



OPEN ACCESS

EDITED BY

Haihan Song,
Shanghai Pudong New Area People's
Hospital, China

REVIEWED BY

Junjie Xing,
Second Military Medical University, China
Juan Yang,
Shanghai Pudong New Area People's
Hospital, China

*CORRESPONDENCE

Liqiang Zheng
✉ liqiangzheng@126.com

[†]These authors share first authorship

[‡]These authors share last authorship

RECEIVED 10 July 2024

ACCEPTED 13 November 2024

PUBLISHED 28 November 2024

CITATION

Bai H, Zhang Y, Tian P, Wu Y, Peng R, Liang B,
Ruan W, Cai E, Lu Y, Ma M and Zheng L (2024)
Serum trimethylamine N-oxide and its
precursors are associated with the
occurrence of mild cognition impairment as
well as changes in neurocognitive status.
Front. Nutr. 11:1461942.
doi: 10.3389/fnut.2024.1461942

COPYRIGHT

© 2024 Bai, Zhang, Tian, Wu, Peng, Liang,
Ruan, Cai, Lu, Ma and Zheng. This is an
open-access article distributed under the
terms of the [Creative Commons Attribution
License \(CC BY\)](https://creativecommons.org/licenses/by/4.0/). The use, distribution or
reproduction in other forums is permitted,
provided the original author(s) and the
copyright owner(s) are credited and that the
original publication in this journal is cited, in
accordance with accepted academic
practice. No use, distribution or reproduction
is permitted which does not comply with
these terms.

Serum trimethylamine N-oxide and its precursors are associated with the occurrence of mild cognition impairment as well as changes in neurocognitive status

He Bai^{1†}, Yao Zhang^{2†}, Peiyong Tian^{3†}, Yani Wu¹, Ruiheng Peng¹, Bin Liang⁴, Wenli Ruan⁵, Enmao Cai⁵, Ying Lu^{5‡}, Mingfeng Ma^{6‡} and Liqiang Zheng^{1,7*}

¹School of Public Health, Shanghai Jiao Tong University School of Medicine, Shanghai, China,

²Department of Endocrinology, Shengjing Hospital of China Medical University, Shenyang, China,

³Department of Gastroenterology, Shanghai Pudong Hospital, Fudan University Pudong Medical Center, Shanghai, China, ⁴Department of Cardiovascular Medicine, Second Hospital of Shanxi Medical University, Taiyuan, China, ⁵Department of Physical and Chemical, Changning District Center for Disease Control and Prevention, Shanghai, China, ⁶Department of Cardiovascular Medicine, Fenyang Hospital of Shanxi Province, Fenyang, China, ⁷Ministry of Education-Shanghai Key Laboratory of Children's Environmental Health, Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China

Background: This study aims to examine the association between gut microbe-dependent trimethylamine N-oxide (TMAO) and its precursors (choline, betaine, and carnitine) levels and mild cognition impairment (MCI), alongside changes in the Chinese version of the Montreal Cognitive Assessment-Basic (Δ MoCA-BC) score in rural adults.

Methods: Drawing data from a large-scale epidemiological study conducted in rural areas of Fuxin County, Liaoning Province, China, 1,535 participants free from brain-related ailments were initially surveyed. MCI was assessed through the MoCA-BC score. Logistic regression models and restricted cubic spline were used to investigate the association between TMAO and its precursors levels and MCI. Additionally, the association between TMAO and its precursors levels and Δ MoCA-BC was analyzed using a generalized linear model in the longitudinal study.

Results: The average age of the study participants was 58.6 ± 9.4 years and the prevalence rate of MCI was 34.5%. With the second quartile as the reference in the logistic regression model, the OR for risk of MCI in the highest quartile for TMAO, betaine, and carnitine was 1.685 (95% CI: 1.232–2.303, $p = 0.001$), 2.367 (95% CI: 1.722–3.255, $p < 0.001$), and 2.239 (95% CI: 1.742–3.295, $p < 0.001$), respectively. The OR of choline for the highest versus lowest quartile was 2.711 (95% CI: 2.012–3.817, $p < 0.001$) for the risk of MCI. We find a J-shaped association between betaine ($P_{\text{non-linear}} = 0.001$) and carnitine ($P_{\text{non-linear}} = 0.003$) levels and MCI. Furthermore, TMAO and its precursors levels were associated with Δ MoCA-BC in the third and fourth quartiles group (All $p < 0.05$).

Conclusion: The findings suggest the existence of an optimal concentration range for serum levels of TMAO, betaine, and carnitine that mitigates MCI risk, paving the way for enhanced dietary interventions aimed at preventing and treating MCI.

KEYWORDS

trimethylamine N-oxide, TMAO, mild cognition impairment, MCI, MoCA-BC, choline, betaine, carnitine

1 Introduction

Dementia represents a leading cause of disability among individuals aged 65 and older globally (1). In China, approximately 15.07 million individuals aged 60 and above are affected by dementia, which constitutes about 25.5% of the worldwide dementia population (2). Currently, there is no specific pharmacological treatment for dementia (3), underscoring the importance of early prevention and intervention. Mild cognitive impairment (MCI) is recognized as a transitional phase between normal cognitive aging and dementia, placing individuals in this category at a heightened risk for developing dementia (4, 5). Research indicates that the annual conversion rate from MCI to dementia is tenfold higher than that of cognitively normal individuals (4–6). Extensive population surveys have reported a concerning MCI prevalence rate of 15.5% in China, equating to approximately 38.77 million affected individuals (4). Traditional risk factors for MCI, including a family history of dementia, hypertension, and diabetes, account for only a portion of MCI cases (7, 8). Therefore, it is crucial to identify additional factors that may influence the onset of MCI.

Trimethylamine N-oxide (TMAO) is a bioactive compound primarily synthesized through the metabolic activities of gut microbiota in the human body (9). Mounting evidence has linked TMAO and its precursors to neurocognitive disorders, notably MCI (10, 11). Through the enzymatic processing of dietary components such as carnitine, choline (including choline-containing compounds like phosphatidylcholine), and betaine (a product of choline metabolism pathway), the intestinal microbiome generates trimethylamine (TMA) within the intestinal lumen (12–14), subsequently, TMA undergoes absorption within the intestines and is ferried to the liver via the portal venous circulation, where flavin-containing monooxygenase subtypes 3 (FMO3) catalyze its oxidation into TMAO (15, 16).

In recent years, research has suggested that elevated plasma TMAO levels may infiltrate the central nervous system, triggering neuroinflammation and immune responses, and impairing the integrity of the blood–brain barrier (17). However, the findings of published studies are currently inconsistent and the underlying mechanisms are still unclear (18–21). With limited studies conducted in China, where TMAO and its precursors are influenced by diverse factors including geographical variations, dietary habits, and gut microbiota composition, our study aims to explore the association between serum TMAO and its precursors with MCI, and further investigate their potential impact on the dynamic changes in MoCA-BC score. This study not only supplements evidence from large-scale population studies in rural China of the relationship between TMAO and its precursors and MCI but also holds promise for future MCI prevention and management strategies through dietary modulation of TMAO and its precursors.

2 Materials and methods

2.1 Study population

We derived the data from a large-scale epidemiological study in the rural areas of Fuxin County, Liaoning Province, China. The baseline survey was conducted from June to August 2019. The

