

# Relationship between blood levels of methyl donor and folate and mild cognitive impairment in Chinese patients with type 2 diabetes: a case-control study

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**Type 2 diabetes is a risk factor for Alzheimer's disease and mild cognitive impairment. Folate insufficiency fosters a decline in the sole methyl donor, S-adenosylmethionine, and decreases methylation potential, which is associated with Alzheimer's disease in non-diabetic patients. However, little is known in diabetic patients. We analyzed plasma levels of S-adenosylmethionine, S-adenosylhomocysteine and serum level of folate in 100 elderly type 2 diabetic patients with and without mild cognitive impairment. S-adenosylmethionine/S-adenosylhomocysteine ratio was used to reflect the methylation potential. Patients with mild cognitive impairment had significantly lower levels of S-adenosylmethionine, folate and S-adenosylmethionine/S-adenosylhomocysteineratios. Furthermore, logistic regression analysis indicated the plasma S-adenosylmethionine, S-adenosylmethionine/S-adenosylhomocysteine ratio and serum folate (OR, 0.96, 0.698, 0.72, respectively;  $p < 0.05$ ) were negatively associated with risk of mild cognitive impairment, even after adjusting for related covariates. In addition, folate level was positively correlated with S-adenosylmethionine and the S-adenosylmethionine/S-adenosylhomocysteine ratio ( $r = 0.38, 0.46$ , respectively;  $p < 0.05$ ) among patients within the middle tertile of folate levels (6.3–9.1  $\mu\text{g/L}$ ). These findings indicate mild cognitive impairment is associated with lower levels of S-adenosylmethionine, folate and weakened methylation potential; plasma S-adenosylmethionine and methylation potential may be predicted by serum folate within a suitable range of folate concentrations in diabetic patients.**

**Key Words:** methyl donor, folate, mild cognitive impairment, type 2 diabetes

Alzheimer's disease (AD) is the most common type of dementia in the elderly and characterized by progressive loss of memory and other cognitive and executive functions. Mild cognitive impairment (MCI) represents a unique transitional state between normal aging and AD, and it is now recognized as the prodromal stage of AD.<sup>(1)</sup> Several major prospective epidemiological studies have indicated that type 2 diabetes (T2D) is a risk factor for dementia and MCI independent of the risk for vascular dementia, and the relative risk of AD among patients with T2D was approximately twice that of non-diabetics.<sup>(2,3)</sup> The prevalence of both AD and T2D is increasing at an alarming rate, and as the therapeutic treatment for diabetes advances, patients with T2D will likely live longer. We may soon be facing the daunting challenge of dealing with a new population of dementia sufferers

with T2D. Thus, identifying potentially modifiable risk factors for cognitive decline in people with T2D has become a topic of increasing interest.

The etiology of dementia and cognitive impairment in people with T2D is uncertain, but it is most likely multifactorial. Chronic hyperglycemia, cerebral microvascular disease, severe hypoglycemia, increased inflammatory mediators and an increased prevalence of macrovascular disease have all been implicated but are unlikely to explain the entire effect.<sup>(4)</sup> Recent studies have suggested that DNA methylation, a vital component of epigenetic modification, plays an important role in several neurodegenerative disorders, including AD pathology.<sup>(5,6)</sup> Mastroeni *et al.*<sup>(7,8)</sup> have shown that DNA methylation is significantly reduced in neurons, particularly in tangles containing neurons of entorhinal cortex layer II in AD patients. Recently, it has also been reported that aberrant CpG methylation of several AD-related genes promoter is correlated with the related protein such as amyloid precursor protein, presenile 1, microtubule-associated protein tau over-expression in AD.<sup>(9,10)</sup> DNA methylation is catalyzed by DNA methyltransferases, which add methyl groups to the cytosine located within CpG dinucleotides. Folate is the dietary source of methyl groups for the homocysteine (Hcy) and methionine cycle. The methyl group is required for Hcy conversion to methionine and the formation of S-adenosylmethionine (SAM) by the folate-dependent enzyme, methionine synthase. Several clinical studies have shown that individuals with AD are characterized by decreased plasma folate values as well as increased plasma Hcy levels.<sup>(11,12)</sup> Folate depletion can cause genomic DNA hypomethylation, which can be reversed upon dietary folate restoration.<sup>(13)</sup>

