TMAO levels and the prevalence of diabetes mellitus (P for nonlinearity, 0.0738, based on 6 studies). Therefore, the linear model was used in the dose-response meta-analysis. Figure 4 shows that the ORs for the prevalence of stroke were 1.05 (95% CI, 1.01–1.09; P < 0.001) per 1-μmol/L increment, 1.48 (95% CI, 1.16–1.78; P < 0.001) per 5-μmol/L increment, and 2.32 (95% CI, 1.38–3.86; P < 0.001, based on 3 studies) per 10-μmol/L increment of TMAO plasma concentration. These results demonstrated that the stroke risk could increase by 48% per 5-μmol/L and 132% per 10-μmol/L increment of circulating TMAO concentration.

3.5. Publication bias

The publication bias was assessed using funnel plot, and the details of the funnel plot are presented in Figure 5. As a result, we can observe that there was a certain asymmetry in the funnel plot, indicating that there is some degree of publication bias in the literature. However, only 10 studies were included, and the funnel plots may not be very reliable. Moreover, the Begg test (P = 0.90) and Egger test (P = 0.54) revealed that there was no significant difference in the meta-analysis of circulating TMAO concentration and OR of stroke.

4. Discussion

4.1. Main findings

The current meta-analysis reported the association between TMAO plasma levels and the risk of stroke in a large population. This meta-analysis included a total of 4402 participants in 9 eligible studies with observational designs. The findings are summarized as follows:

participants with high TMAO levels had ≈64% increased risk of developing stroke as compared to those with low levels;
the circulating levels of TMAO were higher in patients with stroke than in those without stroke;
there was a dose-dependent, direct relation between TMAO levels and the risk of stroke.

Taken together, our data revealed a positive association between circulating TMAO concentration and the increased risk of stroke.

There was a major challenge to reveal the relation between circulating TMAO level and the risk of stroke due to the high heterogeneity of populations involved in the current meta-analysis. We did not perform a meta-regression analysis to detect the potential heterogeneity because only 9 studies were included in this study. A series of sensitivity and subgroup analyses were conducted to explore the potential sources of heterogeneity in the present study. It can be predicted that the heterogeneity of this study is potentially affected by several factors, especially the sample capacity, sex, smoking status, target population, and proportion of patients with diabetes or hypertension in every study. Previous studies have confirmed that both smoking and diabetes may contribute to variations in the composition of the intestinal microbiota.[22] More important, several independent studies have confirmed the well-documented association between TMAO level and diabetes.[23] It should be noted that if the assumption that circulating TMAO concentrations might be affected

Table 2

Characteristics of study population in the included studies.

Reference	Age (yr)	Male, n (%)	BMI (kg/m²)	TMAO (μmol/L)	Smoking, n (%)	Hypertension (%)	Diabetes (%)	Dyslipidemia (%)	hs-CRP (mg/L)	Blood-glucose (mmol/L)	Total cholesterol (mmol/L)
Hou et al[13]	61.7 ± 9.4	63 (53.4)	NA	2.14 (1.19–3.82)	102 (67.1)	62 (39.1)	97 (64.1)	NA	4.2 (2.0–7.4)	6.5 ± 2.2	4.5 ± 1.1
Wu et al[14]	62.5 ± 10.7	215 (43.0)	24.3 ± 2.7	5.1 (3.3–7.6)	80 (21.1)	257 (68.2)	114 (30.2)	61 (16.2)	NA	5.3 (4.6–5.9)	NA
Zhu et al[15]	65.2 ± 11.1	139 (54.3)	NA	3.304 (1.335–8.178)	110 (81.1)	148 (57.8)	71 (27.7)	NA	4.5 (2.6–7.0)	NA	3.9 ± 1.1
Yin et al[17]	61 ± 19	220 (68.3)	23.62 (3.90)	2.70 (3.47)	NA	286 (51.7)	145 (26.2)	NA	NA	NA	5.07 (1.28)
Haghikia et al[18]	59 ± 14	69 (88.4)	NA	2.31 (1.25–4.23)	NA	NA	NA	NA	NA	NA	NA
Rexidamu et al[19]	62 (53–68)	136 (53.3)	25.2 (23.5–27.4)	5.8 (3.3–10.0)	47 (18.3)	186 (72.6)	67 (26.1)	NA	13 ± 29	NA	3.88 (3.23–5.05)
Nie et al[20]	62.2 (7.3)	584 (46.9)	NA	24.8 (3.4)	340 (27.3)	NA	195 (15.6)	NA	0.43 (0.15–0.98)	5.9 (1.7)	5.6 (1.2)
Liang et al[21]	64.1 ± 13.3	104 (58.1)	NA	5.68 (3.184–10.140)	73 (40.1)	83 (46.3)	24 (13.4)	NA	NA	NA	4.49 ± 1.06
Zhai et al[16]	66.5 ± 11.2	124 (55.1)	NA	4.27 (1.07–17.07)	83 (36.8)	155 (68.8)	62 (27.5)	NA	7.5 (2.4–15.5)	6.3 ± 1.5	4.1 ± 1.0

