Original Article

A systematic review and meta-analysis of the gut microbiota-dependent metabolite trimethylamine N-oxide with the incidence of atrial fibrillation

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Background: The gut microbiota-dependent metabolite trimethylamine N-oxide (TMAO) has recently been recognized as one of the novel marker for adverse cardiovascular events and risk of death. However, data on the relationship between TMAO and atrial fibrillation (AF) is limited. The current study was performed to quantify and evaluate the relationship between circulating TMAO levels and AF occurrence.

Methods: The electronic databases PubMed, Cochrane Library, and Embase were systematically searched to March 20, 2021. Research studies were considered that recorded or analyzed the prevalence of AF in individuals in specific populations as well as their circulating TMAO levels. A meta-analysis of two-class variables was used to obtain pooled effects. A dose-response meta-analysis was used to investigate the dose-response relationship between TMAO levels and the risk of AF.

Results: Six studies with a total of 8,837 individuals and 1,668 AF cases were included in the present meta-analysis. Compared with a lower circulating TMAO level, a higher TMAO level was associated with a higher prevalence of AF [odds ratio (OR): 1.40; 95% confidence interval (CI): 1.23, 1.59; I^2=19.8%]. The dose-response analysis revealed the risk of AF increased by 6% per 1-μmol/L increment (OR: 1.06; 95% CI: 1.00, 1.11), 32% per 5-μmol/L increment (OR: 1.32; 95% CI: 1.03, 1.70), and 73% per 10-μmol/L increment (OR: 1.73; 95% CI: 1.05, 2.86) of the circulating TMAO level.

Discussion: This is the first systematic literature review and meta-analysis to demonstrate a significant dose-dependent relationship between increased AF risk and circulating TMAO levels.

Keywords: Gut microbiota metabolite; atrial fibrillation (AF); risk factor; meta-analysis; trimethylamine N-oxide (TMAO)

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Introduction

In clinical practice, atrial fibrillation (AF) is a common heart arrhythmia associated with an elevated risk of adverse effects, such as thromboembolic strokes, heart failure (HF), systemic embolism (SE), and all-cause mortality (1,2). It is estimated that 33.5 million individuals worldwide are affected with AF (3), and its prevalence is projected to increase in the coming years (4). Several traditional risk

factors promote the development and maintenance of AF, such as hypertension (HTN), diabetes mellitus, congestive HF, obesity, alcohol abuse, and obstructive sleep apnea (2).

Recent studies suggest that patients with AF have a disorder of the gut microbiota and show variations in correlated metabolic patterns (5). Changes in the gut microbiome and metabolome are related to the risk of recurrence after radiofrequency ablation of AF (6). The duration of persistent AF is also associated with gut microbiota disorder (7). Therefore, altered gut microbiota may contribute to the initiation and maintenance of AF. The pathophysiological mechanism of AF is complicated, involving pro-inflammatory reactions and atrial fibrosis, leading to atrial restructuring and electrophysiological remodeling. The net result is an electrophysiological substrate for arrhythmogenesis (8,9). Intestinal microbes transform particular dietary nutrients (choline, betaine, phosphatidylcholine, and L-carnitine) into trimethylamine (TMA), which is absorbed by the intestine and transported to the liver via the portal circulation. TMA is subsequently oxidized to trimethylamine N-oxide (TMAO) by the hepatic enzyme flavin-containing monooxygenase 3 (10-12). Animal experiments have shown that elevated TMAO levels may directly contribute to myocardial inflammation and fibrosis (13-16), resulting in structural cardiac remodeling and may increase the instability of atrial electrophysiology by exacerbating autonomic remodeling (17). Several meta-analyses have recently been published suggesting that plasma TMAO levels are associated with the risk of HTN (18), diabetes (19), and stroke (20). However, although some studies have shown an association between TMAO and AF (21), others have not (22). These discrepancies require a comprehensive study to elucidate the actual relationship between TMAO and AF. Therefore, we performed a systematic review and meta-analysis to assess the relationship between AF and TMAO. The current research aims to provide a better understanding of this correlation for readers and clinical researchers.

We present the following article in accordance with the Preferred Reporting Items for Systematic Reviews (PRISMA) reporting checklist (23) (available at https://dx.doi.org/10.21037/apm-21-2763).

Methods

Search strategy

The review protocol is registered in PROSPERO, number CRD42021260294. The electronic databases PubMed, Cochrane Library, and Embase were systematically searched by two authors (WY and RY) to March 20, 2021. We chose the following medical subject headings (MESH) for the literature retrieval: (Trimethylamine N-oxide or TMAO) and (patient or participant or subject). There was no language limitation. Additionally, the original references of the selected studies were reviewed to find additional data sources that potentially met the requirements. The initial selection of studies was based on the titles and abstracts. The final decision was made after the full text was reviewed according to the inclusion criteria.

Inclusion and exclusion criteria

Studies were included if at least one of the following criteria was fulfilled: (I) studies exploring the relationship between TMAO bloodstream levels and incidence of AF; (II) studies that reported or analyzed the percentage of AF patients in a given population and their TMAO bloodstream levels. Exclusion criteria were listed as follows: (I) any duplicate publication data or conference abstracts; (II) articles where the effect size could not be extracted or calculated; (III) animal experiment studies, review articles, commentaries, or case reports. Two authors (WY and QZ) independently selected the eligible studies, and any disagreements were discussed until all authors reached a consensus.

Data extraction

The data extraction was conducted independently by two reviewers (WY and RY). A third reviewer (QZ) was invited to reach a consensus by discussion. The primary information extracted included *the first* author's name, publication date, location of the research projects, sample size, study design and time frame, demographic characteristics of the study population, clinical data of the study population, TMAO measurement method, and circulating TMAO concentration.