SAM is the universal methyl donor in many important methylation reactions in the nervous system, including DNA methylation and synthesis, activation of neurotransmitters, and membrane phospholipid synthesis.<sup>(14)</sup> By donating its methyl group, SAM is converted to S-adenosylhomocysteine (SAH), which in turn inhibits cellular methylation. Subsequently, SAH can be hydrolyzed by Hcy, and this hydrolyzation is reversible. The SAM/SAH ratio reflects the methylation potential. Previous studies have shown that the SAM level is lower in the cerebrospinal fluid (CSF)<sup>(15)</sup> and within the brains of AD patients.<sup>(16)</sup> Although both plasma levels of SAM and SAH of AD patients are higher than those of control individuals, the plasma SAM/SAH ratio in AD

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patients is lower.<sup>(17)</sup> These studies imply that the methylation potential may be weakened in AD patients. On the other hand, epigenetic mechanisms may play a substantial role in the pathophysiology of diabetes and associated vascular complications.<sup>(18)</sup> Disturbed methyl group metabolism is found in diabetes,<sup>(19)</sup> but little is known about the association between plasma methyl donor levels and methylation potential and cognitive decline in diabetic patients.

Therefore, in the current study, we measured peripheral blood levels of folate, methyl donor SAM, its demethylated product, SAH, and their ratios in elderly patients with T2D with or without MCI, and we analyzed the relationship between blood folate, methyl donor levels and MCI in Chinese patients with T2D.

## Materials and Methods

**Subjects and cognitive assessment.** One hundred elderly, hospitalized patients with T2D ( $\geq 65$  years old) who met the WHO diagnostic criteria<sup>(20)</sup> [50 subjects with MCI and 50 age- ( $\pm 3$  years), sex- and education-matched controlled subjects without MCI] were recruited from the Metabolic Disease Hospital of Tianjin Medical University between June 2012 and December 2012. Exclusion criteria were neurological disease, including epilepsy, Parkinson's disease, or any apparent past history of a cerebrovascular event(s); psychiatric illness, such as depression or substance abuse; visual or hearing disabilities; history of severe hypoglycemia; or a recent acute diabetic complication, such as diabetes ketosis, acute myocardial infarction, or acute or chronic infection within the previous three months; or moderate or severe hepatic (serum alanine or aspartate aminotransferase  $>100$  U/L) or renal disease (estimated creatinine clearance  $<60$  ml/min); or cancer. Subjects with an established diagnosis of dementia were also excluded.

Cognitive function was evaluated by a trained interviewer using the Mini-Mental State Examination (MMSE)<sup>(21)</sup> and a short memory questionnaire.<sup>(22)</sup> MMSE assesses global cortical functions including orientation, attention, memory, calculation, language, and the ability to follow simple verbal and written commands. The short memory questionnaire was used to detect whether the patients had a subjective memory impairment complaint. All the patients were also asked to report whether they had any difficulty with activities of daily living (ADL)<sup>(23)</sup> including 16 items (toileting, eating, dressing, grooming, general movement, walking up and down stairs, bathing, cutting toenails, using the telephone, daily shopping, preparing meals, doing housekeeping, doing laundry, taking the bus, taking medicine, and handling personal finances). According to Petersen's diagnostic standard for MCI,<sup>(24)</sup> the patients who met the following criteria were identified as MCI: (1) subjective memory complaint; (2) objective memory impairment adjusted for age and education; (3) the MMSE scores were above 13 and  $\leq 18$  with no education, or  $\leq 20$  with 1 to 6 years of education, or  $\leq 24$  with more than 6 years of education; (4) no or minimal impairment of ADL and (5) not fulfilling the DSM-III-R (Diagnostic and Statistical Manual of Mental Disorders, 3rd edition, revised) criteria of dementia.<sup>(25)</sup> The MMSE scores of the elderly control subjects were higher than the levels described above, according to the corresponding education level.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human patients were approved by Tianjin Medical University Ethics Committee Review Board (TMUHMEC2012020). Written informed consent was obtained from all patients.

**Socioeconomic, clinical and anthropometric assessment.** Information on marital status (married/divorced or widowed), education (no education/1–6 years/6–12 years/ $>12$  years), family per capita income ( $\leq \text{¥}2,000/\text{¥}2,000\text{--}\text{¥}3,000/\geq \text{¥}3,000$ ), living alone (yes/no), smoking status (yes/no), drinking status (yes/no), reading

(yes/no), habit of regular exercise (yes/no), and sleep disorders (yes/no) were collected by self-reported questionnaire. Information on diabetes duration, diabetic complications, including history of hypertension, hyperlipidemia, diabetic retinopathy, and diabetic nephropathy, and hypoglycemic medication history were obtained from medical records in the hospital.

Body mass index (BMI) was calculated as weight/height<sup>2</sup> (in meters<sup>2</sup>). An average systolic blood pressure (SBP) and diastolic blood pressure (DBP) were calculated from two measurements with the subjects in the recumbent position after 15 min of rest.

