


RESEARCH

Open Access



# Dietary intakes and biomarker patterns of folate, vitamin B<sub>6</sub>, and vitamin B<sub>12</sub> can be associated with cognitive impairment by hypermethylation of redox-related genes NUDT15 and TXNRD1

Yu An<sup>1</sup>, Lingli Feng<sup>1,2</sup>, Xiaona Zhang<sup>1</sup>, Ying Wang<sup>1</sup>, Yushan Wang<sup>1</sup>, Lingwei Tao<sup>1</sup>, Zhongsheng Qin<sup>3</sup> and Rong Xiao<sup>1\*</sup> 

## Abstract

**Background:** B vitamins in the one-carbon metabolism pathway (folate, vitamin B<sub>6</sub>, and vitamin B<sub>12</sub>) have been implicated in DNA methylation, and their deficiency may contribute to cognitive decline through increased homocysteine (Hcy) levels and subsequent oxidative damage. The aim of this study was to investigate whether B vitamin deficiency and increased Hcy could interact with DNA methylation of oxidative-related genes and exacerbate cognitive impairment.

**Methods:** Participants were selected from a large cohort study entitled the Effects and Mechanism Investigation of Cholesterol and Oxysterol on Alzheimer's disease (EMCOA) study. We included 2533 participants who completed a selection of comprehensive cognitive tests and a semiquantitative food frequency questionnaire (FFQ) and were followed for an average of 2.3 years. The longitudinal effects of B vitamin intake on cognitive decline were examined using linear mixed-effect models. Seven mild cognitive impairment (MCI) patients, in the prodementia stage of Alzheimer's disease (AD), and five healthy controls were selected for the discovery of genome-wide differentially methylated CpG sites. Candidate oxidative stress-related genes significantly correlated with serum levels of B vitamins were selected for validation in 102 MCI patients and 68 controls. The correlations between DNA methylation levels and serum concentrations of B vitamins and oxidative biomarkers were analyzed with Spearman's correlation. The interactive effects of DNA methylation and B vitamins on cognitive performance were further evaluated by multiple linear regression.

**Results:** In the prospective analysis, inadequate dietary intake of vitamin B<sub>12</sub> was significantly associated with accelerated cognitive decline, whereas adequate folate, vitamin B<sub>6</sub>, and vitamin B<sub>12</sub> intakes were significantly associated with better cognitive reserve. In the case-control analysis, the DNA methylation levels of NUDT15 and TXNRD1 were examined, and significantly hypermethylated sites were identified in MCI patients. Significant correlations of hypermethylated sites with serum levels of folate, homocysteine (Hcy), and oxidative biomarkers were observed, and interactive effects of B vitamins and hypermethylated sites were significantly associated with cognitive performance.

(Continued on next page)

\* Correspondence: [xiaor22@ccmu.edu.cn](mailto:xiaor22@ccmu.edu.cn)

<sup>1</sup>School of Public Health, Capital Medical University, No.10 Xitoutiao, You An Men Wai, Beijing 100069, China

Full list of author information is available at the end of the article



(Continued from previous page)

**Conclusion:** Adequate dietary folate at baseline predicted a better cognitive reserve, while decreased serum levels of B vitamins may contribute to cognitive impairment by affecting methylation levels of specific redox-related genes.

**Trial registration:** EMCOA, ChiCTR-OOC-17011882, Registered 5th, July 2017-Retrospectively registered, <http://www.medresman.org/uc/project/projectedit.aspx?proj=2610>

**Keywords:** Folate, Vitamin B<sub>12</sub>, DNA methylation, Oxidative stress, Cognitive impairment

## Background

Alzheimer's disease (AD) is a chronic and progressive disorder causing deterioration of cognitive function beyond the normal aging process among older people worldwide, ranging in severity from predementia stages such as mild cognitive impairment (MCI) to clinical stages of dementia [1]. As a leading chronic disease contributing to disability and dependence, AD is characterized by progressive cognitive decline and growing functional impairment, beginning with mild difficulties with instrumental activities of daily living (ADL), such as using a telephone and managing medication, and ending with the loss of basic ADL, such as bathing, eating, and dressing [2].

MCI is considered to be a transitional stage between healthy aging and dementia, characterized by cognitive deficits in several domains greater than expected for an individual's age but slight or no impairment in instrumental ADL [3]. Dementia refers to severe brain disorders associated with largely generalized cognitive dysfunction, behavioral disturbances, loss of basic ADL, disability and dependency associated with personal, social, and economic burden [4]. Such concomitant cognitive and functional difficulties increase dependence to negatively affect quality of life (QOL), which is a multidimensional construct integrating cognitive function, physical function, social interactions, mental well-being, and mood [5].