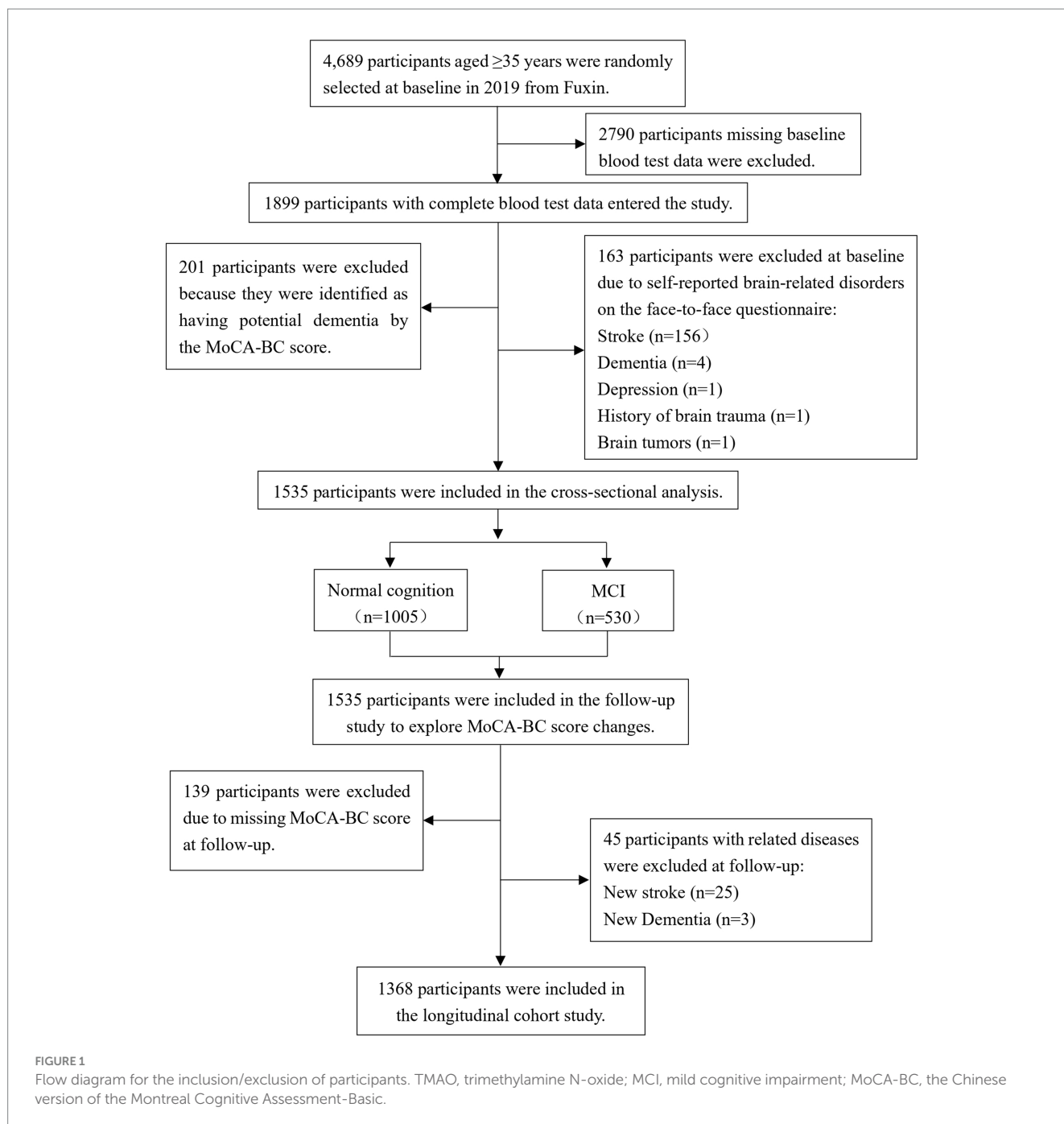
selection of natural villages, questionnaire survey, physical examinations, and other information for this study can all be referenced from the previously published literature by our research group (22–24). Participants were eligible if: (1) they were 35 years of age or older; (2) residency in the study area for a minimum of 5 years; (3) they were willing to sign informed consent to participate in the study. Participants who were pregnant, suffered from severe liver or kidney dysfunction, or were unwilling to participate in this study were excluded (22). Finally, 4,689 participants were recruited as the study population. The follow-up survey was conducted between June and August 2021, adhering to the same inclusion and exclusion criteria as established during the baseline survey. Written informed consent was obtained from all participants. This study was approved by the human experimentation committee of China Medical University [(2018)083] (23, 24).

The flowchart detailing the inclusion and exclusion criteria for this study is presented in Figure 1. Out of a total of 4,689 participants, 2,790 were excluded due to the absence of data on TMAO and its precursors. Additionally, 163 participants were excluded based on self-reported histories of stroke, dementia, depression, brain trauma, and brain tumors, while 201 individuals diagnosed with potential dementia were also excluded. Consequently, 1,535 participants were retained for the cross-sectional study. To further assess changes in cognitive function scores among the participants, 139 individuals lacking MoCA-BC scores were excluded during the follow-up phase, along with 28 participants who developed new cases of stroke and dementia. Ultimately, 1,368 participants were included in the longitudinal study.

2.2 Measurements of TMAO and its precursors

High-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS, Shimadzu, Japan) was employed to quantify serum levels of TMAO and its precursors. Fasting venous blood samples were collected from subjects and subsequently centrifuged at 3000 rpm for 10 min. A volume of 500 μ L of the upper serum layer was extracted and stored at -80°C . For the analytical procedure, 10 μ L of serum was diluted 50-fold with a standard solution, vortexed for 3 min to ensure thorough mixing, and then centrifuged at 15,000 rpm for 15 min at 4°C to isolate the supernatant. The supernatant was then filtered through a 0.22 μ m hydrophobic nylon membrane. Finally, 100 μ L of the filtered serum was transferred into a 1.5 mL sample vial for analysis via HPLC-MS/MS.

Chromatographic separations were performed using an ACQUITY UPLC HSS T3 column (1.7 μ m, 2.1 mm \times 100 mm; Waters, United States). The injection volume was set at 2 μ L, and the column temperature was maintained at 40°C . The mobile phases comprised phase A, consisting of 10 mmol/L ammonium formate, 0.1% formic acid, and a solvent mixture of acetonitrile and water in a 9:1 ratio, and phase B, which contained 5 mmol/L ammonium formate, 0.1% formic acid, and a solvent mixture of acetonitrile and water in a 1:1 ratio. Phase B, the aqueous component, was gradually increased over time to achieve gradient elution. Specifically, from 0.0 to 2.0 min, phase B was maintained at 10%; from 2.0 to 6.0 min, phase B was linearly increased from 10 to 45%; from 6.0 to 6.1 min, phase B was linearly increased from 45 to 100%; from 6.1 to 8.1 min, phase B was held at



100%; from 8.1 to 8.2 min, phase B was decreased from 100% back to 10%; and from 8.2 to 10.0 min, phase B was maintained at 10%. The system was equilibrated for 2.5 min, resulting in a total runtime of 10 min per sample, with a flow rate of 0.4 mL/min (split ratio 5:3).

Reference standards for each metabolite were utilized to accurately determine the chromatographic retention times and to optimize the mass spectrometry parameters. The electrospray ionization source was employed with an impact voltage of 20 eV in positive ion mode. Multiple reaction monitoring (MRM) was implemented, with operating conditions that included a drying gas flow rate of 3 L/min, an atomizer pressure of 50 psi, a drying temperature of 350°C, and a capillary voltage of 3,500 V. The linear range of detection was established between 0.16 and 20.00 μmol/L, demonstrating an

excellent correlation coefficient ($r^2=0.999$). Furthermore, the recovery rates for the metabolites ranged from 90.2 to 102.1%.

2.3 MoCA-BC test and diagnosis of MCI

The MoCA-BC is utilized as a screening instrument for MCI among elderly Chinese individuals with varying levels of educational attainment (25, 26). This assessment evaluates nine cognitive domains: executive function, language, orientation, calculation, conceptual thinking, memory, visual perception, attention, and concentration, with a maximum total score of 30 points (26). The scoring framework classifies individuals into categories of normal cognition (NC), MCI,

and potential dementia, stratified by years of education (22, 27): (1) For individuals with ≤ 6 years of education, scores ranging from 19 to 30 indicate NC, scores from 13 to 18 suggest MCI, and scores from 0 to 12 are indicative of potential dementia; (2) For those with 7 to 12 years of education, scores of 22 to 30 denote NC, scores from 15 to 21 indicate MCI, and scores from 0 to 14 suggest potential dementia; (3) For individuals with over 12 years of education, scores ranging from 24 to 30 represent NC, scores from 16 to 23 indicate MCI, and scores from 0 to 15 are indicative of potential dementia.

In the cross-sectional study, we used the MoCA-BC to screen for NC, MCI, and potential dementia, excluding patients with potential dementia. Simultaneously, this study also focused on Δ MoCA-BC during the follow-up period, and the Δ MoCA-BC was calculated by subtracting the baseline MoCA-BC score from the follow-up MoCA-BC score.

2.4 Covariates

We collected information on participants' age, gender, ethnicity, education level, family income, physical labor level, smoking, and drinking habits through face-to-face questionnaires (23). Because the local is a Mongolian autonomous county, the proportion of Mongolian is relatively high and the living habits are different from those of the Han. Therefore, the nationalities were divided into three groups: Han, Mongolian, and Others. Income was divided into three levels: less than 10,000 yuan (low-income group, lower than the average income of local residents), 10,000 to 30,000 yuan (middle-income group), and more than 30,000 yuan (high-income group, lower than the average income of local residents). Educational level was divided into three categories: primary school and below, middle school, and high school or above. Physical labor level was classified into three tiers: light, moderate, and heavy. Smokers were categorized as individuals who consumed at least one cigarette daily for a minimum of 6 months. According to the Dietary Guidelines for Chinese Residents and considering the long-term effects of alcohol consumption, drinkers were defined as individuals who consumed alcohol at least 3 times per week for at least 6 consecutive months.