**Laboratory measurements.** On the morning following hospital admission, blood samples were drawn after a 12 h fast by vein puncture; three tubes were collected for each patient, two contained ethylene diamine tetraacetic acid (EDTA) anticoagulant and one contained coagulant. The tube containing EDTA anticoagulant was used to measure glycated hemoglobin A1c (GHbA1c) using HPLC. Another tube was immediately centrifuged at 2,500 g for 10 min at 4°C, then the plasma samples were obtained and stored frozen at  $-80^\circ\text{C}$  for subsequent measurement of Hcy, SAM and SAH. The tube containing coagulant was centrifuged at 3,000 g for 10 min, and the serum samples were obtained. The levels of serum triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL) cholesterol and fasting plasma glucose (FPG) were measured enzymatically on a Modular Analytics P800 analyzer (Roche Diagnostics, Indianapolis, IN). At the same time, a portion of each serum sample was stored frozen at  $-80^\circ\text{C}$  for the subsequent measurement of serum folate levels.

The levels of serum folate were determined by chemiluminescence assay (Access Immuno Systems, Beckman Coulter, Brea, CA). Plasma levels of Hcy, SAM, and SAH were analyzed with HPLC as described by Poirier *et al.*<sup>(26)</sup> with slight modifications. Separations of plasma Hcy, SAM or SAH were all accomplished by HPLC with a Waters 700 HPLC Pump and a reversed-phase C18 column (5  $\mu\text{m}$  bead size; 4.6  $\times$  250 mm) (Waters, Milford, MA). For Hcy, the mobile phase consisted of 0.08 M acetate buffer and 5% (v/v) methanol adjusted to pH 4.0 by addition of concentrated acetic acid and then filtered through a 0.45- $\mu\text{m}$  membrane filter. However, for SAM and SAH, the mobile phase consisted of 50 mmol/L sodium phosphate monobasic, monohydrate; 10 mmol/L heptanesulfonic acid and 20% (v/v) methanol adjusted to pH 4.38 by addition of concentrated phosphoric acid and then filtered through a 0.45- $\mu\text{m}$  membrane filter. The isocratic elution was performed using a flow rate of 1.0 mL/min at 30°C and a pressure of 100–110 kgf/cm<sup>2</sup> (1,500–1,800 psi). A fluorescence detector with excitation at 390 nm and emission at 470 nm was used to detect Hcy. The detector wavelength was 254 nm for detection of SAM and SAH. Before analysis of Hcy, the system was calibrated with authentic DL-homocysteine standards in the range of 50 to 4,000 ng. SAM and SAH in samples were identified according to their retention times with authentic standards. Plasma Hcy, SAM and SAH levels were quantified relative to the standard obtained from Sigma Chemical Co. (St. Louis, MO).

**Statistical analysis.** The data are expressed as means and standard deviations for normally distributed continuous variables; medians and inter-quartile ranges for skewed continuous variables; and frequencies and percentages for categorical variables. Differences between the MCI and control groups were tested using a paired-sample *t* test or Wilcoxon rank-sum test for continuous variables and a chi-square test for categorical variables.

Logistic regression was used to estimate the association between folate, methyl donor and Hcy (treated as continuous) and MCI risk (compared to the controls). Two models were created: a crude model and an adjusted model. The latter was adjusted for duration of diabetes, GHbA1c, diabetic retinopathy, and cardiovascular disease risk factors, including BMI, smoking status, SBP, TG and TC. Pearson correlation analysis was used to assess associations between folate and methyl donor.

**Table 1.** Differences in demographic and socioeconomic data

Variables		Control group (n = 50)	MCI group (n = 50)	p value
Sex	Men	17 (34.0)	17 (34.0)	>0.05
	Women	33 (66.0)	33 (66.0)	
Education	No education	12 (24.0)	12 (24.0)	>0.05
	1–6 years	13 (26.0)	13 (26.0)	
	6–12 years	19 (38.0)	19 (38.0)	
	≥12 years	6 (12.0)	6 (12.0)	
Age (years)		74.36 ± 4.54	75.16 ± 4.65	>0.05
MMSE		26.30 ± 2.97	20.40 ± 4.00	<0.01
ADL		22.14 ± 2.98	22.82 ± 3.91	>0.05
Family per capita income	≤¥2,000	17 (34.0)	14 (28.0)	>0.05
	¥2,000–3,000	24 (48.0)	33 (66.0)	
	≥¥3,000	9 (20.0)	3 (8.9)	
Marital status	Married	31 (62.0)	29 (58.0)	>0.05
	Divorced/widowed	19 (38.0)	21 (42.0)	
Smoking status	Yes	16 (32.0)	15 (30.0)	>0.05
	No	34 (68.0)	35 (70.0)	
Drinking status	Yes	13 (26.0)	7 (14.0)	>0.05
	No	37 (74.0)	43 (86.0)	
Reading	Yes	27 (54.0)	20 (40.0)	>0.05
	No	23 (46.0)	30 (60.0)	
Habit of regular exercise	Yes	7 (14.0)	6 (12.0)	>0.05
	No	43 (86.0)	44 (88.0)	
Sleep disorders	Yes	23 (46.0)	20 (40.0)	>0.05
	No	27 (54.0)	30 (60.0)	