The World Alzheimer Report 2018 has estimated that 46.8 million people were suffering from dementia globally in 2015, and the number is predicted to triple by 2050 [6]. Consequently, AD presents continuous social and economic challenges with an ever-increasing aging population. It has been acknowledged that the neurodegenerative process during AD is inevitably irreversible, but early intervention in MCI, including decreasing the risk factors, is promising [7]. As a result, the exploration of strategies to prevent or delay the onset of AD, including identification of risk factors of MCI, has become a major priority of global public health.

There is no doubt that the development of MCI/AD in later life is affected by a series of modifiable risk factors associated with lifestyle and nutritional status [8], such as smoking, education, reading, and dietary nutrient patterns [9]. Potential underlying mechanisms have been proposed, including antioxidant defense, anti-inflammatory effects, decreased vascular burden, and altered DNA methylation

in the central nervous system [10]. In particular, a copious amount of epidemiological evidence has suggested that suboptimal status of B vitamins contributes to cognitive dysfunction in the elderly. The deficiency of B vitamins has been commonly reported in the elderly, which may be caused by inadequate intake, drug-nutrient interactions, and increased requirements due to the negative effects of aging on the absorption, transport, and metabolism of B vitamins [11]. Epidemiological findings of the associations of B vitamin intakes with domain-specific cognitive decline as well as risk of MCI/AD are incongruent. Kim et al. [12] investigated the relationship between intakes of B vitamins and cognitive function in 100 subjects with MCI, 100 with AD, and 121 normal subjects. The findings revealed that total B vitamin intake was associated with better global and domain-specific cognitive function in the AD and MCI groups. However, a large prospective study that enrolled a total of 3718 residents aged 65 years and older found that higher intake of folate may be harmful in relation to cognitive decline [13], which may be attributed to much higher intake of total folate (from food and supplements) than the dietary reference intakes (DRIs) after the implementation of a folic acid fortification program in the USA and residual confounding caused by factors associated with higher folate intake and slower cognitive decline. Moreover, there has been evidence in randomized controlled trials (RCT) to show different effects of B vitamin supplementation on cognitive function in the elderly, and a recent meta-analysis concluded that B vitamin supplementation had no effect on cognition [14].

Although no clear mechanisms have been well established, several biologically plausible mechanisms have been proposed to explain the role of one-carbon metabolism-related B vitamins, including folate, vitamin B<sub>12</sub>, and vitamin B<sub>6</sub>, in cognitive dysfunction [15]. Deficiencies in any of these B vitamins might raise the blood concentration of homocysteine (Hcy) by perturbing one-carbon metabolism and leading to low enzymatic activities for the remethylation or trans-sulfuration of homocysteine [16]. Methylation is a key mechanism by which the body deals with toxins, stress, and infections. The results of ineffective methylation reactions may contribute to numerous diseases, including neurological disorders. B vitamins are essential in the synthesis of *S*-adenosyl-methionine (SAM), which is required

for methylation of DNA [17]. It has been well established that vitamin B<sub>6</sub>, folate, and vitamin B<sub>12</sub> in the diet can reduce serum Hcy level and promote its remethylation to methionine. Consequently, lower intake of these B vitamins and elevated serum Hcy level are linked to altered DNA methylation patterns, which has been observed in AD patients [18]. As a neurotoxin, an increased level of Hcy has also been shown to affect redox signaling pathways in neurons by generating reactive oxygen species (ROS) and decreasing endogenous antioxidants [19]. It is therefore of interest to see whether DNA methylation patterns in redox-related genes could modulate cognitive impairment conferred by B vitamin deficiency and hyperhomocysteinemia.

The present study was designed to investigate the effects of dietary intakes of these one-carbon metabolism-related B vitamins on global and domain-specific cognitive decline in a large Chinese cohort and then explore the possible role of DNA methylation of genes in oxidative stress pathways as a mediator in a case-control design. The results of this study will provide a theoretical basis and scientific data for uncovering the potential interactions between B vitamin nutritional status and the genetic background of cognition.