In addition, Height and weight were measured using a calibrated domestic height and weight measurement instrument (Sitai Corporation, China). Body mass index (BMI) was calculated in terms of weight (kg)/height (m)². Fasting blood glucose was determined using the glucose oxidase method, triglycerides (TG) were measured using colorimetric method, and total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were measured using enzymatic colorimetric method. The above blood parameters were analyzed using the Cobas 8,000 C701 fully automated biochemical analyzer (Roche, Switzerland). Blood pressure was measured using the HEM-8102A/K electronic blood pressure monitor (Omron Corporation, Japan). Three measurements were taken, with an interval of more than 1 min between each measurement, the systolic blood pressure (SBP) and diastolic blood pressure (DBP) were calculated as the average of the three measurements.

2.5 Statistical analysis

Both TMAO and its precursors were converted into categorical variables by the interquartile range and grouped as follows: TMAO (Q1:

< 2.24 μ mol/L, Q2: 2.24–4.26 μ mol/L, Q3: 4.27–7.75 μ mol/L, Q4: > 7.75 μ mol/L), choline (Q1: < 118.11 μ mol/L, Q2: 118.11–162.40 μ mol/L, Q3: 162.41–270.17 μ mol/L, Q4: > 270.17 μ mol/L), betaine (Q1: < 69.24 μ mol/L, Q2: 69.24–92.50 μ mol/L, Q3: 92.51–119.67 μ mol/L, Q4: > 119.67 μ mol/L), carnitine (Q1: < 40.09 μ mol/L, Q2: 40.09–51.18 μ mol/L, Q3: 51.19–70.90 μ mol/L, Q4: > 70.90 μ mol/L).

Continuous variables are represented as the mean \pm standard deviation (SD) or medians (interquartile ranges, IQR), and categorical variables are reported as frequencies (percentages). The comparison of baseline characteristics between MCI and NC groups was conducted using Student's *t*-test, Mann–Whitney *U* test, or chi-square test. We used a binary logistic regression model to analyze the association between the levels of TMAO and its precursors and MCI, adjusting for covariates including age, sex, BMI, smoking, drinking, education level, SBP, DBP, fasting glucose, triglyceride, and total cholesterol. Odds ratios (OR) and their 95% confidence intervals (CI) were calculated. TMAO and its precursors were entered into the model as categorical variables, with Q2 (lowest prevalence) as the reference group for TMAO, betaine, and carnitine, and Q1 as the reference group for choline. Additionally, we used restricted cubic spline (RCS) based on the logistic regression model, adjusting for the same confounding factors, and using the 25th, 50th, and 75th percentiles as the nodes for the concentration levels of TMAO and its precursors. We also conducted subgroup analyses based on gender and age among the study participants. In the longitudinal study, we used generalized linear models to investigate the association between TMAO and its precursors and Δ MoCA-BC. All statistical analyses were performed using R software (version 2.2.3) and IBM SPSS statistical software (version 25.0).

3 Results

3.1 Characteristics of the study population

Table 1 presents a detailed summary of the key demographic and biochemical characteristics of the study. Among the 1,535 participants at baseline, the mean age was 58.6 ± 9.4 years, with 1,038 individuals (67.6%) identified as female. A total of 530 participants were diagnosed with MCI, yielding a prevalence rate of 34.5%. Comparative analyses indicated notable differences between the MCI and NC groups, with MCI participants generally being older, having a higher proportion of females, lower BMI, reduced educational attainment, a greater prevalence of smoking, elevated fasting blood glucose levels, increased SBP, and higher total cholesterol levels. Although no significant difference in TMAO levels [median (IQR)] was observed between the NC and MCI groups [3.84 (2.24, 7.06) vs. 4.40 (2.14, 8.08), $p=0.084$], quartile transformation revealed that TMAO levels were significantly higher in the MCI group ($p=0.012$). In contrast, levels of choline, betaine, and carnitine were consistently higher in the MCI group, regardless of whether they were analyzed as continuous or categorical variables (see Table 1 and Figure 2).

3.2 Association between TMAO and its precursors with MCI

As shown in Table 2, the prevalence of MCI exhibited a distinct trend across quartiles for TMAO, betaine, carnitine, and choline.

TABLE 1 Demographic and biochemical characteristics of the participants between controls and cases of MCI at baseline.