Data are presented as means ± SD or n (%). MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; ADL, activities of daily living.

Statistical analyses were performed using SPSS (ver. 13.0, Chicago, IL). All reported *p* values were two tailed, and *p*<0.05 was considered statistically significant.

## Results

**Demographic and socioeconomic data.** The demographic and socioeconomic data and MMSE scores of the subjects are shown in Table 1. The 50 subjects with T2D and MCI were matched to controls (1:1) based on age (± 3 years), sex and education. The MCI group had significantly lower MMSE scores (20.40 ± 4.00) than the control group (26.30 ± 2.97; *p*<0.01). There were no significant differences in ADL scores, family per capita income, marital status, smoking status, drinking status, reading, habit of regular exercise, or sleep disorders between the two groups.

**Clinical and biochemical indexes.** The mean TC level in the MCI group (5.51 ± 1.11 mmol/L) was significantly higher than that in the control group (4.94 ± 1.03 mmol/L; *p*<0.01). The percent of diabetic retinopathy in the MCI group (42.0%) was significantly higher than that in the control group (20.0%; *p*<0.05). There were no significant differences in BMI, SBP, DBP, duration of diabetes, TG, HDL, FPG, GHbA1c, or the percentages of hypertension, hyperlipidemia or diabetic nephropathy between the two groups (Table 2).

**Levels of methyl donor, folate and Hcy.** The differences in the levels of methyl donor, folate and Hcy between the MCI group and control group were shown in Fig. 1. It was observed that the mean levels of folate, SAM and SAM/SAH ratio were 7.39 ± 3.27 µg/L, 113.86 ± 24.10 and 6.96 ± 2.42 in the MCI group, which were significantly lower than those in the control group (*p*<0.05 or *p*<0.01). Whereas, the mean levels of Hcy and SAH in the MCI group were significantly higher than those in the control group (*p*<0.05) (Hcy: 12.70 ± 1.99 µmol/L vs 11.71 ± 2.72 µmol/L; SAH: 17.36 ± 3.58 nmol/L vs 16.11 ± 3.31 nmol/L).

**ORs of MCI for folate, methyl donor and Hcy.** ORs of

MCI for serum folate, plasma Hcy, SAM, and SAH and the SAM/SAH ratio are presented in Table 3. In the crude model, the ORs of MCI for serum folate (per µg/L increase), plasma SAM (per nmol/L increase) and the SAM/SAH ratio were 0.87 (95% CI 0.78, 0.98; *p*<0.05), 0.97 (95% CI 0.95, 0.99; *p*<0.01), and 0.71 (95% CI 0.56, 0.89; *p*<0.01), respectively. In other words, the levels of serum folate and SAM and the SAM/SAH ratio were negatively associated with the risk of MCI. These significant associations persisted even after adjustment for duration of diabetes, GHbA1c, diabetic retinopathy, and cardiovascular disease risk factors, including BMI, smoking status, SBP, TG and TC. The levels of plasma Hcy (per µg/L increase) and SAH (per nmol/L increase) were positively associated with the risk of MCI (Hcy OR 1.28; 95% CI 1.02, 1.59; *p*<0.05; SAH 1.20, 95% CI 1.02, 1.42; *p*<0.05) in crude model. However, the significant associations disappeared after adjustment for the above covariates (*p*>0.05).

**The correlations between plasma methyl donor levels and serum folate.** Pearson correlation analysis showed that the level of folate was significantly positively correlated with that of SAM (*r* = 0.24, *p*<0.05) and the SAM/SAH ratio (*r* = 0.35, *p*<0.01) and negatively correlated with that of SAH (*r* = -0.32, *p*<0.01). Then, we divided all the subjects into three subgroups according to serum folate level tertiles and reevaluated the correlation between serum folate and methyl donors among the three subgroups. The significant correlations mentioned above between folate and SAM (*r* = 0.38, *p*<0.05), the SAM/SAH ratio (*r* = 0.46, *p*<0.01) and SAH (*r* = -0.37, *p*<0.05) only persisted among those within the middle tertile of folate levels (6.3–9.1 µg/L) [vs Fig. 2 (d–f)]. On the contrary, there was no significant correlation among those in the lower (<6.3 µg/L) (*p*>0.05, Fig. 2 (a–c)) or upper (≥9.1 µg/L) tertile of folate levels [*p*>0.05, Fig. 2 (g–i)].