Qualitative analysis

The methodological quality of each included study was independently assessed by two reviewers (WY and XL). The Newcastle-Ottawa Quality Assessment Scale (NOS) for assessing the quality of nonrandomized studies was used to assess the risk of bias in the case-control and cohort studies (24). It contains three items; selection, comparability,

and outcome (Tables S1,S2). According to the NOS scores, the quality of the included studies was subdivided into low quality (scores 1–3), moderate quality (scores 4–6), and high quality (scores 7–9). The Agency for Healthcare Research and Quality (AHRQ) checklist was used to assess the quality of the cross-sectional studies (Table S3). An item was scored "0" if the answer was "no" or "unclear"; otherwise, the item was scored "1". The final quality scores were assessed as follows: low quality (scores 0–3), moderate quality (scores 4–7), and high quality (scores 8–11).

Statistical analysis

Individuals with high TMAO concentrations (above the median TMAO concentration) and those with low TMAO concentrations (below the median TMAO concentration) were compared in terms of AF prevalence. If the original studies reported the results per quintile in TMAO levels, we calculated the effect size according to the number of cases in the highest two quantiles and the lowest two quantiles. We used the odds ratio (OR) and 95% confidence interval (CI) as the "effect size". Heterogeneity was assessed using I^2 statistics. An I^2 value >50% was defined as significant heterogeneity. A fixed-effects model was used when $I^2 \leq 50\%$ indicated little heterogeneity; otherwise, a random-effects model was selected.

To analyze the sources of heterogeneity, we performed a series of subgroup analyses, including the study region, sample size, demographic, and clinical characteristics. A sensitivity analysis was conducted to assess the constancy of outcomes. We assessed publication bias using a funnel plot and the Egger test (where P>0.1 was considered to have no publication bias).

If the original studies included in the dose-response scrutiny provided a minimum of three TMAO exposure levels, the numbers of cases and non-cases in each stratification and the effect value and its 95% CI in each stratification were calculated. If the original studies did not report the number of cases in every stratification of TMAO, we assumed that the number of participants was equal. If neither median nor mean levels of TMAO in the bloodstream were available in the study, we utilized the median of every range in its place. If the lowermost or uppermost category was open-ended, the median of this category was projected by assuming the width of the category to be the same as the closest category. The lowermost category of bloodstream TMAO levels was defined as the overall reference category. Nonlinear and linear relationships were observed using a random-effects dose-response meta-analysis. We used limited cubic splines with 3 knots to calculate study-specific OR approximates per 1-μmol/L of TMAO increments. Statistical analyses were performed using Stata 14.0 (Stata Corp.) and Review Manager (RevMan, version 5.3; The Cochrane Collaboration).

Results

We searched 943 articles from the PubMed, EMBASE, and Cochrane databases and ten articles from other resources. After removing duplications and title/abstract selections, 87 full texts of potentially relevant articles were considered for further evaluation. Of these, 79 reports lacked relevant data, and two articles were excluded because of data duplication. Ultimately, four cohort studies (21,25-27), one nested case-control study (22), and one cross-sectional study (28) were included in our meta-analysis, with a total of 8,837 patients, including 1,668 patients with AF. The flow chart for the study selection is shown in *Figure 1*.

Study characteristics and qualitative evaluation

The general characteristics of the included studies in this review are illustrated in *Table 1*.

Reports in the current meta-analysis mainly investigated the role of TMAO in the bloodstream of patients who had different diseases, including kidney disease (28), acute and chronic heart failure (CHF) (25-27), and suspected stable angina pectoris (21). Two studies did not directly give the average circulating TMAO concentration of patients (21,22). Three studies were conducted in Europe (21,22,25), two in Asia (26,27), and one study involved 22 countries (28). In four studies, plasma TMAO was estimated (21,22,25,26), while in one study, serum TMAO was investigated (28), and one study did not specify whether the TMAO was from plasma or serum (27). The sample sizes of the individual studies ranged from 146 to 4,141. Three studies had a relatively small sample size (<1,200) (22,25,27), while the remaining three studies had a relatively large sample size (>1,200) (21,26,28). The NOS was applied to the cohort and case-control studies to further evaluate the quality of included studies considered to be of medium to high quality. The individual NOS scores ranged from 6 to 9 (Tables S1,S2). The AHRQ checklist was used to assess the quality of the cross-sectional studies, and the one cross-sectional study included in the meta-analysis scored 8