BMI = body mass index, hs-CRP = hypersensitive c-reactive protein, NA = not available, TMAO = trimethylamine N-oxide.

by the smoking and diabetes-induced variation in microbiota is true, heterogeneity should have been explored in our meta-analysis. It is known that obesity and dietary habits like sodium intake and alcohol consumption strongly influence blood pressure, which is the most important independent risk factor for stroke.[24] Previous evidence has shown that moderate reductions in dietary salt and dietary fiber intake have a positive effect on blood pressure control.[25] Moreover, recent reports showed that diet not only modulates the microbiota but may also impact the plasma concentrations of TMAO.[26] Dietary choices and habits may affect blood pressure through alteration of gut bacterial metabolites and products. Thus, it is worth noting that diet-induced disruption and variations in gut microbiota, rather than genes, primarily influenced the TMAO concentration in individuals. Altogether, these findings highlight the potential role of dietary habits in blood TMAO concentration, and the differences in diet culture (e.g., mainland compared with fremdness diets) could cause the variability in TMAO concentrations observed in studies conducted in different locations. In our study, although all of the population is from China, there are still regional differences in diet within the mainland. Subgroup analyses according to sex ratio and the population disease status elicited consistent results although substantial heterogeneity of patient populations existed in the current study. Generally, the results remained consistent across all the subgroups, which further authenticated the significant positive correlation between circulating TMAO concentration and the prevalence of stroke.

A demand for a heavy economic burden arises due to the increasing number of patients with stroke and the continuous growth of the incidence of chronic diseases. Our dose-response meta-analysis suggested that the OR for the prevalence of stroke increased by 48% per 5-μmol/L increment and by 132% per 10-μmol/L increment in circulating TMAO concentration. It should be noted that an early risk assessment with an estimate of disease severity and prognosis is crucial in providing effective interventions and allocating health care resources to improve the outcomes of stroke.[27] The promising results of this study will encourage us to discover the potential confounders and strategies that can be employed to reduce the circulating TMAO concentrations, thereby developing new and effective ways to control stroke in the future. Researchers recently showed that the intake of L-carnitine and phosphatidylcholine, a major component of lecithin, temporarily raises the blood levels of TMAO.[28] It was suggested that excessive routine consumption of dietary phosphatidylcholine and choline should be avoided especially in patients with cardiovascular diseases. Conversely, eating fruits and foods rich in polyphenols, such as pomegranates and raspberries, can effectively reduce the intake of choline.

4.2. Potential mechanisms

Our data suggest that TMAO may be an important risk factor for stroke, but potential biological mechanisms underlying the deleterious effects of TMAO levels on the outcome of stroke are not well understood. However, previous experimental studies may provide some clues:

TMAO in the blood can upregulate the scavenger receptors in the macrophages and promote the accumulation of cholesterol and the formation of foam cells in macrophages, thereby promoting vascular plaque formation. TMAO can also promote the inflammatory response by mitogen-activated protein kinase and nuclear factor kappa B pathway.[29]

Elevated plasma TMAO levels could cause vascular endothelial dysfunction, exacerbate prothrombotic changes in platelet reactivity and/or endothelial activation, induce abnormal lipid metabolism, and increase inflammatory factors.[30] These lines of evidence indicate the potential importance of TMAO in the cardiovascular system. Endothelial dysfunction, in