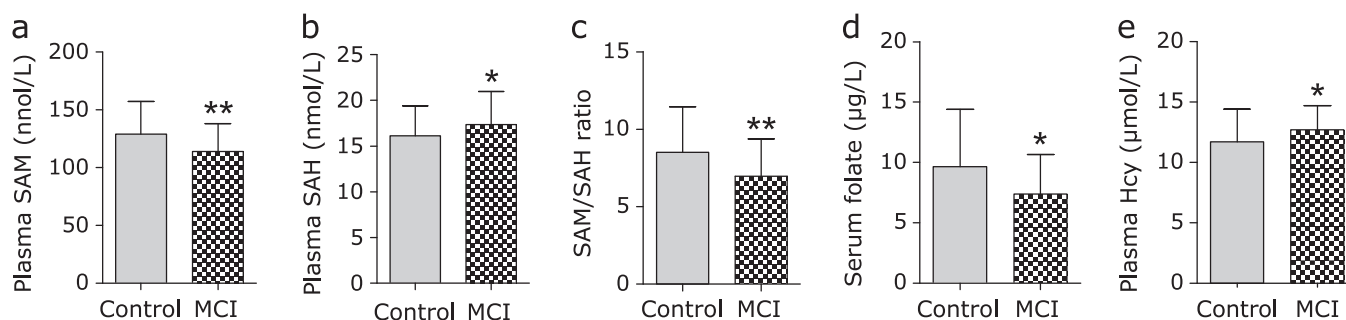
## Discussion

Older age, male sex, and less education are significantly associated with cognitive decline in stroke-free patients;<sup>(27)</sup> the

**Table 2.** Differences in clinical and biochemical indexes

Variables	Control group (n = 50)	MCI group (n = 50)	p value
BMI (kg/m <sup>2</sup> )	25.56 ± 2.88	26.72 ± 3.91	>0.05
Systolic blood pressure (mmHg)	130.8 ± 15.9	135.3 ± 15.3	>0.05
Diastolic blood pressure (mmHg)	74.2 ± 8.6	75.9 ± 7.5	>0.05
Duration of diabetes (years)	17.0 (10.0, 22.0)	15.0 (9.05, 20.0)	>0.05
Triglycerides (mmol/L)	1.32 (1.01, 2.01)	1.66 (1.13, 2.26)	>0.05
Total cholesterol (mmol/L)	4.94 ± 1.03	5.51 ± 1.11	<0.01
HDL (mmol/L)	1.35 ± 0.37	1.40 ± 0.29	>0.05
Fasting plasma glucose (mmol/L)	8.27 ± 2.68	9.20 ± 2.83	>0.05
Glycated hemoglobin A1c (%)	8.42 ± 2.08	8.89 ± 2.45	>0.05
Hypertension			
Yes	35 (70.0)	38 (76.0)	>0.05
No	15 (30.0)	12 (24.0)	
Hyperlipidemia			
Yes	25 (50.0)	31 (62.0)	>0.05
No	25 (50.0)	19 (38.0)	
Diabetic retinopathy			
Yes	10 (20.0)	21 (42.0)	<0.05
No	40 (80.0)	29 (58.0)	
Diabetic nephropathy			
Yes	15 (30.0)	19 (38.0)	>0.05
No	35 (70.0)	31 (62.0)	

Data are presented means ± SD, median (inter-quartile range), or n (%). MCI, mild cognitive impairment. HDL: high-density lipoprotein.



**Fig. 1.** Differences in plasma SAM (a), SAH (b), SAM/SAH ratio (c), serum folate (d) and Hcy (e) between the MCI and control groups. MCI, mild cognitive impairment; SAM, S-adenosylmethionine; SAH, S-Adenosylhomocysteine; Hcy, homocysteine. Values represent the means ± SD. \**p*<0.05; \*\**p*<0.01 vs the controls.