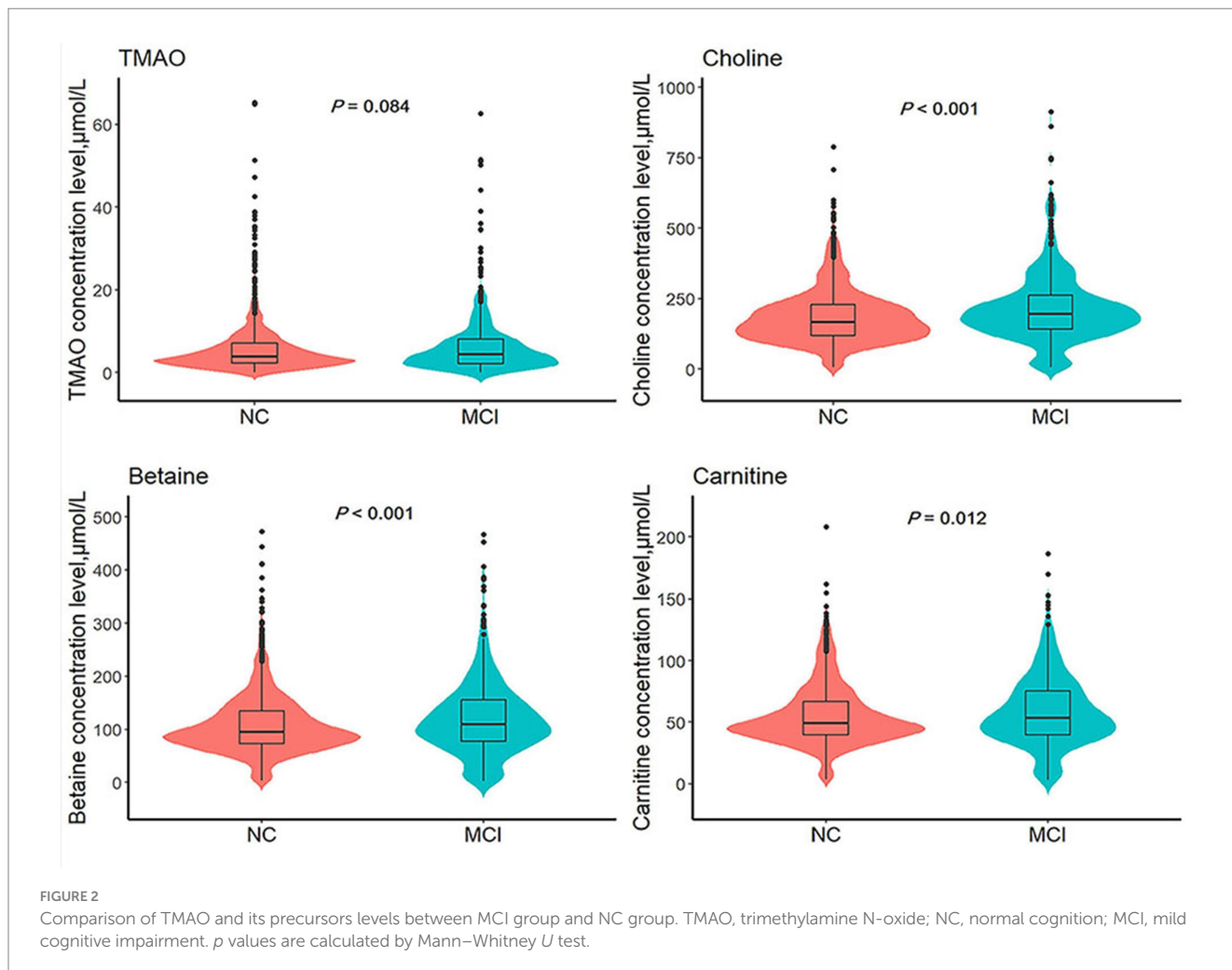
	Total	NC	MCI	<i>p</i>
	<i>n</i> = 1,535	<i>n</i> = 1,005	<i>n</i> = 530	
Age ^a , y	58.6 ± 9.4	57.4 ± 9.7	60.8 ± 8.5	<0.001
Female, <i>n</i> (%)	1,038 (67.6)	708 (70.4)	330 (62.3)	0.001
BMI ^a , kg/m ²	24.9 ± 3.7	25.1 ± 3.8	24.5 ± 3.5	0.004
Education level, <i>n</i> (%)				0.006
Illiterate or primary school	591 (38.5)	360 (35.8)	231 (43.6)	
Junior high school	690 (45.0)	464 (46.2)	226 (42.6)	
Tertiary high school or higher	254 (16.5)	181 (18.0)	73 (13.8)	
Ethnicity, <i>n</i> (%)				0.780
Han ethnicity	992 (64.6)	646 (64.3)	346 (65.3)	
Mongolian	487 (31.7)	320 (31.8)	167 (31.5)	
Others	56 (3.6)	39 (3.9)	17 (3.2)	
Income, <i>n</i> (%)				0.940
<10,000 yuan	1,099 (71.6)	721 (71.7)	378 (71.3)	
10,000–30,000 yuan	361 (23.5)	234 (23.3)	127 (24.0)	
≥30,000 yuan	75 (4.9)	50 (5.0)	25 (4.7)	
Physical labor level, <i>n</i> (%)				0.156
Low	421 (27.4)	263 (26.2)	158 (29.8)	
Moderate	1,050 (68.4)	695 (69.2)	355 (67.0)	
High	64 (4.2)	47 (4.7)	17 (3.2)	
Smoker, <i>n</i> (%)	496 (32.3)	302 (30.0)	194 (36.6)	0.011
Drinker, <i>n</i> (%)	311 (20.3)	190 (18.9)	121 (22.8)	0.080
Fasting glucose ^b , mmol/L	5.5 [5.1,6.0]	5.4 [5.1,6.0]	5.6 [5.2,6.1]	0.001
SBP ^a , mmHg	133.5 ± 20.3	132.4 ± 20.5	135.8 ± 20.0	0.002
DBP ^a , mmHg	80.3 ± 10.7	80.1 ± 10.9	80.7 ± 10.5	0.372
LDL-C ^a , mmol/L	3.3 ± 0.8	3.3 ± 0.8	3.3 ± 0.8	0.259
HDL-C ^a , mmol/L	1.2 ± 0.2	1.2 ± 0.2	1.2 ± 0.3	0.696
Triglyceride ^b , mmol/L	1.3 [0.9,1.9]	1.3 [0.9,1.8]	1.3 [0.9,2.0]	0.711
Total cholesterol ^b , mmol/L	5.2 ± 1.0	5.2 ± 0.9	5.3 ± 1.0	0.041
TMAO ^b , μmol/L	4.05 [2.20,7.43]	3.84 [2.24,7.06]	4.40 [2.14,8.08]	0.084
Choline ^b , μmol/L	179.12 [125.44,239.80]	167.52 [118.79,229.72]	195.34 [142.88,262.14]	<0.001
Betaine ^b , μmol/L	99.40 [74.26,140.99]	95.57 [73.45,134.56]	109.72 [76.96,155.72]	<0.001
Carnitine ^b , μmol/L	50.43 [39.91,69.81]	49.47 [39.96,66.83]	53.71 [39.78,75.39]	0.012
TMAO, <i>n</i> (%)				0.012
Q1 (<2.24 μmol/L)	394 (25.7)	253 (25.2)	141 (26.6)	
Q2 (2.24–4.26 μmol/L)	403 (26.3)	289 (28.8)	114 (21.5)	
Q3 (4.27–7.75 μmol/L)	382 (24.9)	247 (24.6)	135 (25.5)	
Q4 (>7.75 μmol/L)	356 (23.2)	216 (21.5)	140 (26.4)	
Choline, <i>n</i> (%)				<0.001
Q1(<118.11 μmol/L)	414 (27.0)	306 (30.4)	108 (20.4)	
Q2(118.11–162.40 μmol/L)	390 (25.4)	260 (25.9)	130 (24.5)	
Q3(162.41–270.17 μmol/L)	387 (25.2)	245 (24.4)	142 (26.8)	
Q4(>270.17 μmol/L)	344 (22.4)	194 (19.3)	150 (28.3)	

(Continued)

TABLE 1 (Continued)

	Total	NC	MCI	<i>p</i>
	<i>n</i> = 1,535	<i>n</i> = 1,005	<i>n</i> = 530	
Betaine, <i>n</i>(%)				
Q1(<69.24 μmol/L)	404 (26.3)	276 (27.5)	128 (24.2)	<0.001
Q2(69.24–92.50 μmol/L)	407 (26.5)	296 (29.5)	111 (20.9)	
Q3(92.51–119.67 μmol/L)	377 (24.6)	237 (23.6)	140 (26.4)	
Q4(>119.67 μmol/L)	347 (22.6)	196 (19.5)	151 (28.5)	
Carnitine, <i>n</i> (%)				
Q1(<40.09 μmol/L)	392 (25.5)	257 (25.6)	135 (25.5)	<0.001
Q2(40.09–51.18 μmol/L)	408 (26.6)	300 (29.9)	108 (20.4)	
Q3(51.19–70.90 μmol/L)	367 (23.9)	231 (23.0)	136 (25.7)	
Q4(>70.90 μmol/L)	368 (24.0)	217 (21.6)	151 (28.5)	

NC, normal cognition; MCI, mild cognitive impairment; BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TMAO, trimethylamine N-oxide; Ref, reference. Data are mean (SD), median (interquartile range), or *n* (%); *Mean (SD). ^aMedian (interquartile range). *p* values of categorical variables are calculated by χ^2 test, and continuous variables are calculated by student's *t*-test or Mann–Whitney *U* test.



Following adjustment for pertinent confounding variables, with Q2 serving as the reference for TMAO, participants in the Q1 group faced a 50.4% higher MCI risk (OR: 1.504, 95% CI: 1.107–2.045, *p* = 0.009).

Similarly, elevated MCI risks were evident in Q3 (OR: 1.406, 95% CI: 1.031–1.917, *p* = 0.031) and Q4 (OR: 1.685, 95% CI: 1.232–2.303, *p* = 0.001) groups for TMAO. Concerning betaine and carnitine,

TABLE 2 Association between serum TMAO and its precursors levels and prevalent MCI.

	Total	MCI <i>n</i> (%)	Model 1		Model 2	
			OR (95% CI)	<i>P</i> value	OR (95% CI)	<i>P</i> value
TMAO, $\mu\text{mol/L}$						
Q1(<2.24)	394	141 (35.8)	1.413 (1.048, 1.905)	0.023	1.504 (1.107,2.045)	0.009
Q2(2.24–4.26)	403	114 (28.3)	1.000 (Ref.)		1.000 (Ref.)	
Q3(4.27–7.75)	382	135 (35.3)	1.386 (1.025, 1.873)	0.034	1.406 (1.031,1.917)	0.031
Q4(>7.75)	356	140 (39.3)	1.643 (1.213, 2.226)	0.001	1.685 (1.232,2.303)	0.001
<i>P</i> trend				0.012		0.008
Choline, $\mu\text{mol/L}$						
Q1(<118.11)	414	108 (26.1)	1.000 (Ref.)		1.000 (Ref.)	
Q2(118.11–162.40)	390	130 (33.3)	1.417 (1.045, 1.920)	0.025	1.555 (1.139,2.124)	0.005
Q3(162.41–270.17)	387	142 (36.7)	1.642 (1.215, 2.220)	0.001	2.040 (1.489,2.794)	<0.001
Q4(>270.17)	344	150 (43.6)	2.191 (1.613, 2.974)	<0.001	2.711 (2.012,3.817)	<0.001
<i>P</i> trend				<0.001		<0.001
Betaine, $\mu\text{mol/L}$						
Q1(<69.24)	404	128 (31.7)	1.237 (0.914, 1.674)	0.169	1.275 (0.933,1.741)	0.127
Q2(69.24–92.50)	407	111 (27.3)	1.000 (Ref.)		1.000 (Ref.)	
Q3(92.51–119.67)	377	140 (37.1)	1.575 (1.165, 2.131)	0.003	1.711 (1.251,2.339)	0.001
Q4(>119.67)	347	151 (43.5)	2.054 (1.515, 2.785)	<0.001	2.367 (1.722,3.255)	<0.001
<i>P</i> trend				<0.001		<0.001
Carnitine, $\mu\text{mol/L}$						
Q1(<40.09)	392	135 (34.4)	1.459 (1.078, 1.975)	0.015	1.474 (1.077,2.017)	0.015
Q2(40.09–51.18)	408	108 (26.5)	1.000 (Ref.)		1.000 (Ref.)	
Q3(51.19–70.90)	367	136 (37.1)	1.645 (1.205, 2.219)	0.002	1.768 (1.289,2.425)	<0.001
Q4(>70.90)	368	151 (41.0)	1.933 (1.428, 2.616)	<0.001	2.239 (1.742,3.295)	<0.001
<i>P</i> trend				<0.001		<0.001

