Editorial

Shusi Ding, Jing Xue, Qi Zhang and Lemin Zheng*

Trimethylamine-N-oxide is an important target for heart and brain diseases

https://doi.org/10.1515/mr-2022-0026

Trimethylamine-N-oxide (TMAO) is a metabolite produced from dietary nutrients by the gut microbiome, which was first discovered in 2011 and used to predict the risk of cardiovascular disease [1]. The precursors of TMAO, including phosphatidylcholine [1], choline [2], and L-carnitine [3], are commonly found in cheese, red meat, seafood, egg yolks, and other foods. To date, four different microbial enzyme systems have been identified that can convert precursor substances into trimethylamine (TMA), including choline-TMA lyase (cutC/D) [4], carnitine monooxygenase (cntA/B) [5], betaine reductase, and TMAO reductase [6]. In addition, yeaW/X, which is homologous to cntA/B and can utilize a variety of substrates such as choline, betaine, carnitine and γ-butyrobetaine, can also promote the synthesis of TMA. The cooperation between yeaW/X and cutC/D was the most well studied [7].

Most of the TMA produced by gut metabolism enters the circulatory system; TMA is absorbed into the bloodstream and enters the liver, where it is converted to TMAO by flavin monooxygenases (FMOs) [8]. The FMO family contains five functional enzymes, of which FMO3 is the key restriction enzyme for the conversion of TMA to TMAO, with the highest conversion efficiency. Excess TMA is directly decomposed into dimethylamine. Previous studies have shown that TMAO can accumulate in the heart, kidneys, brain or other tissues and participate in a variety of biological processes, such as activating platelet aggregation, increasing foam cell formation, inducing inflammatory responses, and reducing reverse cholesterol transport. In most cases, most of TMAO is eliminated by the kidneys, and the rest is reduced to TMA by TMAO reductase in the intestine [9].

In 2013, based on a nontargeted metabolomics analysis, researchers established a direct association between TMAO and cardiovascular event risk. TMAO has been found to have a certain correlation with atherosclerosis, heart failure, hypertension, chronic kidney disease, tumors, obesity-related diseases, diabetes and other diseasesin nearly a decade [10–13].

However, it seems that the effect of TMAO on various diseases is controversial. If there is no rule out whether there are other ways to produce TMAO, the effect of TMAO on diseases is often a process involving genes and environment, and it is obviously individualized. Multiple studies have concluded that TMAO does not exacerbate the development of atherosclerosis in mice, and a 2018 study on Apoe –/– mice showed that choline supplementation promoted an increase in TMAO levels in mice but had no effect on the absolute size of aortic plaques [14]. There are also clinical studies showing that in healthy young adults, TMAO may not significantly increase the risk of early atherosclerotic disease [15]. The probability that TMAO raises the risk of disease varies from person to person, is associated with different genotypes, and cannot be generalized to the entire population [16]. Due to large population variations, plasma TMAO is sometimes negatively correlated with the severity of the disease [17].

Notably, the prognostic value of TMAO is not corrected by traditional risk factors, which highlights its potential as a biomarker that can be used for risk stratification beyond traditional risks. While not all studies observed an association between elevated TMAO levels and the risk of new-onset cardiovascular disease (CVD) tests conducted on existing clinical studies have shown an association between elevated TMAO levels and the risk of new-onset CVD. Multiple meta-analyses concluded that there was indeed a strong correlation between elevated circulating TMAO levels and CVD risk and mortality in multiple cohorts across different continents [18, 19]. In many studies, plasma TMAO cutoffs > 6 μmol/L predicted an increased risk of adverse cardiac

***Corresponding author: Lemin Zheng**, China National Clinical Research Center of Neurological Diseases, Beijing Tiantan Hospital, Capital Medical University, Beijing 100050, China; School of Basic Medical Sciences, Health Sciences Center, The Institute of Cardiovascular Sciences and Institute of Systems Biomedicine, Peking University, Xueyuan Road NO. 38, Haidian District, Beijing 100871, China, E-mail: zhengl@bjmu.edu.cn