**Table 3.** Odds ratios of mild cognitive impairment for folate, methyl donor and Hcy

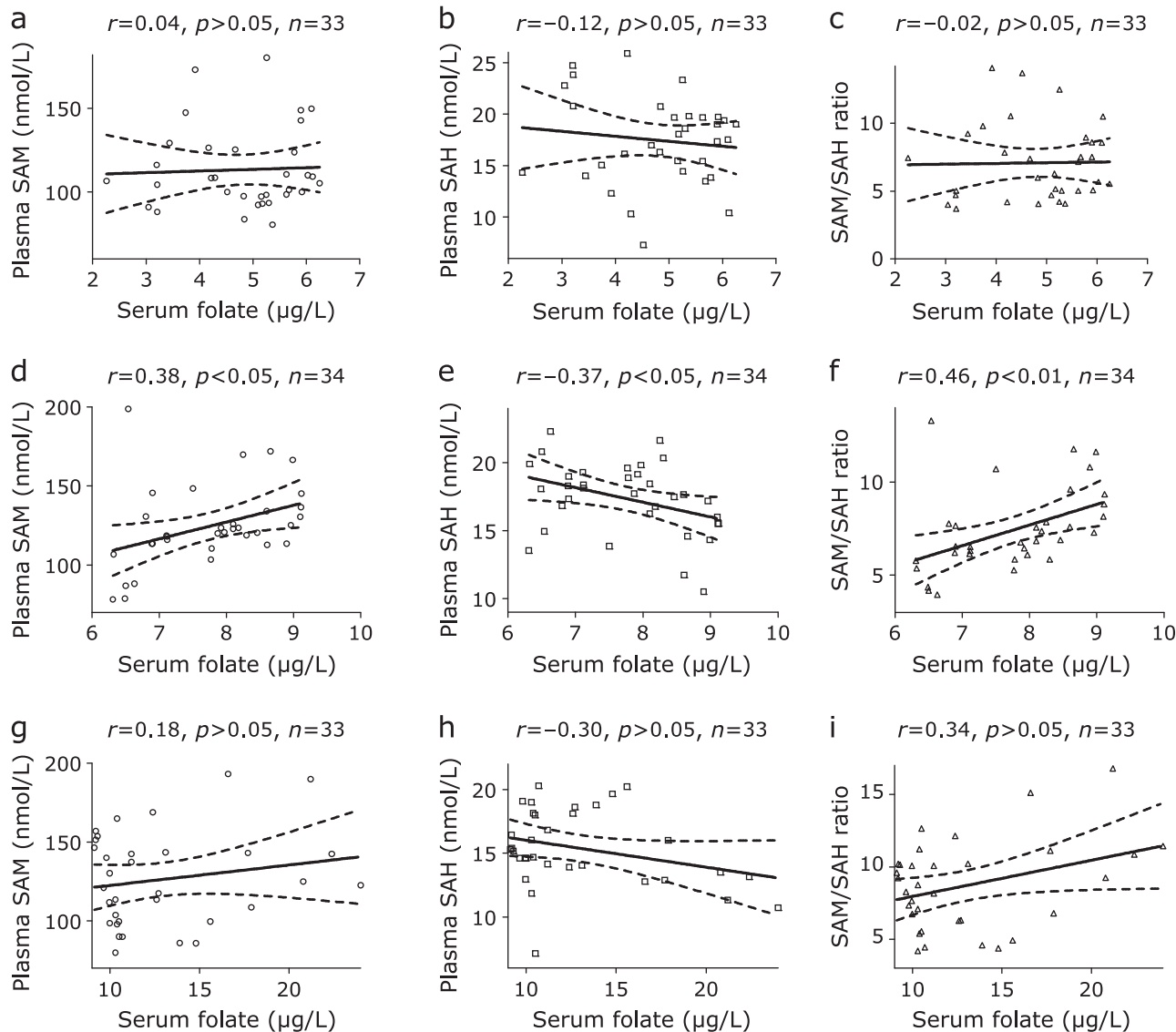
Variables	unadjusted		Adjusted †	
	OR (95% CI)	p value	OR (95%CI)	p value
SAM (per nmol/L increase)	0.97 (0.9, 50.99)	0.005	0.96 (0.93, 0.99)	<0.05
SAH (per nmol/L increase)	1.20 (1.02, 1.42)	0.03	1.22 (0.98, 1.53)	>0.05
SAM/SAH ratio	0.71 (0.56, 0.89)	0.004	0.68 (0.50, 0.91)	<0.05
Folate (per µg/L increase)	0.87 (0.78, 0.98)	0.02	0.72 (0.56, 0.93)	<0.05
Hcy (per µg/L increase)	1.28 (1.02, 1.59)	0.034	1.34 (0.97, 1.85)	>0.05

† Adjusted for duration of diabetes (years), GHbA1c (%), diabetic retinopathy (yes/no), and cardiovascular disease risk factors, including body mass index (kg/m<sup>2</sup>), smoking status (yes/no), systolic blood pressure (mmHg), triglycerides (mmol/L), and total cholesterol (mmol/L). SAM, S-adenosylmethionine; SAH, S-Adenosylhomocysteine; Hcy, homocysteine.