## Materials and Methods

### Participants

Participants between the ages of 50 and 70 years were selected from a large-scale community-based study entitled the Effects and Mechanism investigation of Cholesterol and Oxysterol on Alzheimer's disease (EMCOA) study, an ongoing multicenter epidemiological survey funded by the State Key Program of National Natural Science Foundation of China [20]. This study was registered at the Chinese Clinical Trial Registry as ChiCTR-OOC-17011882. The medical Ethics Committee of Capital Medical University (No. 2013SY35) approved the study protocol, and written informed consent was obtained from all subjects. The baseline examination took place between January 2014 and December 2015, and follow-up examinations took place approximately every 2 years. Face-to-face interviews were performed at each examination, with the collection of sociodemographic information (e.g., age, sex, and education years), medical history of chronic diseases (e.g., hypertension, diabetes, and heart disease), lifestyle (e.g., smoking and drinking), and a broad range of neuropsychological tests and dietary surveys. Fasting venous blood was collected during all the interviews following standardized protocols for storage of blood samples. A survey team consisted of clinical neuropsychologists, and research surveyors were trained in the details of the measurements and questionnaires before starting the examination. We adopted outreach efforts through oral and written advertisements,

flyers, word of mouth, and educational presentations provided in the community. The exclusion criteria for the original study included suffering from severe diseases or conditions known to affect cognitive function (e.g., depression, malignant tumors, a history of traumatic brain injury, cerebral infarction or cerebrovascular disease, long-term frequent intake of drugs and medications, or dietary supplements to improve cognitive function). Consequently, the longitudinal association of dietary B vitamins with cognitive decline was investigated among the 2533 participants, who were followed for an average of 2.3 years after the dietary assessment at baseline.

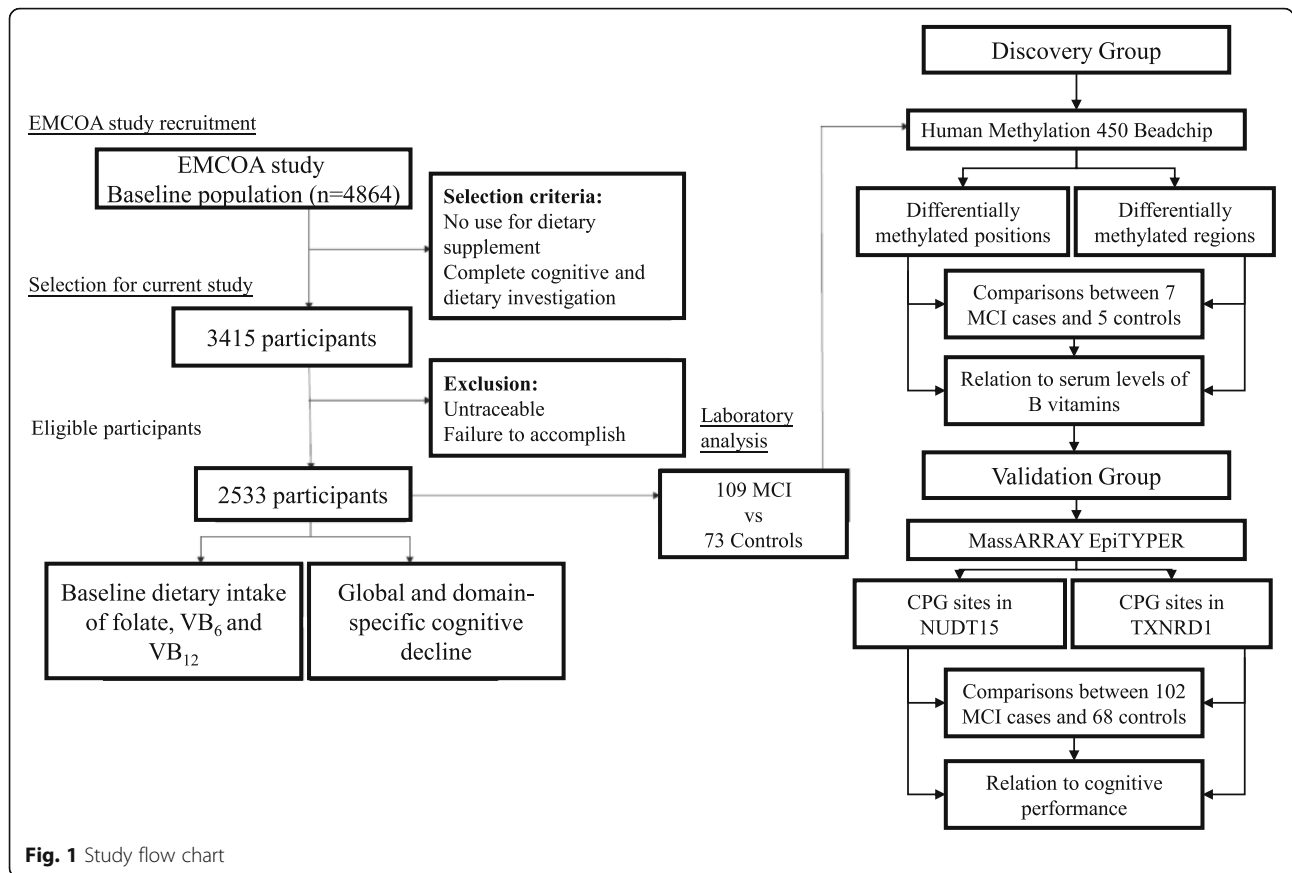
Next, a subgroup of 109 newly diagnosed MCI patients and 73 controls were selected for DNA methylation and biochemical analyses among participants who were the first to complete the follow-up in 2016 in one center. To further exclude potential confounders and obtain a relatively homogenous study population, the predefined selection criteria included the following: no reported changes in the use of dietary supplements containing B vitamins during the study, no heavy alcohol use at baseline or during follow-up, and no gastrointestinal diseases. Figure 1 describes the procedure for the current study.