Shusi Ding and Jing Xue, China National Clinical Research Center of Neurological Diseases, Beijing Tiantan Hospital, Capital Medical University, Beijing, China. https://orcid.org/0000-0002-0360-9896 (S. Ding)

Qi Zhang, School of Basic Medical Sciences, The Institute of Cardiovascular Sciences and Institute of Systems Biomedicine, Peking University, Beijing, China

events [20]. In a recent meta-analysis of 25,000 participants, every 10 μmol/L increase in TMA increased all-cause mortality by 7.6% [18].

There are individual differences in metabolites of intestinal flora in both human beings and experimental animals, which requires the experimenter to pay attention to keep the collection and preservation methods of fecal samples unchanged and use relatively unified sequencing platform and data analysis method when doing research. These differences may affect the consistency of the gut microbiota and lead to changes in the gut microbiota. In human clinical cohort studies, many parameters of subjects may affect the consistency of microbiota findings, such as dietary habits, lifestyle, and medications. In animal studies, different ages, living conditions, or diets and feeding periods will also affect the intestinal flora. In a recent report by Hazen et al., the gut microbial pathways that produce TMA from γ-butyrobetaine were revealed and helped explain how diets rich in red meat led to elevated TMAO levels and increased risk of CVD [21]. But they also note that, according to a previous report on the provision of l-carnitine supplements to vegans and omnivores, mean TMAO levels increased in both groups, but TMA(O) levels did not change in a significant proportion of vegans [22]. This indicates that the metabolic capacity of gut microbes has a certain stability, which may remain unchanged over time, which can explain some doubts about the role of TMAO. The broad role of TMAO is unquestionable, so properly designing studies to make reliable discoveries is critical for future research.

In addition, because of the progress of brain-intestine axis research, TMAO and neurological-related diseases are not in the minority. *In vivo* studies have shown the presence of TMAO in the cerebrospinal fluid of mice and humans [23, 24]. This means that hepatic TMAO can penetrate the blood-brain barrier (BBB) to reach the nerve center, but the specific mechanism of penetration is still unclear [25]. There is another idea that TMAO found in cerebrospinal fluid is partly converted from TMA by FMOs in the brain, rather than completely from the penetration of the BBB [26].

Similarly, there is much debate about whether the effects of TMAO on the brain are positive or harmful. Clinical studies have shown that patients with Alzheimer's disease (AD) dementia have higher levels of TMAO in cerebrospinal fluid than individuals with normal cognitive function, but the specific effects of TMAO on brain cognitive function cannot be clarified. High-circulation TMAO may also promote neuroinflammation by increasing intrabrainous nuclear factor kappa-B (NF-κB) and proinflammatory cytokines, which are recognized mediators of cognitive aging and nerve function [27].

In a mice study, TMAO can induce brain aging and aging-related cognitive dysfunction in senescence-resistant 1 (SAMR1) mice, and aggravate the brain aging process in senescence-prone 8 (SAMP8) mice, which may provide a new idea for the effect of gut microbiota on brain aging process and help to delay aging by regulating gut microbiota metabolites [28]. Regarding the relationship between TMAO and ischemic stroke, in a large Chinese cohort, elevated TMAO levels were associated with an increased risk of recurrent stroke in patients with small-artery occlusion subtypes [29]. However, TMAO appears to affect the brain in a dose-dependent manner. Some studies have shown that TMAO may exert a neuroprotective effect within the normal physiological range (plasma concentration of 0.5–5.0 μmol/L) [30].