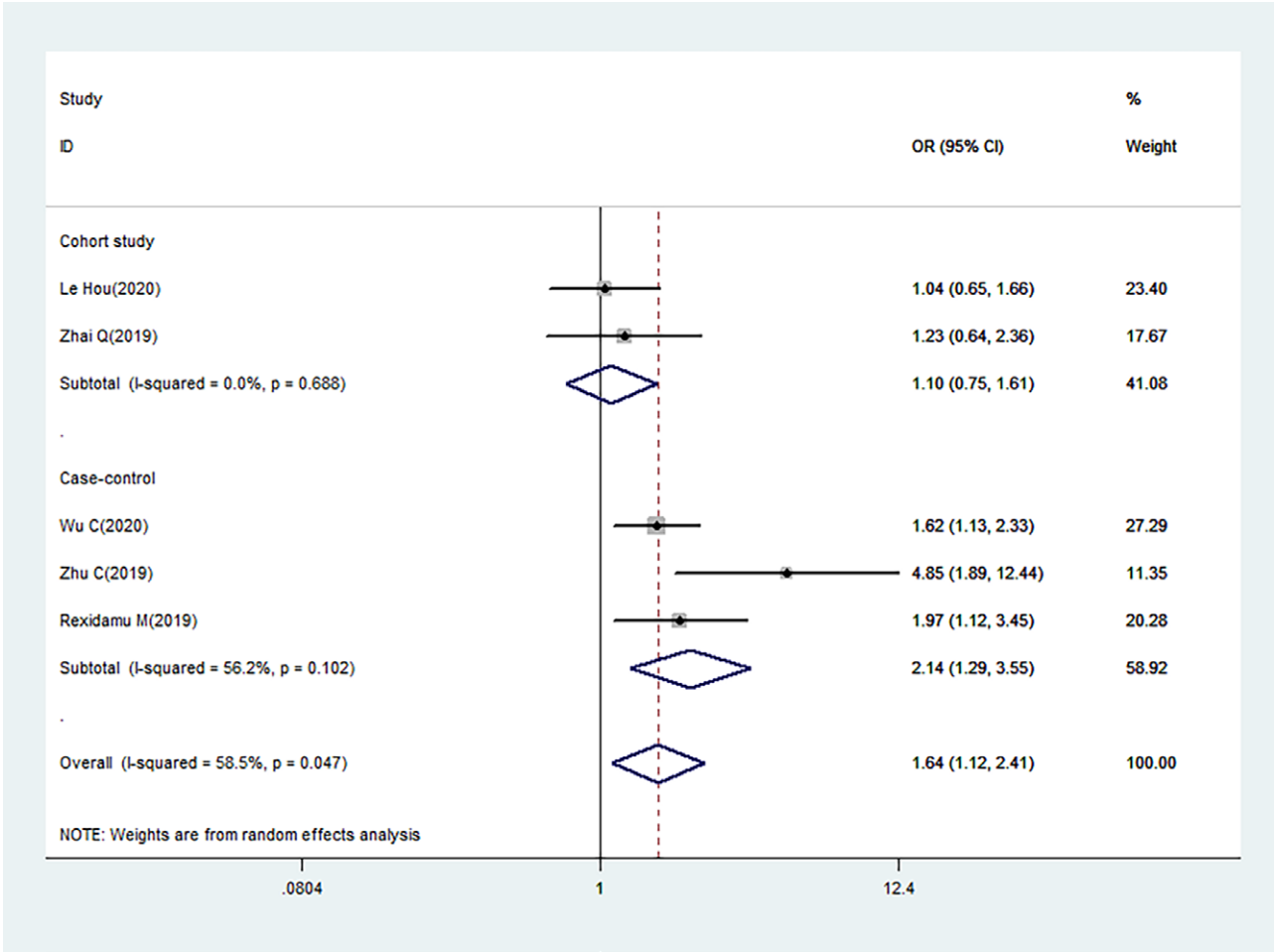

Figure 2. The prevalence of stroke was increased in the high-level trimethylamine N-oxide group. ID = included literature, OR = odds ratio.