levels of SAM and SAH and the SAM/SAH ratio are higher in the brains of female AD patients than those of males<sup>(28)</sup> and change with increasing age in older mice.<sup>(29)</sup> We randomly selected the controls who matched with each case's gender, education level and age (within 3 years). We found lower serum levels of folate and higher plasma levels of Hcy in subjects with MCI than in controls, and the serum folate level (per µg/L increase) was negatively associated with MCI diagnosis after adjustments for age, duration of diabetes, GHbA1c, diabetic retinopathy, and cardiovascular disease risk factors, including BMI, smoking status, SBP,

TG and TC in T2D. The results are consistent with previous studies on diabetic<sup>(30)</sup> and non-diabetic populations.<sup>(11,31)</sup> The association between hyperhomocysteinemia, which is often related to folate deficiency, and AD is well established.

Despite the fact that previous studies determined SAM and SAH levels in CSF, brain tissue or plasma of AD patients,<sup>(15-17,32)</sup> brain tissue and CSF are believed to give more reliable data. However, sources of postmortem AD patient brains are scarce and the CSF procedure requires a lumbar puncture, which is invasive, and carries risks of infection, epidural bleeding and tissue damage.



**Fig. 2.** The correlations between folate and methyl donor levels by tertiles of folate levels. SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; Hcy, homocysteine. The level of folate was significantly positively correlated with that of SAM and the SAM/SAH ratio and negatively correlated with that of SAH among patients with folate levels between 6.3 and 9.1 µg/L ( $p<0.05$ , d-f), but no significant correlation existed among patients with folate levels less than 6.3 µg/L ( $p>0.05$ , a-c) or over 9.1 µg/L ( $p>0.05$ , g-i).

Moreover, patients, especially those with MCI, do not generally accept this method. Administration of SAM either intravenously or orally is associated with a significant increase in both plasma and CSF SAM levels,<sup>(15)</sup> indicating that SAM crosses the blood-brain barrier in humans either by diffusion or carrier-mediated transport.<sup>(33)</sup> Therefore, the level of plasma SAM can, at least in part, reflect CSF levels. In the present study, the diabetic patients with MCI were characterized by significantly lower SAM levels and higher SAH levels in plasma compared with controls, which led to a lower SAM/SAH ratio for MCI patients. Our results are consistent with previous data showing that the CSF SAM level in AD patients is significantly lower than that of non-diabetic controls.<sup>(15,32)</sup> Similarly, the level of SAM was severely decreased in brain tissue of AD patients in a postmortem analysis.<sup>(16)</sup> On the contrary, Mulder *et al.*<sup>(34)</sup> did not observe any significant differences in CSF levels of SAM or SAH or their ratios between 30 “probable” AD patients and 28 controls without dementia. However, this study may have been limited because the control

group consisted of 20 people with subjective memory complaints and two with a positive family history for AD. Selley<sup>(17)</sup> has shown that the plasma SAM/SAH ratio of AD patients was lower than that of controls, which, in agreement with our results, indicated the methylation potential may be weakened in patients with cognitive decline. However, the plasma SAM and SAH levels were higher among AD patients. The most likely reason for the different results for the relative SAM level in Selley’s study compared to our study may be the different populations used. Our subjects were all diabetic patients, whereas Selley’s study excluded individuals with a history of diabetes. In diabetic rat models, methionine synthase activity and hepatic levels of methionine are decreased.<sup>(35)</sup> The fact that diabetes is characterized by disturbed methyl group metabolism may explain, in part, the different results for the two studies. However, as all our study participants had diabetes, we were not able to formally analyze the methyl donor for a differential effect size in diabetes.

We also found that the SAM level (per nmol/L increase) and

SAM/SAH ratio were negatively associated with MCI diagnosis after adjustments for age, duration of diabetes, GHbA1c, diabetic retinopathy, and cardiovascular disease risk factors, including BMI, smoking status, SBP, TG and TC in T2D ( $p < 0.05$ ). Due to SAM's role as the sole methyl group donor in many important methylation reactions in the nervous system and its demethylated product SAH's role as a strong methyltransferases inhibitor, lower SAM levels and a lower SAM/SAH ratio are supposed to result in reduced methylation capacity.<sup>(14)</sup> Therefore, our data indicate lower methyl donor levels and reduced methylation capacity may be associated with cognitive decline among diabetic patients. In the AD mouse model, the level of methyl donor was able to modulate DNA methylase and demethylase activities.<sup>(36)</sup> The SAM decrease induced DNA demethylation responsible for the activation and overexpression of genes involved in AD pathology.<sup>(37)</sup> Previous studies have suggested the low SAM level induced by folate and vitamin B12 reduction can increase the express of several AD genetic risk factors, such as presenilin-1 and  $\beta$ -secretase, which may promote amyloid  $\beta$  production *in vitro* in human neuroblastoma cells and mouse models. Supplemental SAM can restore normal gene expression, thus reducing AB levels, which is directly related to methylation in the promoter of the related gene.<sup>(6,38)</sup> A recent clinical study has shown that administration of SAM adjunct to antidepressants in depressed patients enhances their cognitive symptoms and ability.<sup>(39)</sup>