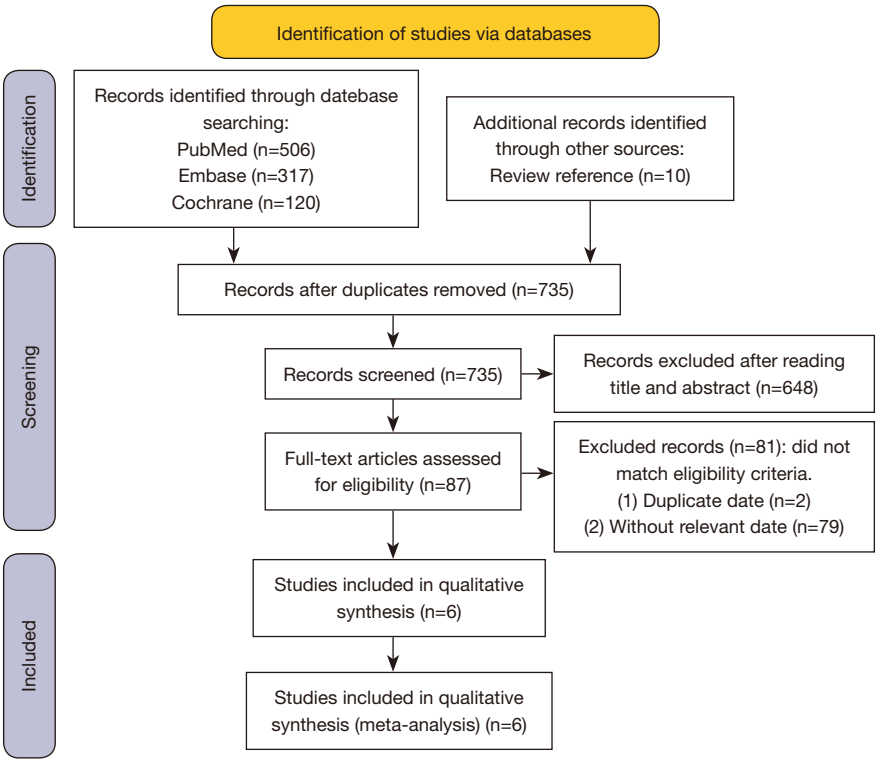

Figure 1 Flow chart of the study selection for the meta-analysis.

points, indicating high quality (28) (Table S3).

Results from the two-class meta-analysis

The two-class meta-analysis was implemented to explore the correlation between the OR of AF and the TMAO levels in the 1,668 AF cases out of 8,837 participants. The outcomes revealed that increased TMAO levels were correlated with a higher occurrence rate of AF. The pooled OR of the number of AF patients with high TMAO levels compared with low TMAO levels was 1.40 (95% CI: 1.23, 1.59; I^2=19.8%; P-heterogeneity =0.284; fixed-effects model; *Figure 2*). These results were consistent across subgroups stratified by age, sex, and diabetes (*Table 2*). Moreover, our results suggest that the higher levels of TMAO in the bloodstream were correlated with a higher prevalence of AF in the European group, HF group, lower prevalence of HTN group, and higher smoking group.

Findings from the dose-response meta-analysis

Two articles (21,26) with a total of 5,349 participants were used to investigate if there was a dose-response relationship between TMAO bloodstream levels and AF occurrence rates (21,26). The outcomes revealed a pooled OR of 1.06 (95% CI: 1.00, 1.11; *Figure 3A*) per 1-μmol/L increase, an OR of 1.32 (95% CI: 1.03, 1.70; *Figure 3B*) per 5-μmol/L increase, and an OR of 1.73 (95% CI: 1.0; 2.86, *Figure 3C*) per 10-μmol/L increase in TMAO levels in plasma, which demonstrates that the AF risk rose by 32% per 5-μmol/L increase and by 73% per 10-μmol/L increase in TMAO levels in plasma. A non-linear dose-response is presented in *Figure 4*.

Publication bias and sensitivity analysis

The Egger test (P=0.417) indicated no statistically significant publication bias in this meta-analysis. The funnel plot is presented in *Figure 5*. The sensitivity analysis showed that all of the consolidated results were stable (*Figure 6*).

Discussion

To the best of our knowledge, this report is the first meta-analysis to investigate the association between TMAO levels in the bloodstream and AF occurrence rates in a large

Table 1 Baseline characteristics of the studies included in the systematic review of the association between TMAO and AF

Study	Location	Sample size, n	Disease status	Sample	TMAO measure method	TMAO, μmol/L	Design	Study quality	Study period	Age (y)	Male, %	Diabetes, %	AF, %	HTN, %	Current smoking, %
Suzuki, 2016 (25)	Europe	972	Acute HF	Plasma	HPLC-MS/MS	5.6 (3.4–10.5)	Cohort study	High	2006–2011	78 [69–84]	61	33.8	45.4	58.3	9.6
Svingen, 2018 (WECAC) (21)	Europe	4,141	Suspected stable angina pectoris	Plasma	LC-MS/MS	N/A	Cohort study	High	2000–2009	58 [51–65]	66.5	8.0	8.3	39.5	35.7
Stubbs, 2019 (28)	EVOLVE trial	1,243	ESKD	Serum	HPLC-MS/MS	(2.5–1,103.1)	Cross-sectional	High	N/A	54±14	60	32	12.2	92	26
Zhou, 2020 (26)	China	1,208	CHF patients after MI	Plasma	HPLC-MS/MS	4.5 (2.83–7.92)	Cohort study	Moderate	2014–2018	73 [64–80]	68.5	28.6	18.6	39.7	N/A
Papandreou, 2021 (22)	Europe	1,127	AF/non-AF	Plasma	LC-MS/MS	N/A	Nested case-control	High	2003–2017	60–70	50.6	48.9	45.2	85.4	13.8
Kinugasa, 2021 (27)	Japan	146	Acute HF	N/A	HPLC-MS/MS	20.37 (10.45–38.31)	Cohort study	High	2012–2017	80 [73–85]	46.4	40.4	50	80.1	N/A

TMAO, trimethylamine N-oxide; AF, atrial fibrillation; HTN, hypertension; WECAC, Western Norway Coronary Angiography Cohort; EVOLVE, Evaluation of Cinacalcet Hydrochloride Therapy to Lower Cardiovascular Events; HF, heart failure; ESKD, end-stage kidney disease; CHF, chronic heart failure; MI, myocardial infarction; N/A, not applicable; HPLC-MS/MS, high-performance liquid chromatography-atmospheric pressure chemical ionization tandem mass spectrometry; LC-MS/MS, stable isotope dilution liquid chromatography with online tandem mass spectrometry.