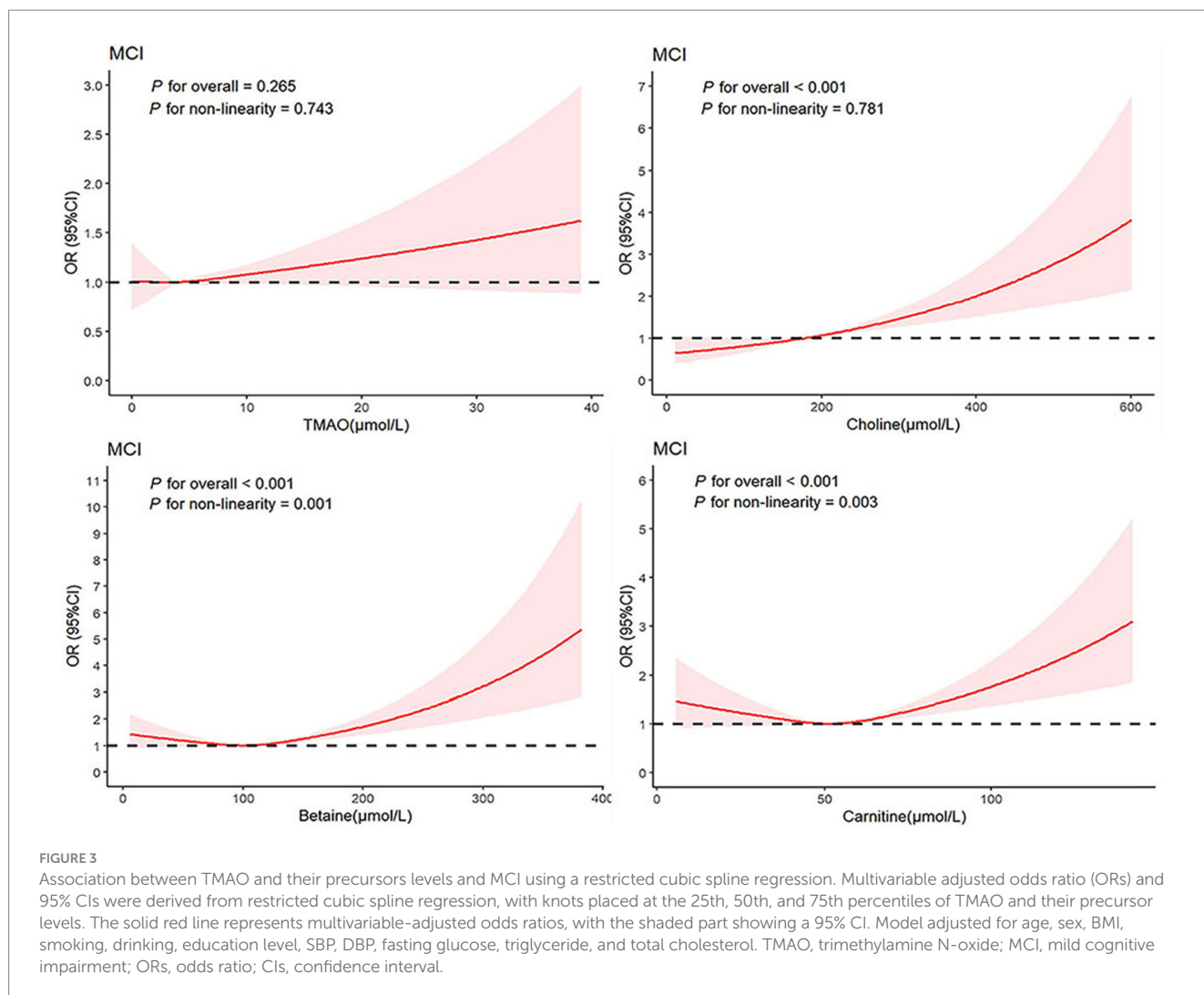
OR, odds ratio; CI, confidence interval; TMAO, trimethylamine N-oxide; Ref, reference; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MCI, mild cognitive impairment. Model 1: unadjusted; Model 2: adjusted for age, sex, BMI, smoking, drinking, education level, SBP, DBP, fasting glucose, triglyceride, and total cholesterol. ORs and CIs were calculated using binary logistic regression models.

individuals within the Q4 group exhibited a 136.7% higher risk of MCI for betaine (OR: 2.367, 95% CI: 1.722–3.255, $p < 0.001$) and a 123.9% higher risk of MCI for carnitine (OR: 2.239, 95% CI: 1.742–3.295, $p < 0.001$) compared to those in the Q2 group. The risk of MCI associated with betaine and carnitine levels corresponded consistently with that of TMAO. These findings suggest an optimal concentration range for TMAO, betaine, and carnitine in MCI prevention, with concentrations below or above this range potentially escalating MCI risk. In contrast, choline showcased a distinctive pattern, with progressively escalating MCI risk across quartiles. Compared to the lowest Q1 group, the MCI risk surged in the Q2 (OR: 1.555, 95% CI: 1.139–2.124, $p = 0.005$), Q3 (OR: 2.040, 95% CI: 1.489–2.794, $p < 0.001$), and Q4 (OR: 2.711, 95% CI: 2.012–3.817, $p < 0.001$) groups in the adjusted model.

Additionally, employing multivariable-adjusted restricted cubic spline plots (refer to Figure 3), no significant association was observed between TMAO and MCI ($P_{\text{overall}} = 0.265$). While choline exhibited a linear relationship with MCI ($P_{\text{non-linear}} = 0.781$), both betaine ($P_{\text{non-linear}} = 0.001$) and carnitine ($P_{\text{non-linear}} = 0.003$) showcased a J-shaped association with MCI, indicating complex dynamics.

3.3 Association between TMAO and its precursors with $\Delta\text{MoCA-BC}$

The $\Delta\text{MoCA-BC}$ value (mean \pm SD) was -1.6 ± 4.5 points over a two-year follow-up period. Analysis presented in Figure 4 revealed significant variations in $\Delta\text{MoCA-BC}$ across different levels of TMAO, choline, betaine, and carnitine. Utilizing a generalized linear model, we examined the complex relationship between TMAO and its precursor levels and $\Delta\text{MoCA-BC}$ (see Table 3). Relative to the Q2 group, the $\Delta\text{MoCA-BC}$ demonstrated a significant increase of 0.682 in the Q1 group for TMAO levels, with similar increases of 0.679 and 0.976 observed in the Q3 and Q4 groups, respectively, all of which were statistically significant. These results highlight the intricate interplay between TMAO levels and alterations in neurocognitive function, suggesting the existence of an optimal range of TMAO levels that exerts minimal impact on $\Delta\text{MoCA-BC}$. Furthermore, $\Delta\text{MoCA-BC}$ increased by 0.903 for betaine and 1.301 for carnitine in the Q4 group compared to the Q2 group. In contrast, choline exhibited a significant positive increase in $\Delta\text{MoCA-BC}$ of 0.777 and 1.384 for the Q3 and Q4 groups, respectively, when compared to the lowest Q1 group.



3.4 Subgroup analysis

Subgroup analysis further delineated the impact of TMAO and its precursors on MCI risk (refer to [Supplementary Figures S1–S4](#)), stratified by gender (male, female) and age (< 60 years, ≥ 60 years), and no interaction was found between TMAO and its precursors and gender ($P_{\text{interaction}} > 0.05$). In males, the MCI risk in the Q1 group for TMAO was higher than that in the Q2 group, with an OR of 2.069 (95% CI: 1.219–3.513), while there was no significant difference in females. Conversely, no significant difference was observed in females. These findings were consistent with the results of the unstratified analysis. Furthermore, the association between MCI risk and the levels of TMAO and its precursors was statistically significant in the cohort aged 60 years and older.