Folate serves as a methyl group carrier for the methylation cycle, and dietary folic acid deficiency may induce SAM and SAH imbalance in animals.<sup>(38,40)</sup> We supposed there may be some relationship between serum folate and methyl donor, and we found that the level of folate was significantly positively correlated with that of SAM and the SAM/SAH ratio ( $r$  value 0.24, 0.35, respectively,  $p < 0.05$ ) and negatively correlated with that of SAH ( $r$  value  $-0.32$ ,  $p < 0.05$ ) in diabetic patients. However, Herrmann *et al.*<sup>(41)</sup> have not found a correlation between serum folate and SAM, SAH or their ratios in patients with diabetic nephropathy. At the same time, they have found plasma concentrations of SAM and SAH increased significantly with worsening renal function, whereas serum concentrations of B vitamins (folate, vitamins B12 and B6) did not differ appreciably between groups with different renal damage. Therefore, renal dysfunction may influence the relationship between folate and methyl donor levels in blood. In the present study, patients with moderate or severe renal diseases (estimated creatinine clearance  $< 60$  ml/min) were excluded. Taking account of metabolic factors such as the availability of methionine or choline could increase the levels of methyl donors when the folate was insufficiency, whereas excessive folate supplementation could not increase the SAM level to improve the methylation ability.<sup>(40,42)</sup> Therefore, we divided all the subjects into three subgroups according to serum folate level to observe the trend of the correlation between serum folate and methyl donors in different folate levels. The above significant correlations between folate and SAM, SAM/SAH ratio or SAH only persisted among those within the middle tertile of folate levels (6.3–9.1  $\mu\text{g/L}$ ). Thus, the data of the present study indicate that lower folate in serum may predict lower plasma SAM and weakened methylation potential

within a suitable range of serum folate concentrations (such as 6.3–9.1  $\mu\text{g/L}$ ) in diabetic patients without apparent renal dysfunction. In fact, some other methyl group nutrients, such as choline, can provide labile methyl groups to regenerate methionine from Hcy by betaine-homocysteine methyltransferase to maintain methylation capacity under conditions of folate or Vitamin B12 deficiency.<sup>(42)</sup> A previous study has shown plasma betaine concentrations are more variable in diabetic patients compared with controls.<sup>(43)</sup> Altogether, the ability of lower folate levels to predict weakened methylation potential may be modified under conditions of relative folate insufficiency in diabetic patients. On the other hand, although serum folate levels increased with increasing folic acid intake, excessive supplementation failed to elevate SAM level and enhance the methylation potential in animals.<sup>(40)</sup> There are no significant correlations between serum folate and plasma SAM or the SAM/SAH ratio in diabetic patients when the serum folate level is too low ( $< 6.3$   $\mu\text{g/L}$ ) or too high ( $\geq 9.1$   $\mu\text{g/L}$ ).

In conclusion, the present study suggests that MCI is associated with lower SAM and folate levels in peripheral blood and weakened methylation potential (SAM/SAH) in diabetic patients. We also observed that serum folate is positively correlated with plasma SAM and methylation potential within a suitable range of serum folate concentrations (6.3–9.1  $\mu\text{g/L}$ ) in diabetic patients with normal renal function, which indicates moderate folate intake may help to protect against cognitive impairment by improving methylation status.

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## Abbreviations

AD	Alzheimer's disease
ADL	activities of daily living
BMI	body mass index
CSF	cerebrospinal fluid
DBP	diastolic blood pressure
FPG	fasting plasma glucose
Hcy	homocysteine
HDL	high-density lipoprotein
MCI	mild cognitive impairment
MMSE	Mini-Mental State Examination
SAH	S-adenosylhomocysteine
SAM	S-adenosylmethionine
SBP	systolic blood pressure
T2D	type 2 diabetes
TC	total cholesterol
TG	triglycerides

## Conflict of interest

No potential conflicts of interest were disclosed.

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