### Cognitive tests

The cognitive tests were administered and scored according to a standard procedure by nurses or researchers who had attended unified training several times before. This comprehensive neuropsychological battery evaluated global and multiple cognitive domains, and the time required was approximately 40 min. The Mini-Mental State Examination (MMSE) and Montreal Cognitive Assessment (MoCA) were used for global cognitive evaluation [21]. The Symbol Digit Modalities Test (SDMT) [22] was used to assess processing speed. The Auditory Verbal Learning Test (AVLT) [23], including five trials of the recall of a 12-word list, measured immediate recall (AVLT-IR), short recall (AVLT-SR), and long recall (AVLT-LR) of memory. The Logical Memory Test (LMT) [24] and the digit span forwards (DSF) and digit span backwards (DSB) [25] of the Wechsler Memory Scale-Revised, Chinese version (WMS-RC) were used to measure attention.

### Diagnosis of MCI

MCI patients were diagnosed through a two-stage procedure. First, the cognitive function of subjects was assessed by MoCA, which was a 30-point global cognitive screening instrument used for screening MCI patients [26]. The cut-off points used for MCI screening applied to the elderly Chinese population were as follows: 13/14 for illiterate individuals, 19/20 for individuals with 1 to 6 years of education, and 24/25 for individuals with seven or more years of education [27]. Second, participants suspected of having MCI based on their MoCA



**Fig. 1** Study flow chart

performance were secondarily examined by neurologists to establish a clinical diagnosis.

**Dietary assessment**

Dietary information was collected from a 33-item interviewer-administered Food Frequency Questionnaire (FFQ). The participants were requested to state the frequency (per year, month, week, or day) and amount (in grams, bowls, etc.) of food intake for each food item during the 1 year before the interview. For each food reported, food models and an album with over 50 photos of the most common dietary products were used as helpful tools to determine the amounts of food portions. To calculate dietary intakes of the relevant B vitamins, the B vitamin composition of each food was multiplied by the frequency of consumption and then summed over all food items. B vitamin composition was obtained from the China Food Composition Database [9]. The validity and reproducibility of the FFQ has been reported previously; it is a useful tool to estimate B vitamins in the Chinese population [28–30]. The FFQ used in our study was slightly revised by specialists from the National Institute for Nutrition and Health, Chinese Center for Disease Control and Prevention, and was validated by a pilot study within our cohort.

**Covariates** Sociodemographic information included age at study baseline, gender, education (in years), and residential status (categorized as solitude or not). Lifestyle factors included smoking status (determined by self-report and dichotomized as current smoker or not). Risk factors for cognitive decline included body mass index (the ratio of weight to squared height, BMI), diabetes (fasting glucose  $\geq 7.0$  mmol/L or antidiabetic medication), hypertension (measured blood pressure  $> 140/90$  mmHg or antihypertensive medication), and coronary heart disease (CHD).

**Laboratory analysis**

The blood samples for biochemical parameters were drawn via vein puncture after an overnight fast into a tube containing coagulant. The tube was then immediately centrifuged at 3000 g for 8 min at 4 °C, and the serum samples were collected and stored at - 80 °C until further analysis. Serum levels of B vitamins and metabolites (folate, vitamin B<sub>6</sub>, vitamin B<sub>12</sub>, and Hcy) were measured using an Abbott Architect i2000 immunoassay analyzer. Concentrations of oxidative biomarkers, including reactive oxygen species (ROS), malondialdehyde (MDA), 8-hydroxy-deoxyguanosine (8-OHdG), and 8-iso-prostaglandin F<sub>2</sub> $\alpha$  (8-iso-PGF<sub>2</sub> $\alpha$ ), were measured

using commercially available ELISA kits from Nanjing Jiancheng Biotechnology Institute Co., Ltd. according to the manufacturer's instructions.