Gut microbiota, as a complex microecosystem, constantly affects the health of the host, and the physiological and pathological processes in which it participates have gradually become the most important position for new drug discovery and research. The mechanism of TMAO's effect on cardio-cerebral, renal, and systemic metabolic disorders still requires further study. Because intestinal flora metabolites are heavily influenced by the environment, individual variability, and heterogeneity of experimental design, this makes it necessary for experimenters to understand these highly complex systems, especially as we turn more to human studies, and need to continuously improve computational and statistical methods to obtain and implement the multiomics and longitudinal data required for integrated methods. The studies on the gut microbiota-TMA-TMAO metabolic pathway and various organ diseases are the most typical examples of gut microbiota metabolites. It is reasonable to believe that microecological agents, small molecule drugs, fecal microbiota transplantation and other intervention methods can become a new means of disease prevention and treatment in the future.

Research funding: This work was supported by the National Natural Science Foundation of China (91639108, 81770272, 81970425), and by the National High Technology Research and Development Program of China (2020YFA0803700), and by Hangzhou Qianjiang Distinguished Expert Project (Prof. Lemin Zheng).

Competing interests: The authors declare no competing interests.

References

1. Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature 2011;472:57–63.

2. Romano KA, Vivas EI, Amador-Noguez D, Rey FE. Intestinal microbiota composition modulates choline bioavailability from diet and accumulation of the proatherogenic metabolite trimethylamine-N-oxide. mBio 2015;6:e2481.
3. Koeth RA, Wang Z, Levison BS, Buffa JA, Org E, Sheehy BT, et al. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. Nat Med 2013;19:576–85.
4. Craciun S, Marks JA, Balskus EP. Characterization of choline trimethylamine-lyase expands the chemistry of glycyl radical enzymes. ACS Chem Biol 2014;9:1408–13.
5. Zhu Y, Jameson E, Crosatti M, Schafer H, Rajakumar K, Bugg TD, et al. Carnitine metabolism to trimethylamine by an unusual Rieske-type oxygenase from human microbiota. Proc Natl Acad Sci U S A 2014;111:4268–73.
6. Pascal MC, Burini JF, Chippaux M. Regulation of the trimethylamine N-oxide (TMAO) reductase in Escherichia coli: analysis of tor:Mud1 operon fusion. Mol Gen Genet 1984;195: 351–5.
7. Koeth RA, Levison BS, Culley MK, Buffa JA, Wang Z, Gregory JC, et al. γ-Butyrobetaine is a proatherogenic intermediate in gut microbial metabolism of L-carnitine to TMAO. Cell Metabol 2014; 20:799–812.
8. Bennett BJ, de Aguiar VT, Wang Z, Shih DM, Meng Y, Gregory J, et al. Trimethylamine-N-oxide, a metabolite associated with atherosclerosis, exhibits complex genetic and dietary regulation. Cell Metabol 2013;17:49–60.
9. Zhu W, Buffa JA, Wang Z, Warrier M, Schugar R, Shih DM, et al. Flavin monooxygenase 3, the host hepatic enzyme in the metaorganismal trimethylamine N-oxide-generating pathway, modulates platelet responsiveness and thrombosis risk. J Thromb Haemostasis 2018;16:1857–72.
10. Organ CL, Li Z, Sharp TR, Polhemus DJ, Gupta N, Goodchild TT, 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:e16223.
11. Zhang W, Miikeda A, Zuckerman J, Jia X, Charugundla S, Zhou Z, et al. Inhibition of microbiota-dependent TMAO production attenuates chronic kidney disease in mice. Sci Rep 2021;11: 518.