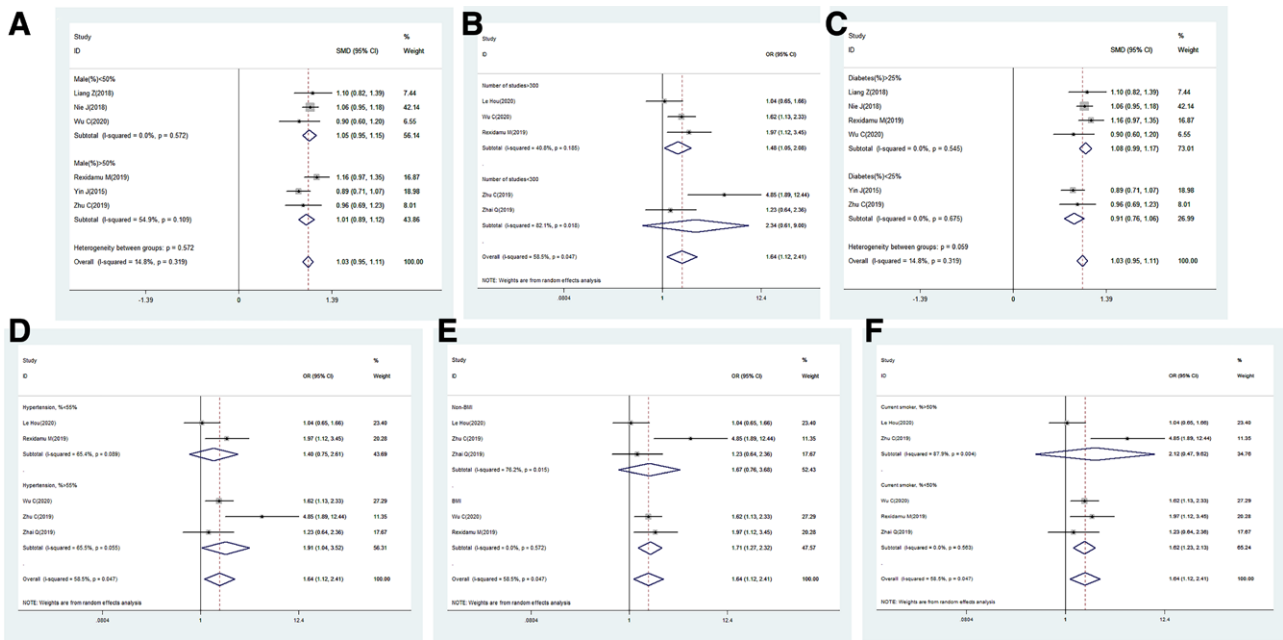

Figure 3. Subgroup analysis of high concentration trimethylamine N-oxide for the prevalence of stroke according to male rate (A), number of cases (B), diabetic patients (C), hypertensive patients (D), body mass index (E), and current smokers' rate (F). ID = included literature, OR = odds ratio, SMD = standardized mean difference.

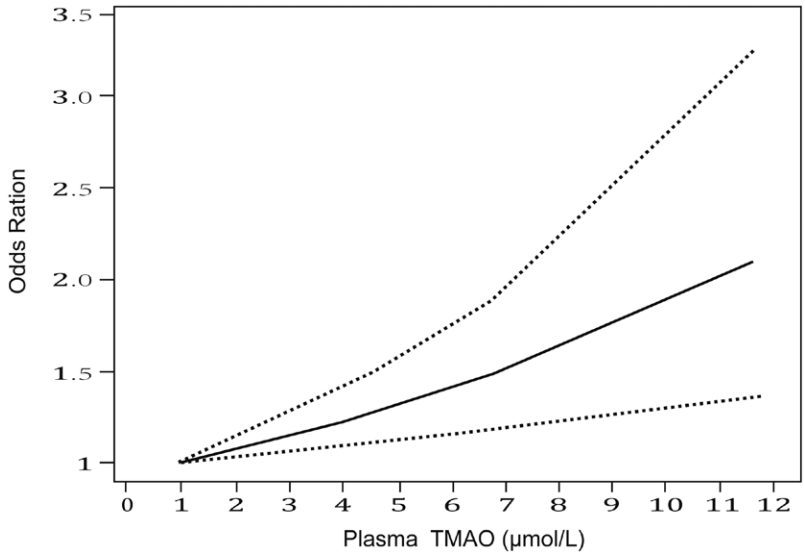

Figure 4. Dose-response analysis: increased TMAO levels augment the prevalence of stroke. TMAO = trimethylamine N-oxide.