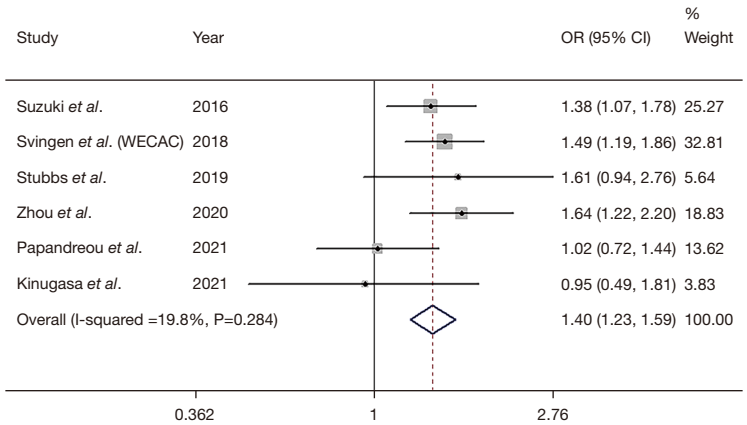

Figure 2 Forest plot demonstrating the pooled OR for AF in the high versus low category of TMAO levels. I^2 represents the degree of heterogeneity. OR, odds ratio; AF, atrial fibrillation; TMAO, trimethylamine N-oxide; CI, confidence interval; WECAC, Western Norway Coronary Angiography Cohort.

population. This study compared individuals with high and low TMAO bloodstream levels and found that those with elevated TMAO levels had a 40% greater incidence of AF. Furthermore, there was a dose-dependent correlation between TMAO amounts in the bloodstream and AF incidence. In conclusion, the current work demonstrates that TMAO bloodstream levels are positively correlated with an increased incidence of AF.

TMAO is a gut microbiota-derived metabolite and is closely related to diet (29,30). Previous meta-analyses showed that increased TMAO levels were correlated with an increased risk of several diseases, including HTN (18), diabetes (19), obesity (31), and inflammation (32), all of which are known risk factors for AF. The present meta-analysis showed that individuals with a high concentration of circulating TMAO at baseline were more likely to develop AF than those with a low concentration of circulating TMAO, and the heterogeneity was within the acceptable range. Further subgroup analysis and sensitivity analysis showed that the results were stable. To date, there have only been a few reports on the association between circulating TMAO concentrations and AF risk. The main findings are summarized as follows: Yu *et al.* indicated that TMAO could induce AF susceptibility by promoting autonomic nerve remodeling (17). A study of two cohorts showed that a baseline elevated plasma TMAO concentration was associated with AF [hazard ratio (HR): 1.16, 95% CI: 1.05, 1.28 and HR: 1.10, 95% CI: 1.004, 1.19] (21). In addition, compared with patients with non-AF, normal flora and microbial metabolite alterations have been found among patients with AF (5), and more enzymes are involved in driving TMAO precursor TMA formation (33). The underlying mechanism of the relationship between increased TMAO concentration and increased risk of AF is unclear; TMAO has been shown to aggravate cardiac dysfunction, myocardial fibrosis, and inflammation in mice with HF (15). Conversely, inhibition of TMAO improves cardiac function and remodeling in a murine model of HF (34,35). In addition, TMAO can accelerate the differentiation of fibroblasts into myofibroblasts and induce cardiac fibrosis (13). The remodeling of the left atrial matrix is an important pathophysiological mechanism in the development of AF. Therefore, we speculate that a high concentration of TMAO may advance the formation of the AF-susceptible matrix by promoting inflammation and fibrosis of the left atrium, but this hypothesis will require further study.

Our dose-response analysis showed a non-linear dose-dependent relationship between TMAO bloodstream levels and AF. Our dose-response meta-analysis indicated that the OR for AF occurrence was amplified by 32% per 5-μmol/L increase and by 73% per 10-μmol/L increase in the TMAO bloodstream levels. In addition, in the older subgroup (≥70 years old), an increased TMAO concentration demonstrated a higher risk of AF, suggesting that more attention should be paid to the effect of circulating TMAO concentrations on AF in the elderly population. Indeed, previous studies suggest that circulating TMAO levels increase with age (36). As the aging of the worldwide population increases, the incidence of AF will significantly

Table 2 Subgroup analyses of high-level TMAO for AF prevalence according to study characteristics

Subgroups	Overall			Heterogeneity	
	Studies, n	OR (95% CI)	P value	I^2, %	P value
All	6	1.40 (1.23, 1.59)	<0.001	19.8	0.284
Location					
Europe	3	1.33 (1.09, 1.62)	0.005	39.2	0.193
EVOLVE trial	1	1.61 (0.94, 2.76)	0.083	–	–
Asia	2	1.35 (0.81, 2.26)	0.246	55.1	0.135
Sample size (n)					
<1,200	3	1.19 (0.94, 1.50)	0.139	19.5	0.289
≥1,200	3	1.55 (1.31, 1.83)	<0.001	0	0.869
Study quality					
High	5	1.47 (1.28, 1.68)	<0.001	0	0.626
Moderate	1	1.02 (0.72, 1.44)	0.911	–	–
Sample					
Plasma	4	1.39 (1.18, 1.65)	<0.001	35.1	0.202
Serum	1	1.61 (0.94, 2.76)	0.083	–	–
N/A	1	0.95 (0.49, 1.83)	0.878	–	–
Disease status					
ESKD	1	1.61 (0.94, 2.76)	0.083	–	–
HF	3	1.43 (1.15, 1.76)	0.001	17.0	0.300
AF	1	1.02 (0.72, 1.44)	0.911	–	–
Suspected stable angina pectoris	1	1.49 (1.19, 1.86)	<0.001	–	–
Age (y)					
<70	3	1.33 (1.02, 1.75)	0.036	45.4	0.160
≥70	3	1.43 (1.15, 1.76)	0.001	17.0	0.300
Male (%)					
<65%	4	1.24 (1.01, 1.53)	0.036	12.5	0.330
≥65%	2	1.54 (1.29, 1.84)	<0.001	0	0.611
HTN (%)					
<80%	3	1.49 (1.29, 1.72)	<0.001	0	0.686
≥80%	3	1.13 (0.85, 1.52)	0.395	11.7	0.322
Diabetes (%)					
≥30%	4	1.24 (1.01, 1.53)	0.036	12.5	0.330
<30%	2	1.54 (1.29, 1.84)	<0.001	0	0.611