4 Discussion

This study demonstrates that both low and high levels of TMAO, betaine, and carnitine are associated with an elevated risk of MCI, whereas moderate concentrations (Q2 group) of these metabolites may confer protective effects. Furthermore, elevated levels of TMAO and its precursors correlate with an increase in $\Delta\text{MoCA-BC}$ score. The

observed bidirectional relationship between TMAO, betaine, carnitine, and MCI risk may be influenced by various factors. The dosage of TMAO could also play a role in cognitive impairment, as typical fasting blood levels in the general population range from 3.6 to 3.7 $\mu\text{mol/L}$ (28), which falls within the Q2 concentration range identified in our study. Animal studies have indicated that TMAO at physiological concentrations can significantly enhance blood–brain barrier integrity following acute inflammation in murine models, thereby improving cognitive function (29). Additionally, TMAO has been shown to mitigate neurological dysfunction in diabetic rat models (20). These findings from animal studies support the notion that TMAO may exert beneficial effects on cognitive function at specific concentration ranges. This suggests that TMAO and its precursors may provide protective effects through the activation of innate defense mechanisms or the facilitation of repair processes at optimal concentrations. Previous research has highlighted that this protective role of TMAO may be mediated through antioxidant pathways. For instance, TMAO can activate the Nrf2 signaling pathway, leading to increased expression of antioxidant genes, reduced muscle cell damage, and enhanced protection of neurons against oxidative stress (30).

Elevated concentrations of TMAO have been identified as a potential risk factor for cognitive impairment, a conclusion

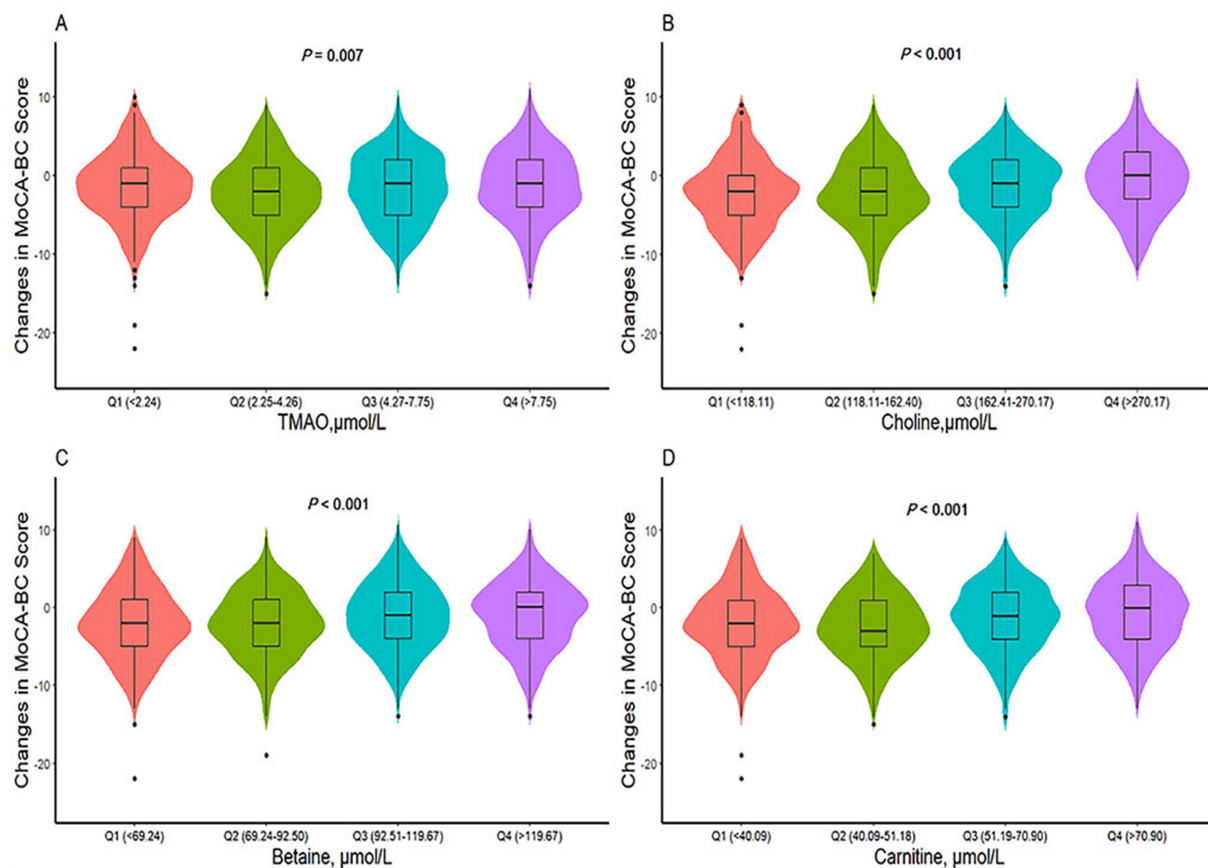


FIGURE 4

Differences in Δ MoCA-BC score of TMAO and its precursors levels among different groups. TMAO, trimethylamine N-oxide; MoCA-BC, the Chinese version of the Montreal Cognitive Assessment-Basic. *p* values are calculated by Kruskal-Wallis test. (A) Differences in Δ MoCA-BC score of TMAO levels among different groups. (B) Differences in Δ MoCA-BC score of choline levels among different groups. (C) Differences in Δ MoCA-BC score of betaine levels among different groups. (D) Differences in Δ MoCA-BC score of carnitine levels among different groups.

supported by numerous studies. Specifically, high TMAO levels serve as a significant independent risk factor for MCI in patients with type 2 diabetes (11). A cross-sectional study has demonstrated increased TMAO levels in the cerebrospinal fluid of individuals with MCI, revealing a direct correlation between TMAO concentrations and markers of cognitive dysfunction and neurodegeneration (31). Furthermore, prospective cohort studies indicate that elevated baseline plasma TMAO levels are significantly associated with cognitive dysfunction following transient ischemic attacks (32). These findings are consistent with our observations that high TMAO levels exacerbate cognitive impairment, thereby increasing the risk of MCI. Investigations into the mechanisms underlying TMAO-induced MCI suggest that elevated TMAO levels may initiate inflammatory cascades, particularly through the activation of the NF- κ B pathway, which heightens the risk of neuroinflammation when the integrity of the blood-brain barrier is compromised, ultimately contributing to cognitive decline (33–35). Additionally, elevated TMAO levels may accelerate brain aging by promoting mitochondrial damage and increasing superoxide production, thereby exacerbating age-related cognitive dysfunction (19, 36, 37). Collectively, these findings underscore the detrimental impact of elevated TMAO concentrations on cognitive function and emphasize the necessity for precise regulation of TMAO and its precursors to preserve cognitive health.

We initially identified a J-shaped association between serum levels of betaine and carnitine and the risk of MCI, suggesting that deviations from optimal concentrations may elevate the risk of MCI. This observation may imply that betaine and carnitine operate through distinct metabolic pathways or signaling mechanisms at varying concentration levels. However, existing literature indicates a negative correlation between plasma betaine levels and cognitive impairment in stroke patients (38). Chen et al. reported an inverse relationship between elevated plasma carnitine levels and cognitive impairment 3 months post-stroke, which is not entirely congruent with our findings (39). Research has demonstrated that the metabolism of betaine and carnitine is significantly influenced by dietary intake and gut microbiota (16). Consequently, their serum levels and the associated relationship with cognitive function may be modulated by a variety of factors. Both excessively low and high concentrations may disrupt metabolic equilibrium and adversely affect cognitive function. Additionally, the positive correlation between choline levels and the risk of MCI was corroborated in this study, aligning with previous research that has reported elevated choline levels in patients with MCI or Alzheimer's disease (AD) (40, 41). While some studies indicate that increased choline levels may confer protective effects on cognitive function under specific conditions, they may also exacerbate neuroinflammation and elevate the risk of MCI, particularly in the context of metabolic imbalances or dysbiosis of the gut microbiota (42, 43).

TABLE 3 Association of TMAO and its precursors levels on changes in MoCA-BC score.