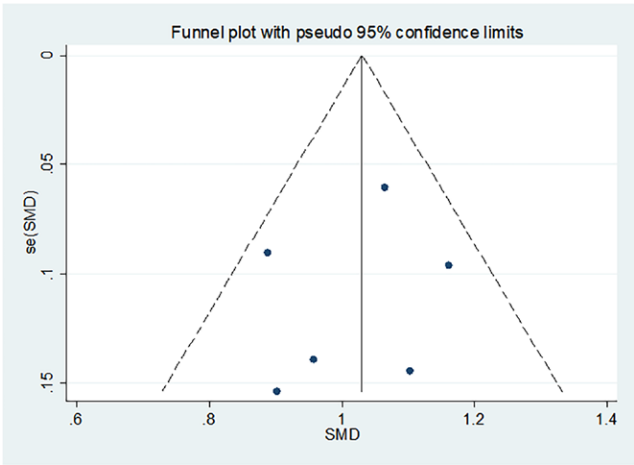

Figure 5. Funnel plot analysis for the assessment of publication bias. SMD = standardized mean difference.

part, resulting from excessive production of reactive oxygen species (ROS), such as superoxide anion, which inactivates nitric oxide, is also an important mechanism.[31]

One study demonstrated that TMAO resulted in an increased mononuclear inflammatory response, decreased cellular immunity, and increased cardiovascular risk.

One study observed that TMAO stimulated the expression of thioredoxin-interactive protein–nucleotide-binding oligomerization domain-like receptor family pyrin domain-containing 3 inflammasomes on the surface of human umbilical vascular endothelial cells, and they found that TMAO induced inflammation and endothelial dysfunction by activating the ROS–thioredoxin-interactive protein–nucleotide-binding oligomerization domain-like receptor family pyrin domain-containing 3 signaling pathway, revealing the possible mechanism of TMAO causing stroke and cardiovascular disease.[32]

4.3. Relationship to previous studies and the strengths in the current study

Numerous reviews and meta-analyses have been published examining the association between TMAO and stroke risk.[33,34] The current study is an updated meta-analysis and provided supportive results that are in line with a previous meta-analysis, which identified that TMAO is a risk factor of stroke.[33] However, several inconsistencies in the relationship between circulating TMAO and stroke risk are a matter of debate. For instance, a study from Guasch-Ferre et al[35] failed to show any significant association between TMAO and risk of stroke. A similar result was found by Mafune et al[36] in 227 patients undergoing elective coronary angiography.

Notably, the present meta-analysis found that patients with stroke had 1.03 μmol/L higher circulating TMAO concentrations compared with nonstroke controls, which was not in line with the data (2.20 μmol/L) from a previous meta-analysis by Farhangi et al.[33] The possible reason is that sample types (serum and plasma) are different.[37] In the study by Farhangi et al, more than half of the studies included were performed with serum TMAO, while all the studies included in the current meta-analysis were performed with plasma TMAO. In addition, day-to-day variability due to, for example, expected variation in diet might also have influenced the TMAO distribution in blood.[38] Thus, our findings differed from that previous reported. But anyway, most of the original articles included in our meta-analysis and previous all detailed the baseline characteristics of the included subjects and were of high quality. As we explore the sources of heterogeneity, one of the main advantages is the abundance of information. Subgroup analyses detailed the nature of heterogeneity in the study.

4.4. Study limitations

There are still many limitations in our meta-analysis. First, the included studies and sample size were small. All the studies included in this meta-analysis included high-risk participants, and most of the participants were from China. These facts suggest that the current meta-analysis may pose potential bias. The relationship between TMAO and the risk of stroke needs to be further explored in a more comprehensive, long-term follow-up population. In addition, some important values, such as dietary intake, that may affect TMAO production, and long-term concentrations of TMAO, are better suited to confirm this relationship than just a single measure. However, this is not currently available in inclusion studies. The included studies confirmed a history of hypertension but did not report the subjects' blood pressure at the time of sample collection, so we were not able to assess the relationship between TMAO concentration and the

severity of hypertension in subgroup analysis. Future research needs to address these deficiencies and explore their association with other clinically significant end points such as the degree of nerve damage and mortality.

5. Conclusion

In summary, the results of the current meta-analysis show that there was a significant association between higher plasma TMAO concentrations and the risk of stroke regardless of the different stratifications. However, more prospective studies are needed to evaluate this relationship and the underlying mechanisms.

Author contributions

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