#### Genome-wide DNA methylation discovery and validation

Genomic DNA was isolated from peripheral blood leukocytes using the QIAamp DNA Blood Kit (Qiagen, CA, USA) and subjected to bisulfite conversion using the EZ DNA Methylation Kit (Zymo Research, CA, USA). Genome-wide DNA methylation using Illumina Infinium Human Methylation450K BeadChip was performed following the methylation protocol. DNA methylation was validated using MassArray® EpiTyper™ in BioMiao Biological Technology, Beijing, China. This method applied matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry, of which the mass spectra were collected using a MassArray Compact MALDI-TOF, and the spectra's methylation ratios were performed with EpiTYPER software (Sequenom, San Diego, CA). The design of polymerase chain reaction (PCR) primers covering 25 CpG sites of the NUDT15 gene and 17 CpG sites of the TXNRD1 gene and calculation of DNA methylation ratio were both done using EpiDesigner software. The  $\beta$  value, representing methylation status of the individual probes, was used to indicate the methylation level at each CpG site, with the range from 0 (fully unmethylated) to 1 (fully methylated).

#### Statistical analysis

Statistical analyses were performed using STATA version 13.0 (STATA, College Station, TX). Prior to analysis, the normality of the data distribution was checked. Continuous variables are expressed as median (interquartile ranges, IQR) when nonnormally distributed or as mean  $\pm$  standard deviation (SD) when normally distributed. Student's *t* test or the Mann–Whitney *U* test was used to compare continuous variables, as appropriate. The differences in the frequencies of the categorical variables were evaluated using the chi-square test or Fisher's exact test. Longitudinal associations of dietary intakes of B vitamins with global and domain-specific cognitive decline were estimated using linear mixed-effect models. Dietary intake of vitamin B<sub>6</sub>, folate, and vitamin B<sub>12</sub> were categorized into quartiles, and the quartile nearest the Chinese recommended nutrient intake (RNI, 2013 edition) was considered the reference. Spearman's or Pearson's correlation coefficient was calculated to analyze the relationships between DNA methylation levels and the biochemical values of participants. Moreover, multiple linear regression models were used to examine the effects of differentially methylated CpG sites and their interaction with B vitamins on cognitive performance in the validation group. All of the models were adjusted for covariates. A two-sided *P* <

0.05 was considered statistically significant, and issues of multiple testing were taken into account by considering *P* values adjusted for the false discovery rate (FDR).

## Results

### Characteristics of the sample

The study participants were 59 years old on average (range 50–70) at the time of the dietary survey, and 58.1% were female (Table 1). Baseline characteristics, including lifestyle, medical history of hypertension, diabetes, CHD, and smoking and drinking status, are also provided in Table 1. The median daily intakes were 2.3 mg/day for vitamin B<sub>6</sub>, 388.1  $\mu$ g/day for folate, and 2.1  $\mu$ g/day for vitamin B<sub>12</sub>. Compared with RNIs for the Chinese population aged 50–70 (vitamin B<sub>6</sub>: 1.6 mg/day; folate: 400  $\mu$ g/day; vitamin B<sub>12</sub>: 2.4  $\mu$ g/day), the average intakes of folate and vitamin B<sub>12</sub> were inadequate in the study participants.

### Effects of B vitamin intake on cognitive decline

The results of fully adjusted mixed-effect linear regression analyses for B vitamin intakes and cognitive decline are shown in Tables 2, 3, and 4. Dietary intake of B vitamins was divided into four quartiles as categorical variables. Multivariate analyses adjusting for confounders took the quartile of each B vitamin nearest the RNI as the reference. Therefore, the 1st quartile of vitamin B<sub>6</sub>, 3rd quartile of folate, and 3rd quartile of vitamin B<sub>12</sub> were used as references. As shown in Table 2, adequate intake of vitamin B<sub>6</sub> higher than the RNI was significantly associated with better performance of verbal memory. However, the 4th quartile of vitamin B<sub>6</sub> was negatively associated with the MoCA and DSF scores, suggesting that vitamin B<sub>6</sub> intake much higher than the RNI may have adverse effects on global cognition and attention. Additionally, adequate intake of folate higher than the RNI was significantly associated with better cognitive reserve for global cognition, verbal memory, and attention, whereas no associations were observed between inadequate intake of folate and cognitive decline (Table 3). In contrast, severe deficiency of vitamin B<sub>12</sub> (1st quartile) was significantly associated with accelerated cognitive decline across all domains, and moderate deficiency (2nd quartile) was associated with decline in most cognitive domains. Beneficial effects of adequate vitamin B<sub>12</sub> intake on global cognitive reserve were observed in both the MMSE and MoCA scores (Table 4).