	β	SE	95% CI	p
TMAO, $\mu\text{mol/L}$				
Q1 (<2.24)	0.682	0.322	(0.052, 1.313)	0.034
Q2 (2.24–4.26)	0.000 (Ref.)			
Q3 (4.27–7.75)	0.679	0.327	(0.039, 1.319)	0.038
Q4 (>7.75)	0.976	0.341	(0.299, 1.635)	0.005
Choline, $\mu\text{mol/L}$				
Q1 (<118.11)	0.000 (Ref.)			
Q2 (118.11–162.40)	0.088	0.320	(–0.539, 0.714)	0.784
Q3 (162.41–270.17)	0.777	0.331	(0.128, 1.426)	0.019
Q4 (>270.17)	1.384	0.350	(0.698, 2.070)	<0.001
Betaine, $\mu\text{mol/L}$				
Q1 (<69.24)	–0.009	0.319	(–0.635, 0.617)	0.978
Q2 (69.24–92.50)	0.000 (Ref.)			
Q3 (92.51–119.67)	0.932	0.329	(0.281, 1.583)	0.005
Q4 (>119.67)	0.903	0.346	(0.216, 1.590)	0.010
Carnitine, $\mu\text{mol/L}$				
Q1 (<40.09)	0.455	0.326	(–0.183, 1.093)	0.163
Q2 (40.09–51.18)	0.000 (Ref.)			
Q3 (51.19–70.90)	0.872	0.330	(0.226, 1.518)	0.008
Q4 (>70.90)	1.301	0.341	(0.632, 1.971)	<0.001

TMAO, trimethylamine N-oxide; MoCA-BC, the Chinese version of the Montreal Cognitive Assessment; SE, standard Error; CIs, confidence interval. Model adjusted for age, sex, BMI, smoking, drinking, education level, SBP, DBP, fasting glucose, triglyceride, and total cholesterol. Data were calculated using a generalized linear model.

In our study, the observed average decline of 1.6 points in the MoCA-BC score during the follow-up period underscores the dynamic nature of cognitive function and emphasizes the necessity of longitudinal assessments for monitoring cognitive decline. Consistent with prior research on MCI and cerebral small vessel disease, we identified that TMAO levels outside the range of 2.24–4.26 $\mu\text{mol/L}$ were associated with an increase in $\Delta\text{MoCA-BC}$ (44), suggesting that TMAO may exert a beneficial effect on cognitive function when present at concentrations outside its optimal range. Additionally, the correlation between elevated levels of choline, betaine, and carnitine and cognitive improvements further supports the potential influence of these metabolites on cognitive function. However, the variability in findings from prospective cohort studies may be attributable to differences in baseline MoCA-BC scores and shorter follow-up durations, necessitating careful interpretation of our results.

This study, conducted on a large cohort from rural Northeast China, provides comprehensive measurements of serum concentrations of choline, betaine, and carnitine, thereby contributing to the population-based evidence regarding the association between TMAO precursors and MCI. It represents the first longitudinal investigation into the relationship between TMAO and its precursors with changes in $\Delta\text{MoCA-BC}$. However, several limitations should be acknowledged. Firstly, the relatively small sample size and the brief follow-up period may hinder the assessment of the long-term effects of TMAO and its precursors on cognitive function. Secondly, the exclusive reliance on the MoCA-BC score for defining MCI may have led to an inflated prevalence rate while compromising specificity. Additionally, the regional and lifestyle characteristics of the sample

may restrict the generalizability of the findings. Future research should focus on validating these findings in larger and more diverse populations, as well as expanding animal studies to elucidate the mechanistic pathways linking TMAO, betaine, carnitine, and choline to MCI. Such investigations will contribute to improved strategies for the prevention and management of MCI.

5 Conclusion

In a large-sample population study conducted in rural areas of Liaoning Province, we found that serum levels of TMAO, choline, betaine, and carnitine are not only associated with MCI but also correlated with changes in $\Delta\text{MoCA-BC}$. Our analysis revealed a J-shaped relationship between betaine and carnitine levels and the incidence of MCI, suggesting the existence of an optimal concentration range for serum levels of TMAO, betaine, and carnitine that may minimize the risk of MCI. Furthermore, elevated levels of TMAO and its precursors were linked to an increase in $\Delta\text{MoCA-BC}$. Future research should focus on the dynamic changes in neurocognitive function, and dietary guidance may play a crucial role in the prevention and management of MCI.

Data availability statement

The original contributions presented in the study are included in the article/[Supplementary material](#), further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by responsible committee on human experimentation of China Medical University. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

HB: Data curation, Formal analysis, Methodology, Software, Writing – original draft, Writing – review & editing. YZ: Data curation, Methodology, Resources, Writing – original draft. PT: Data curation, Methodology, Resources, Writing – original draft. YW: Data curation, Methodology, Software, Writing – original draft. RP: Data curation, Methodology, Software, Writing – original draft. BL: Methodology, Writing – original draft. WR: Methodology, Software, Writing – original draft. EC: Methodology, Software, Writing – original draft. YL: Methodology, Resources, Writing – review & editing. MM: Methodology, Resources, Writing – review & editing. LZ: Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This research was supported by funds from the National Nature Science Foundation of China [no. 82073645]; and from Key Research and Development Project for the Introduction of

References

1. GBD 2019 Dementia Forecasting Collaborators. Estimation of the global prevalence of dementia in 2019 and forecasted prevalence in 2050: an analysis for the global burden of disease study 2019. *Lancet Public Health*. (2022) 7:e105–25. doi: 10.1016/s2468-2667(21)00249-8
2. Jia L, Quan M, Fu Y, Zhao T, Li Y, Wei C, et al. Dementia in China: epidemiology, clinical management, and research advances. *Lancet Neurol*. (2020) 19:81–92. doi: 10.1016/s1474-4422(19)30290-x
3. Tahir MS, Almezgagi M, Zhang Y, Bashir A, Abdullah HM, Gamah M, et al. Mechanistic new insights of Flavonols on neurodegenerative diseases. *Biomed Pharmacother*. (2021) 137:111253. doi: 10.1016/j.biopha.2021.111253
4. Jia L, Du Y, Chu L, Zhang Z, Li F, Lyu D, et al. Prevalence, risk factors, and Management of Dementia and Mild Cognitive Impairment in adults aged 60 years or older in China: a cross-sectional study. *Lancet Public Health*. (2020) 5:e661–71. doi: 10.1016/S2468-2667(20)30185-7
5. Alzheimer's Disease. 2022 Alzheimer's disease facts and figures. *Alzheimers Dement*. (2022) 18:700–89. doi: 10.1002/alz.12638
6. Petersen RC, Lopez O, Armstrong MJ, Getchius TSD, Ganguli M, Gloss D, et al. Practice guideline update summary: mild cognitive impairment: report of the guideline development, dissemination, and implementation Subcommittee of the American Academy of neurology. *Neurology*. (2018) 90:126–35. doi: 10.1212/wnl.00000000000004826
7. Livingston G, Huntley J, Sommerlad A, Ames D, Ballard C, Banerjee S, et al. Dementia prevention, intervention, and care: 2020 report of the lancet commission. *Lancet*. (2020) 396:413–46. doi: 10.1016/s0140-6736(20)30367-6
8. Hendriks S, Ranson JM, Peetoom K, Lourida I, Tai XY, de Vugt M, et al. Risk factors for young-onset dementia in the Uk biobank. *JAMA Neurol*. (2024) 81:134–42. doi: 10.1001/jamaneurol.2023.4929

High-level Talent in Lvliang City [no. 2019115] and [no. 2022RC27].

Acknowledgments

We would like to express our heartfelt gratitude to all the investigators, staff members, and dedicated participants for their invaluable contributions. This article used Open AI's ChatGPT version 3.5 for language touch-ups.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fnut.2024.1461942/full#supplementary-material>

9. Zeisel SH, Warriner M. Trimethylamine N-oxide, the microbiome, and heart and kidney disease. *Annu Rev Nutr*. (2017) 37:157–81. doi: 10.1146/annurev-nutr-071816-064732
10. Zhou S, Liu J, Sun Y, Xu P, Liu JL, Sun S, et al. Dietary choline metabolite Tmao impairs cognitive function and induces hippocampal synaptic plasticity declining through the Mtor/P70s6k/4ebp1 pathway. *Food Funct*. (2023) 14:2881–95. doi: 10.1039/d2fo03874a
11. Xu N, Wan J, Wang C, Liu J, Qian C, Tan H. Increased serum trimethylamine N-oxide level in type 2 diabetic patients with mild cognitive impairment. *Diabetes Metab Syndr Obes*. (2022) 15:2197–205. doi: 10.2147/dmso.S370206
12. Bennett BJ, de Aguiar Vallim TQ, 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 Metab*. (2013) 17:49–60. doi: 10.1016/j.cmet.2012.12.011
13. Fennema D, Phillips IR, Shephard EA. Trimethylamine and trimethylamine N-oxide, a Flavin-containing monooxygenase 3 (Fmo3)-mediated host-microbiome metabolic Axis implicated in health and disease. *Drug Metab Dispos*. (2016) 44:1839–50. doi: 10.1124/dmd.116.070615
14. Zhu Y, Jameson E, Crosatti M, Schäfer 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. doi: 10.1073/pnas.1316569111
15. Socola K, Doboszewska U, Szopa A, Serefko A, Włodarczyk M, Zielińska A, et al. The role of microbiota-gut-brain Axis in neuropsychiatric and neurological disorders. *Pharmacol Res*. (2021) 172:105840. doi: 10.1016/j.phrs.2021.105840
16. Liu D, Gu S, Zhou Z, Ma Z, Zuo H. Associations of plasma Tmao and its precursors with stroke risk in the general population: a nested case-control study. *J Intern Med*. (2023) 293:110–20. doi: 10.1111/joim.13572