12. Gupta N, Buffa JA, Roberts AB, Sangwan N, Skye SM, Li L, et al. Targeted inhibition of gut microbial trimethylamine N-oxide production reduces renal tubulointerstitial fibrosis and functional impairment in a murine model of chronic kidney disease. Arterioscler Thromb Vasc Biol 2020;40:1239–55.
13. Kwee LC, Ilkayeva O, Muehlbauer MJ, Bihlmeyer N, Wolfe B, Purnell JQ, et al. Metabolites and diabetes remission after weight loss. Nutr Diabetes 2021;11:10.
14. Lindskog JA, Caesar R, Akrami R, Reinhardt C, Fak HF, Boren J, et al. Impact of gut microbiota and diet on the development of atherosclerosis in Apoe(-/-) mice. Arterioscler Thromb Vasc Biol 2018;38:2318–26.
15. Meyer KA, Benton TZ, Bennett BJ, Jacobs DJ, Lloyd-Jones DM, Gross MD, et al. Microbiota-dependent metabolite trimethylamine N-oxide and coronary artery calcium in the coronary artery risk development in young adults study (CARDIA). J Am Heart Assoc 2016;5:e003970.
16. Bordoni L, Samulak JJ, Sawicka AK, Pelikant-Malecka I, Radulska A, Lewicki L, et al. Trimethylamine N-oxide and the reverse cholesterol transport in cardiovascular disease: a cross-sectional study. Sci Rep 2020;10:18675.
17. Vernon ST, Tang O, Kim T, Chan AS, Kott KA, Park J, et al. Metabolic signatures in coronary artery disease: results from the BioHEART-CT study. Cells 2021;10:980.
18. Schiattarella GG, Sannino A, Toscano E, Giugliano G, Gargiulo G, Franzone A, et al. Gut microbe-generated metabolite trimethylamine-N-oxide as cardiovascular risk biomarker: a systematic review and dose-response meta-analysis. Eur Heart J 2017;38:2948–56.
19. Heianza Y, Ma W, Manson JE, Rexrode KM, Qi L. Gut microbiota metabolites and risk of major adverse cardiovascular disease events and death: a systematic review and meta-analysis of prospective studies. J Am Heart Assoc 2017;6:e004947.
20. Tang W, Backhed F, Landmesser U, Hazen SL. Intestinal microbiota in cardiovascular health and disease: JACC state-of-the-art review. J Am Coll Cardiol 2019;73:2089–105.
21. Buffa JA, Romano KA, Copeland MF, Cody DB, Zhu W, Galvez R, et al. The microbial gbu gene cluster links cardiovascular disease risk associated with red meat consumption to microbiota L-carnitine catabolism. Nat Microbiol 2022;7: 73–86.
22. Koeth RA, Lam-Galvez BR, Kirsop J, Wang Z, Levison BS, Gu X, et al. l-Carnitine in omnivorous diets induces an atherogenic gut microbial pathway in humans. J Clin Invest 2019;129:373–87.
23. Vogt NM, Romano KA, Darst BF, Engelman CD, Johnson SC, Carlsson CM, et al. The gut microbiota-derived metabolite trimethylamine N-oxide is elevated in Alzheimer's disease. Alzheimers Res Ther 2018;10:124.
24. Del RD, Zimetti F, Caffarra P, Tassotti M, Bernini F, Brighenti F, et al. The gut microbial metabolite trimethylamine-N-oxide is present in human cerebrospinal fluid. Nutrients 2017;9:1053.
25. Vernetti L, Gough A, Baetz N, Blutt S, Broughman JR, Brown JA, et al. Functional coupling of human microphysiology systems: intestine, liver, kidney proximal tubule, blood-brain barrier and skeletal muscle. Sci Rep 2017;7:42296.
26. Zhang J, Cashman JR. Quantitative analysis of FMO gene mRNA levels in human tissues. Drug Metab Dispos 2006;34:19–26.
27. Ownby RL. Neuroinflammation and cognitive aging. Curr Psychiatr Rep 2010;12:39–45.
28. Li D, Ke Y, Zhan R, Liu C, Zhao M, Zeng A, et al. Trimethylamine-N-oxide promotes brain aging and cognitive impairment in mice. Aging Cell 2018;17:e12768.
29. Xu J, Cheng A, Song B, Zhao M, Xue J, Wang A, et al. Trimethylamine N-oxide and stroke recurrence depends on ischemic stroke subtypes. Stroke 2022;53:1207–15.
30. Hoyles L, Pontifex MG, Rodriguez-Ramiro I, Anis-Alavi MA, Jelane KS, Snelling T, et al. Regulation of blood-brain barrier integrity by microbiome-associated methylamines and cognition by trimethylamine N-oxide. Microbiome 2021;9:235.