Table 2 (*continued*)

Table 2 (*continued*)

Subgroups	Overall			Heterogeneity	
	Studies, n	OR (95% CI)	P value	I^2, %	P value
Current smoking (%)					
<20%	2	1.22 (0.91, 1.63)	0.192	47.3	0.168
≥20%	2	1.51 (1.23, 1.85)	<0.001	0	0.795
N/A	2	1.35 (0.81, 2.26)	0.246	55.1	0.135

TMAO, trimethylamine N-oxide; AF, atrial fibrillation; OR, odds ratio; CI, confidence interval; EVOLVE, Evaluation of Cinacalcet Hydrochloride Therapy to Lower Cardiovascular Events; ESKD, end-stage kidney disease; HF, heart failure; HTN, hypertension; N/A, not applicable.

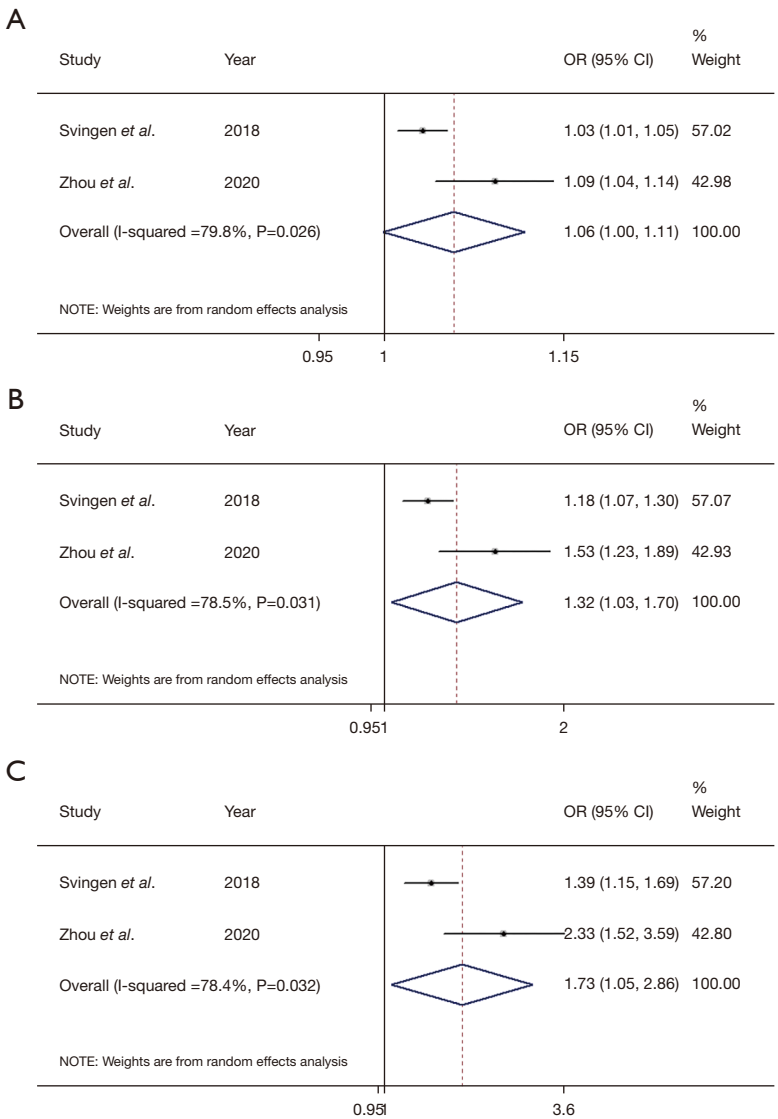

Figure 3 Forest plots of the non-linear dose-response meta-analysis on the relationship between TMAO levels in the bloodstream and AF prevalence. (A) OR per 1-μmol/L augmentation, (B) OR per 5-μmol/L increment, and (C) OR per 10-μmol/L rise. TMAO, trimethylamine N-oxide; AF, atrial fibrillation; OR, odds ratio; CI, confidence interval.

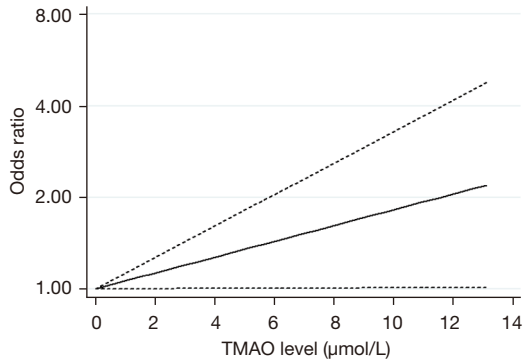

Figure 4 The dose-response analysis: increased TMAO levels increase the occurrence of AF. TMAO, trimethylamine N-oxide; AF, atrial fibrillation.