### Comparison of selected MCI patients and controls in the discovery and validation groups

The general characteristics, cognitive performance, dietary intakes, and serum levels of B vitamins and oxidative biomarkers for selected MCI patients and cognitively normal controls were determined (Table 5). In the

**Table 1** Baseline characteristics and B vitamin intakes of the participants from EMCOA study ( $n = 2533$ )

Baseline characteristics	Overall sample ( $N = 2533$ )	Men ( $N = 1165$ )	Women ( $N = 1368$ )
Demographic characteristics			
Age	59 (55, 62)	59 (56, 63)	58 (55, 62)
Education years	9 (9, 12)	12 (9, 12)	9 (9, 12)
BMI ( $\text{kg}/\text{m}^2$ )	24.5 (22.7, 26.6)	24.9 (23.2, 27.0)	24.2 (22.3, 26.2)
Lifestyle			
Current smoker, $n(\%)$	597 (25.4%)	562 (48.2%)	35 (2.6%)
Current drinker, $n(\%)$	634 (26.9%)	560 (48.1%)	74 (5.4%)
Medical history			
Diabetes, $n(\%)$	381 (15.0%)	215 (18.5%)	166 (12.1%)
Hypertension, $n(\%)$	834 (32.9%)	425 (36.5%)	409 (29.9%)
Coronary heart disease, $n(\%)$	250 (9.9%)	148 (12.7%)	102 (7.5%)
Dietary B vitamin intakes			
Vitamin B <sub>6</sub> (mg/day)	2.3 (1.8, 3.0)	2.4 (1.9, 3.3)	2.2 (1.7~2.8)
1st quartile	0.4~1.8	0.5~1.9	0.4~1.7
2nd quartile	1.8~2.3	1.9~2.4	1.7~2.2
3rd quartile	2.3~3.0	2.4~3.3	2.2~2.8
4th quartile	3.0~17.4	3.3~17.4	2.8~9.5
Folate ( $\mu\text{g}/\text{day}$ )	388.1 (274.8~534.2)	394.8 (277.5, 551.5)	381.1 (271.9, 518.9)
1st quartile	27.0~274.8	31.5~277.5	27.0~271.1
2nd quartile	274.8~388.1	277.5~394.8	271.1~381.1
3rd quartile	388.1~534.2	394.8~551.5	381.1~518.9
4th quartile	534.2~5952.2	551.5~5952.2)	518.9~3132.2
Vitamin B <sub>12</sub> ( $\mu\text{g}/\text{day}$ )	2.1 (1.3, 3.4)	2.5 (1.6, 4.0)	1.9 (1.1, 2.8)
1st quartile	0~1.3	0~1.6	0~1.1
2nd quartile	1.3~2.1	1.6~2.5	1.1~1.9
3rd quartile	2.1~3.4	2.5~4.0	1.9~2.8
4th quartile	3.4~36.5	4.0~36.5	2.8~23.8

BMI body mass index

discovery group, there were no significant differences in sociodemographic or lifestyle factors between MCI patients and controls, whereas MCI patients in the validation group were more likely to be male ( $P = 0.041$ ) and less likely to be current drinkers ( $P < 0.001$ ). With respect to cognitive performance, the scores of MoCA ( $P = 0.005$ ), LMT ( $P = 0.010$ ), and DSF ( $P = 0.010$ ) and serum levels of folate ( $P=0.003$ ) were significantly lower, whereas levels of Hcy ( $P < 0.001$ ) and 8-iso-PGF<sub>2</sub> $\alpha$  ( $P = 0.049$ ) were significantly higher, in MCI patients in the discovery group. These significant differences were replicated in the validation group in addition to the finding that all of the domain-specific cognitive scores and dietary intake of folate were significantly lower, whereas all of the oxidative biomarkers were significantly higher, in MCI patients ( $P < 0.05$ ). Significant negative correlations were observed between folate and oxidative biomarkers,

whereas Hcy was positively correlated with oxidative biomarkers ( $P < 0.001$ , Table 6, Figs. 2 and 3). Overall, MCI patients tended to have folate deficiency, disturbances of B vitamin metabolism, and imbalanced redox status.