17. Zhang Y, Jian W. Signal pathways and intestinal Flora through trimethylamine N-oxide in Alzheimer's disease. *Curr Protein Pept Sci.* (2023) 24:721–36. doi: 10.2174/1389203724666230717125406
18. Mueed Z, Mehta D, Rai PK, Kamal MA, Poddar NK. Cross-interplay between Osmolytes and Mtor in Alzheimer's disease pathogenesis. *Curr Pharm Des.* (2020) 26:4699–711. doi: 10.2174/1381612826666200518112355
19. Li D, Ke Y, Zhan R, Liu C, Zhao M, Zeng A, et al. *Aging Cell.* (2018) 17:e12768. doi: 10.1111/acel.12768
20. Lupachyk S, Watcho P, Stavniichuk R, Shevalye H, Obrosova IG. Endoplasmic reticulum stress plays a key role in the pathogenesis of diabetic peripheral neuropathy. *Diabetes.* (2013) 62:944–52. doi: 10.2337/db12-0716
21. Hoyle 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. doi: 10.1186/s40168-021-01181-z
22. Feng W, Chen Q, Ma M, Xu J, Guo H, Yuan W, et al. Sex-modified association between grip strength and mild cognitive impairment: a cross-sectional and follow-up study in rural China. *BMC Geriatr.* (2023) 23:710. doi: 10.1186/s12877-023-04376-1
23. Xu J, Sun Z, Zhang R, Li R, Yu Z, Zhang Q, et al. Non-linear association between serum spermidine and mild cognitive impairment: results from a cross-sectional and longitudinal study. *Front Aging Neurosci.* (2022) 14:14. doi: 10.3389/fnagi.2022.924984
24. Gu C, Ma M, Xu J, Yuan W, Li R, Guo H, et al. Association between pulmonary Ventilatory function and mild cognitive impairment: a population-based study in rural China. *Front Public Health.* (2022) 10:10. doi: 10.3389/fpubh.2022.1038576
25. Chen KL, Xu Y, Chu AQ, Ding D, Liang XN, Nasreddine ZS, et al. Validation of the Chinese version of Montreal cognitive assessment basic for screening mild cognitive impairment. *J Am Geriatr Soc.* (2016) 64:e285–90. doi: 10.1111/jgs.14530
26. Trzepacz PT, Hochstetler H, Wang S, Walker B, Saykin AJ. Relationship between the Montreal cognitive assessment and Mini-mental state examination for assessment of mild cognitive impairment in older adults. *BMC Geriatr.* (2015) 15:107. doi: 10.1186/s12877-015-0103-3
27. Huang L, Chen KL, Lin BY, Tang L, Zhao QH, Lv YR, et al. Chinese version of Montreal cognitive assessment basic for discrimination among different severities of Alzheimer's disease. *Neuropsychiatr Dis Treat.* (2018) 14:2133–40. doi: 10.2147/ndt.S174293
28. Kühn T, Rohrmann S, Sookthai D, Johnson T, Katzke V, Kaaks R, et al. Intra-individual variation of plasma trimethylamine-N-oxide (Tmao), betaine and choline over 1 year. *Clin Chem Lab Med.* (2017) 55:261–8. doi: 10.1515/cclm-2016-0374
29. Long C, Li Z, Feng H, Jiang Y, Pu Y, Tao J, et al. Association of Trimethylamine Oxide and its Precursors with cognitive impairment: a systematic review and Meta-analysis. *Front Aging Neurosci.* (2024) 16:1465457. doi: 10.3389/fnagi.2024.1465457
30. Zou H, Zhou Y, Gong L, Huang C, Liu X, Lu R, et al. Trimethylamine N-oxide improves exercise performance by reducing oxidative stress through activation of the Nrf2 signaling pathway. *Molecules.* (2024) 29:759. doi: 10.3390/molecules29040759
31. 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. doi: 10.1186/s13195-018-0451-2
32. Wang L, Zhan X, Jiang L, Xu G, Bao Y, Wang J, et al. Association between plasma trimethylamine-N-oxide and cognitive impairment in patients with transient ischemic attack. *Neurol Res.* (2023) 45:634–45. doi: 10.1080/01616412.2023.2176632
33. Lanz M, Janeiro MH, Milagro FI, Puerta E, Ludwig IA, Pineda-Lucena A, et al. Trimethylamine N-oxide (Tmao) drives insulin resistance and cognitive deficiencies in a senescence accelerated mouse model. *Mech Ageing Dev.* (2022) 204:111668. doi: 10.1016/j.mad.2022.111668
34. Fu BC, Hullar MAJ, Randolph TW, Franke AA, Monroe KR, Cheng I, et al. Associations of plasma trimethylamine N-oxide, choline, carnitine, and betaine with inflammatory and Cardiometabolic risk biomarkers and the fecal microbiome in the multiethnic cohort adiposity phenotype study. *Am J Clin Nutr.* (2020) 111:1226–34. doi: 10.1093/ajcn/nqaa015
35. Manor O, Zubair N, Conomos MP, Xu X, Rohwer JE, Krafft CE, et al. A multi-Omic association study of trimethylamine N-oxide. *Cell Rep.* (2018) 24:935–46. doi: 10.1016/j.celrep.2018.06.096
36. Ibi D, Tsuchihashi A, Nomura T, Hiramatsu M. Involvement of Gat2/Bgt-1 in the preventive effects of betaine on cognitive impairment and brain oxidative stress in amyloid B peptide-injected mice. *Eur J Pharmacol.* (2019) 842:57–63. doi: 10.1016/j.ejphar.2018.10.037
37. Ibi D, Hirashima K, Kojima Y, Sumiya K, Kondo S, Yamamoto M, et al. Preventive effects of continuous betaine intake on cognitive impairment and aberrant gene expression in Hippocampus of 3xtg mouse model of Alzheimer's disease. *J Alzheimers Dis.* (2021) 79:639–52. doi: 10.3233/jad-200972
38. Zhong C, Lu Z, Che B, Qian S, Zheng X, Wang A, et al. Choline pathway nutrients and metabolites and cognitive impairment after acute ischemic stroke. *Stroke.* (2021) 52:887–95. doi: 10.1161/strokeaha.120.031903
39. Che B, Chen H, Wang A, Peng H, Bu X, Zhang J, et al. Association between plasma L-carnitine and cognitive impairment in patients with acute ischemic stroke. *J Alzheimers Dis.* (2022) 86:259–70. doi: 10.3233/jad-215376
40. González-Domínguez R, García-Barrera T, Gómez-Ariza JL. Combination of Metabolomic and phospholipid-profiling approaches for the study of Alzheimer's disease. *J Proteome.* (2014) 104:37–47. doi: 10.1016/j.jprot.2014.01.014
41. Peña-Bautista C, Roca M, Hervás D, Cuevas A, López-Cuevas R, Vento M, et al. Plasma metabolomics in early Alzheimer's disease patients diagnosed with amyloid biomarker. *J Proteome.* (2019) 200:144–52. doi: 10.1016/j.jprot.2019.04.008
42. Nurk E, Refsum H, Bjelland I, Drevon CA, Tell GS, Ueland PM, et al. Plasma free choline, betaine and cognitive performance: the Hordaland health study. *Br J Nutr.* (2013) 109:511–9. doi: 10.1017/s0007114512001249
43. Judd JM, Jasbi P, Winslow W, Serrano GE, Beach TG, Klein-Seetharaman J, et al. Inflammation and the pathological progression of Alzheimer's disease are associated with low circulating choline levels. *Acta Neuropathol.* (2023) 146:565–83. doi: 10.1007/s00401-023-02616-7
44. Salvadori E, Poggese A, Pracucci G, Chiti A, Ciolli L, Del Bene A, et al. Longitudinal changes in Moca performances in patients with mild cognitive impairment and small vessel disease. Results from the Vmci-Tuscany study. *Cereb Circ Cogn Behav.* (2021) 2:100008. doi: 10.1016/j.cccb.2021.100008