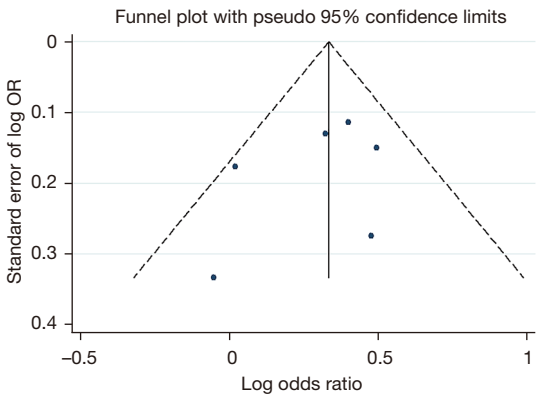

Figure 5 Funnel plot. OR, odds ratio.

increase and become a serious public health problem (37). Our meta-analysis findings are of great significance, highlighting the role of TMAO in the pathogenesis of AF. The current results encourage us to investigate more effective ways to reduce circulating TMAO levels in future studies in the hope of reducing the future prevalence of AF. TMAO is closely related to diets rich in choline, phosphatidylcholine, betaine, and L-carnitine, and red meat (38), eggs (39), and fish (40) are the most common dietary sources of TMAO. This can provide a reference for nutritionists when prescribing a diet to patients. Nutritionists need to be aware that the advantage of the nutrients in these foods is offset by the disadvantage of the metabolites (e.g., TMAO) they contain, especially for those elderly patients with comorbidities such as HTN, diabetes, and HF because these comorbidities themselves increase the risk of AF.

Previous studies have suggested that TMAO affects platelet activation and induces platelet hyperreactivity, which leads to a prothrombotic effect and promotes thrombus formation (41,42). Additionally, TMAO has been linked to thrombus development and identified as a risk marker for ischemic stroke in AF patients (43). Future large-scale prospective cohort studies are needed to establish the function of TMAO in the prognosis of patients with AF.

Study strengths

This meta-analysis is the first to clarify the potential relationship between the gut microbiota-dependent

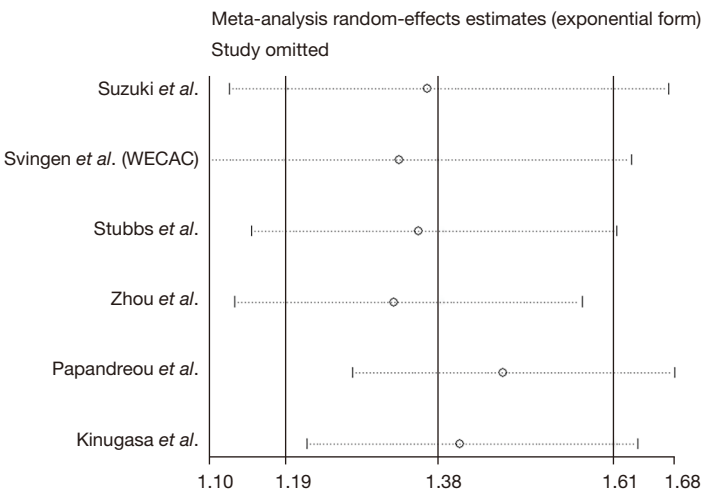

Figure 6 The sensitivity analysis of the consolidated results. WECAC, Western Norway Coronary Angiography Cohort.

metabolite TMAO and the prevalence of AF. The majority of the original reports in our meta-analysis were of excellent quality and included extensive baseline participant information. In our meta-analysis of binary variables, small heterogeneity was an advantage, and reliable outcomes were achieved in the sensitivity analysis. In particular, a dose-response analysis was used to determine the risk of AF associated with specific quantitative TMAO levels.

Study limitations

Numerous potential restrictions of our study must be considered when interpreting the results. First, all the reports included in this meta-analysis recruited participants with existing cardiovascular conditions, which may have potential bias. Therefore, our conclusions cannot be extrapolated to the general population. Second, because the studies included in the meta-analysis are observational, we cannot draw a causal relationship between TMAO and AF. Third, the most important limitation is that the effect values obtained in our study are calculated based on the four-grid table and not adjusted by the model, so the effect values cannot reflect the actual association between TMAO and AF; further high-quality randomized controlled trials or prospective studies that can provide adjusted effect values should be conducted to verify the conclusions of this study. Fourth, few studies have investigated the relationship between circulating TMAO levels and AF, especially for dose-response analysis, which may be a limitation. Uncontrolled confounding variables, such as dietary habits and genetic variations, can also significantly impact TMAO levels. Given these limitations, more research is needed to validate our finding of an association between TMAO levels and AF incidence and investigate its relationship with other clinically relevant outcomes, such as the prevalence of AF in the overall population.

Conclusions

The current meta-analysis found a significant and positive correlation between TMAO blood levels and AF by conducting dose-response and two-class meta-analyses. More research is needed to determine the causality of the correlations and to assess the impact of TMAO level manipulation on AF prognosis. To the best of our knowledge, this study is the first to analyze the correlation between TMAO levels and AF, and it may provide an informative and comprehensive basis for further evaluations.

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Footnote

Reporting Checklist: The authors have completed the PRISMA reporting checklist. Available at https://dx.doi.org/10.21037/apm-21-2763

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://dx.doi.org/10.21037/apm-21-2763). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The review protocol is registered in PROSPERO, number CRD42021260294.

References

1. Virani SS, Alonso A, Benjamin EJ, et al. Heart disease and stroke statistics-2020 update: a report from the American Heart Association. Circulation 2020;141:e139-596.
2. Lee HJ, Kim HK, Kim M, et al. Clinical impact of atrial fibrillation in a nationwide cohort of hypertrophic cardiomyopathy patients. Ann Transl Med 2020;8:1386.
3. Chugh SS, Havmoeller R, Narayanan K, et al. Worldwide epidemiology of atrial fibrillation: a Global Burden of Disease 2010 Study. Circulation 2014;129:837-47.
4. Yu R, Xi H, Lu J, et al. Real-world investigation on discontinuation of oral anticoagulation after paroxysmal atrial fibrillation catheter ablation in China. Ann Palliat Med 2020;9:940-6.