A total of 2277 differentially methylated CpG sites were identified in the discovery group when comparing MCI cases ( $n = 7$ ) versus controls ( $n = 5$ ). The associations between DNA methylation levels of the oxidative stress-related genes and serum B vitamin biomarkers were analyzed. Negative correlations were found between the serum level of folate and DNA methylation levels of CpG sites in the nudix hydrolase 15 (NUDT15) and thioredoxin reductase 1 (TXNRD1) genes. The significant CpG sites were located in TSS1500 for NUDT15 and the 5'UTR for TXNRD1. Forty-two CpG candidate sites located in these genes were selected for further validation. The methylation

**Table 2** Longitudinal associations of dietary intake of vitamin B<sub>6</sub> with global and domain-specific cognitive decline

Cognitive performance		Vitamin B <sub>6</sub> (mg/day)			
		1st quartile	2nd quartile	3rd quartile	4th quartile
MMSE	<i>B</i> (95%CI)	Ref	− 0.04 (− 0.17, 0.10)	− 0.13 (− 0.29, 0.02)	− 0.07 (− 0.27, 0.13)
	<i>P</i> value	Ref	0.586	0.091	0.499
MoCA	<i>B</i> (95%CI)	Ref	− 0.06 (− 0.27, 0.14)	− 0.26 (− 0.56, 0.04)	− 0.31 (− 0.55, − 0.08)
	<i>P</i> value	Ref	0.555	0.094	0.009*
AVLT-IR	<i>B</i> (95%CI)	Ref	0.35 (0.03, 0.68)	0.40 (0.03, 0.77)	0.66 (0.18, 1.14)
	<i>P</i> value	Ref	0.033*	0.034*	0.007*
AVLT-SR	<i>B</i> (95%CI)	Ref	0.33 (0.17, 0.50)	0.30 (0.12, 0.49)	0.46 (0.22, 0.70)
	<i>P</i> value	Ref	< 0.001*	0.002*	< 0.001*
AVLT-LR	<i>B</i> (95%CI)	Ref	0.25 (0.07, 0.43)	0.39 (0.12, 0.65)	0.19 (− 0.02, 0.40)
	<i>P</i> value	Ref	0.007*	0.004*	0.069
SDMT	<i>B</i> (95%CI)	Ref	1.13 (0.44, 1.82)	0.76 (− 0.03, 1.55)	− 0.22 (− 1.23, 0.79)
	<i>P</i> value	Ref	0.001*	0.058	0.669
LMT	<i>B</i> (95%CI)	Ref	0.20 (− 0.24, 0.63)	− 0.06 (− 0.56, 0.43)	− 0.16 (− 0.80, 0.48)
	<i>P</i> value	Ref	0.376	0.799	0.625
DSF	<i>B</i> (95%CI)	Ref	− 0.02 (− 0.11, 0.07)	− 0.04 (− 0.15, 0.06)	− 0.17 (− 0.30, − 0.04)
	<i>P</i> value	Ref	0.664	0.427	0.012*
DSB	<i>B</i> (95%CI)	Ref	0.03 (− 0.06, 0.11)	− 0.03 (− 0.12, 0.07)	− 0.05 (− 0.17, 0.07)
	<i>P</i> value	Ref	0.512	0.604	0.426

MMSE mini-mental state examination, MoCA Montreal Cognitive Assessment, AVLT-IR auditory verbal learning test-immediate recall, AVLT-SR auditory verbal learning test-short recall, AVLT-LR auditory verbal learning test-long recall, SDMT symbol digit modalities test, LMT logical memory test, DSF digit span forwards, DSB digit span backwards

*B*: regression coefficients of linear mixed-effect models

\* $P < 0.05$

of CpG\_17 and CpG\_19 in NUDT15 as well as CpG\_9 CpG\_11 in TXNRD1 showed significant differences between MCI patients and controls (FDR adjusted  $P < 0.05$ , Table 7, Figs. 4 and 5).

#### Analysis of correlations and regression models

Table 8 demonstrates that the DNA methylation levels of CpG\_19 of NUDT15 and CpG\_11 of TXNRD1 had significantly negative correlations with folate ( $r = -0.219$ , FDR adjusted  $P = 0.028$  for NUDT15;  $r = -0.192$ , FDR adjusted  $P = 0.041$  for TXNRD1) and positive correlations with Hcy ( $r = 0.251$ , FDR adjusted  $P = 0.010$  for NUDT15;  $r = 0.225$ , FDR adjusted  $P = 0.045$  for TXNRD1). In addition, CpG\_19 of NUDT15 had significant correlations with ROS ( $r = 0.230$ , FDR adjusted  $P = 0.020$ ) and 8-OHdG ( $r = 0.259$ , FDR adjusted  $P = 0.008$ ) and CpG\_11 of TXNRD1 with 8-iso-PGF2 $\alpha$  ( $r = 0.192$ , FDR adjusted  $P = 0.042$ , Table 8, Figs. 6 and 7).