5. Zuo K, Li J, Li K, et al. Disordered gut microbiota and alterations in metabolic patterns are associated with atrial fibrillation. Gigascience 2019;8:giz058.
6. Li J, Zuo K, Zhang J, et al. Shifts in gut microbiome and metabolome are associated with risk of recurrent atrial fibrillation. J Cell Mol Med 2020;24:13356-69.
7. Zuo K, Li J, Wang P, et al. Duration of persistent atrial fibrillation is associated with alterations in human gut microbiota and metabolic phenotypes. mSystems 2019;4:00422-19.
8. Wijesurendra RS, Casadei B. Mechanisms of atrial fibrillation. Heart 2019;105:1860-7.
9. Korantzopoulos P, Letsas KP, Tse G, et al. Inflammation and atrial fibrillation: a comprehensive review. J Arrhythm 2018;34:394-401.
10. Lang DH, Yeung CK, Peter RM, et al. Isoform specificity of trimethylamine N-oxygenation by human flavin-containing monooxygenase (FMO) and P450 enzymes: selective catalysis by FMO3. Biochem Pharmacol 1998;56:1005-12.
11. Wang Z, Klipfell E, Bennett BJ, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature 2011;472:57-63.
12. Tang WH, Wang Z, Levison BS, et al. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. N Engl J Med 2013;368:1575-84.
13. Yang W, Zhang S, Zhu J, et al. Gut microbe-derived metabolite trimethylamine N-oxide accelerates fibroblast-myofibroblast differentiation and induces cardiac fibrosis. J Mol Cell Cardiol 2019;134:119-30.
14. Li Z, Wu Z, Yan J, et al. Gut microbe-derived metabolite trimethylamine N-oxide induces cardiac hypertrophy and fibrosis. Lab Invest 2019;99:346-57.
15. Shuai W, Wen J, Li X, et al. High-choline diet exacerbates cardiac dysfunction, fibrosis, and inflammation in a mouse model of heart failure with preserved ejection fraction. J Card Fail 2020;26:694-702.
16. Li X, Geng J, Zhao J, et al. Trimethylamine N-oxide exacerbates cardiac fibrosis via activating the NLRP3 inflammasome. Front Physiol 2019;10:866.
17. Yu L, Meng G, Huang B, et al. A potential relationship between gut microbes and atrial fibrillation: Trimethylamine N-oxide, a gut microbe-derived metabolite, facilitates the progression of atrial fibrillation. Int J Cardiol 2018;255:92-8.
18. Ge X, Zheng L, Zhuang R, et al. The gut microbial metabolite trimethylamine N-oxide and hypertension risk: a systematic review and dose-response meta-analysis. Adv Nutr 2020;11:66-76.
19. Zhuang R, Ge X, Han L, et al. Gut microbe-generated metabolite trimethylamine N-oxide and the risk of diabetes: a systematic review and dose-response meta-analysis. Obes Rev 2019;20:883-94.
20. Farhangi MA, Vajdi M, Asghari-Jafarabadi M. Gut microbiota-associated metabolite trimethylamine N-Oxide and the risk of stroke: a systematic review and dose-response meta-analysis. Nutr J 2020;19:76.
21. Svingen GFT, Zuo H, Ueland PM, et al. Increased plasma trimethylamine-N-oxide is associated with incident atrial fibrillation. Int J Cardiol 2018;267:100-6.
22. Papandreou C, Bulló M, Hernández-Alonso P, et al. Choline metabolism and risk of atrial fibrillation and heart failure in the PREDIMED study. Clin Chem 2021;67:288-97.
23. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71.
24. Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. Eur J Epidemiol 2010;25:603-5.
25. Suzuki T, Heaney LM, Bhandari SS, et al. Trimethylamine N-oxide and prognosis in acute heart failure. Heart 2016;102:841-8.
26. Zhou X, Jin M, Liu L, et al. Trimethylamine N-oxide and cardiovascular outcomes in patients with chronic heart failure after myocardial infarction. ESC Heart Fail 2020;7:188-93.
27. Kinugasa Y, Nakamura K, Kamitani H, et al. Trimethylamine N-oxide and outcomes in patients hospitalized with acute heart failure and preserved ejection fraction. ESC Heart Fail 2021;8:2103-10.
28. Stubbs JR, Stedman MR, Liu S, et al. Trimethylamine N-oxide and cardiovascular outcomes in patients with ESKD receiving maintenance hemodialysis. Clin J Am Soc Nephrol 2019;14:261-7.
29. Croci S, D'Apolito LI, Gasperi V, et al. Dietary strategies for management of metabolic syndrome: role of gut microbiota metabolites. Nutrients 2021;13:1389.
30. Mei Z, Chen GC, Wang Z, et al. Dietary factors, gut microbiota, and serum trimethylamine-N-oxide associated with cardiovascular disease in the Hispanic Community Health Study/Study of Latinos. Am J Clin Nutr 2021;113:1503-14.
31. Dehghan P, Farhangi MA, Nikniaz L, et al. Gut microbiota-derived metabolite trimethylamine N-oxide (TMAO) potentially increases the risk of obesity in adults:

An exploratory systematic review and dose-response meta-analysis. Obes Rev 2020;21:e12993.
32. Farhangi MA, Vajdi M. Novel findings of the association between gut microbiota-derived metabolite trimethylamine N-oxide and inflammation: results from a systematic review and dose-response meta-analysis. Crit Rev Food Sci Nutr 2020;60:2801-23.
33. Zuo K, Liu X, Wang P, et al. Metagenomic data-mining reveals enrichment of trimethylamine-N-oxide synthesis in gut microbiome in atrial fibrillation patients. BMC Genomics 2020;21:526.
34. Wang G, Kong B, Shuai W, et al. 3,3-Dimethyl-1-butanol attenuates cardiac remodeling in pressure-overload-induced heart failure mice. J Nutr Biochem 2020;78:108341.
35. Organ CL, Li Z, Sharp TE 3rd, et al. Nonlethal inhibition of gut microbial trimethylamine N-oxide production improves cardiac function and remodeling in a murine model of heart failure. J Am Heart Assoc 2020;9:e016223.
36. Ke Y, Li D, Zhao M, et al. Gut flora-dependent metabolite Trimethylamine-N-oxide accelerates endothelial cell senescence and vascular aging through oxidative stress. Free Radic Biol Med 2018;116:88-100.
37. Kornej J, Börschel CS, Benjamin EJ, et al. Epidemiology of atrial fibrillation in the 21st century: novel methods and new insights. Circ Res 2020;127:4-20.
38. Koeth RA, Wang Z, Levison BS, et al. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. Nat Med 2013;19:576-85.
39. Miller CA, Corbin KD, da Costa KA, et al. Effect of egg ingestion on trimethylamine-N-oxide production in humans: a randomized, controlled, dose-response study. Am J Clin Nutr 2014;100:778-86.
40. Hamaya R, Ivey KL, Lee DH, et al. Association of diet with circulating trimethylamine-N-oxide concentration. Am J Clin Nutr 2020;112:1448-55.
41. Gong D, Zhang L, Zhang Y, et al. Gut microbial metabolite trimethylamine N-oxide is related to thrombus formation in atrial fibrillation patients. Am J Med Sci 2019;358:422-8.
42. Zhu W, Gregory JC, Org E, et al. Gut microbial metabolite TMAO enhances platelet hyperreactivity and thrombosis risk. Cell 2016;165:111-24.
43. Liang Z, Dong Z, Guo M, et al. Trimethylamine N-oxide as a risk marker for ischemic stroke in patients with atrial fibrillation. J Biochem Mol Toxicol 2019;33:e22246.

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Table S1 NOS for cohort studies included in the systematic review and meta-analysis of the association between TMAO and AF

Authors, year	Selection				Comparability	Outcome			Final score
	Representativeness of the exposed cohort	Selection of the non-exposed cohort	Ascertainment of exposure	Demonstration that outcome of interest was not present at start of study	Comparability of cohorts based on the design or analysis	Assessment of outcome	Was follow-up long enough for outcomes to occur	Adequacy of follow up of cohorts	
Suzuki, 2016 (25)	*	*	*	*	*	*	*	–	7
Svingen, 2018 (WECAC) (21)	*	*	*	*	**	*	*	*	9
Zhou, 2020 (26)	–	*	*	*	*	*	*	–	6
Kinugasa, 2021 (27)	*	*	*	*	*	*	*	–	7

One star represents a score of 1, and a study can be awarded a maximum score of 9 in total. "*" represents score of 1; "**" represents score of 2; "–" represents score of 0. NOS, Newcastle-Ottawa Quality Assessment Scale; TMAO, trimethylamine N-oxide; AF, atrial fibrillation; WECAC, Western Norway Coronary Angiography Cohort.

Table S2 NOS for case-control studies included in the systematic review and meta-analysis of the association between TMAO and AF

Authors, year	Selection				Comparability	Exposure			Final score
	Is the case definition adequate?	Representativeness of the cases	Selection of controls	Definition of controls	Comparability of cases and controls based on the design or analysis	Ascertainment of exposure	Same method of ascertainment for cases and controls	Non-response rate	
Papandreou, 2021 (22)	*	*	*	*	**	*	*	–	8

One star represents a score of 1, and a study can be awarded a maximum score of 9 in total. "*" represents score of 1; "**" represents score of 2; "–" represents score of 0. NOS, Newcastle-Ottawa Quality Assessment Scale; TMAO, trimethylamine N-oxide; AF, atrial fibrillation.

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Table S3 AHRQ checklist to assess the quality of the cross-sectional studies included in the meta-analysis of the association between TMAO and AF

ARHQ methodology checklist items for cross-sectional study	Stubbs, 2019 (28)
(I) Define the source of information (survey, record review)	1
(II) List inclusion and exclusion criteria for exposed and unexposed subjects (cases and controls) or refer to previous publications	1
(III) Indicate time period used for identifying patients	0
(IV) Indicate whether or not subjects were consecutive if not population-based	1
(V) Indicate if evaluators of subjective components of study were masked to other aspects of the status of the participants	1
(VI) Describe any assessments undertaken for quality assurance purposes (e.g., test/retest of primary outcome measurements)	1
(VII) Explain any patient exclusions from the analysis	1
(VIII) Describe how confounding was assessed and/or controlled.	1
(IX) If applicable, explain how missing data were handled in the analysis	1
(X) Summarize patient response rates and completeness of data collection	0
(XI) Clarify what follow-up, if any, was expected and the percentage of patients for which incomplete data or follow-up was obtained	0
Final score	8

If the answer was "no" or "unclear", the item was scored "0"; otherwise, the item was scored "1". The maximum score for the scale is 11 points. The final quality scores were as follows: 0 to 3 was considered low quality, 4 to 7 moderate quality, and 8 to 11 high quality. AHRQ, Agency for Healthcare Research and Quality (available online: https://www.ncbi.nlm.nih.gov/books/NBK35156/); TMAO, trimethylamine N-oxide; AF, atrial fibrillation.

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