We used multiple linear regression models to investigate the associations of B vitamin-related hypermethylated CpG sites in NUDT15 and TXNRD1 with cognitive performance (Table 9). These analyses allowed us to test whether hypermethylated CpG sites in NUDT15 and TXNRD1 were each associated with global or domain-specific cognitive performance. Next, we

investigated whether serum levels of folate, vitamin B<sub>12</sub>, and Hcy and hypermethylated CpG sites in NUDT15 and TXNRD1 were synergistically associated with cognitive performance. Synergistic effects were tested in models that included a two-way interaction term; this allowed us to assess whether the impact of B vitamins on cognitive performance was mediated by DNA methylation of NUDT15 or TXNRD1. These models were repeated for all of the cognitive tests. Analyses revealed no significant associations of NUDT15 or TXNRD1 with cognitive performance (all  $P > 0.05$ ) but significant synergism between DNA methylation of NUDT15 and Hcy, TXNRD1 and folate, as well as TXNRD1 and Hcy, such that the combination of NUDT15 and Hcy was associated with lower scores of MoCA ( $B = -0.325$ ;  $P < 0.001$ ), LMT ( $B = -0.187$ ;  $P = 0.015$ ), and DSF ( $B = -0.221$ ;  $P < 0.005$ ). The interaction between TXNRD1 and Hcy was also selectively associated with MoCA ( $B = -0.345$ ;  $P < 0.001$ ), LMT ( $B = -0.269$ ;  $P < 0.001$ ), and DSF ( $B = -0.252$ ;  $P = 0.001$ ) in addition to SDMT ( $B = -0.137$ ;  $P = 0.049$ ). In contrast, the DNA methylation of TXNRD1 and serum level of folate were synergistically associated with better cognitive performance on MMSE ( $B = 0.147$ ;  $P = 0.048$ ), MoCA ( $B = 0.268$ ;  $P < 0.001$ ), and AVLT-IR ( $B = 0.163$ ;  $P = 0.042$ ).

**Table 3** Longitudinal associations of dietary intake of folate with global and domain-specific cognitive decline

Cognitive performance		Folate ( $\mu\text{g}/\text{day}$ )			
		1st quartile	2nd quartile	3rd quartile	4th quartile
MMSE	<i>B</i> (95%CI)	-0.11 (-0.25, 0.04)	0.09 (-0.05, 0.23)	Ref	0.06 (-0.09, 0.22)
	<i>P</i> value	0.155	0.188	Ref	0.418
MoCA	<i>B</i> (95%CI)	-0.17 (-0.40, 0.06)	-0.04 (-0.25, 0.17)	Ref	0.25 (0.01, 0.48)
	<i>P</i> value	0.14	0.732	Ref	0.039*
AVLT-IR	<i>B</i> (95%CI)	0.32 (-0.04, 0.67)	0.09 (-0.24, 0.42)	Ref	-0.07 (-0.44, 0.30)
	<i>P</i> value	0.081	0.604	Ref	0.7
AVLT-SR	<i>B</i> (95%CI)	0.07 (-0.1, 0.24)	0.01 (-0.18, 0.20)	Ref	0.23 (0.05, 0.41)
	<i>P</i> value	0.405	0.939	Ref	0.013*
AVLT-LR	<i>B</i> (95%CI)	0.12 (-0.07, 0.32)	-0.10 (-0.29, 0.08)	Ref	-0.01 (-0.21, 0.20)
	<i>P</i> value	0.219	0.286	Ref	0.966
SDMT	<i>B</i> (95%CI)	0.57 (-0.18, 1.33)	0.56 (-0.14, 1.27)	Ref	0.51 (-0.28, 1.30)
	<i>P</i> value	0.138	0.119	Ref	0.207
LMT	<i>B</i> (95%CI)	-0.03 (-0.51, 0.45)	0.01 (-0.44, 0.46)	Ref	0.60 (0.10, 1.10)
	<i>P</i> value	0.903	0.963	Ref	0.019*
DSF	<i>B</i> (95%CI)	-0.01 (-0.11, 0.08)	-0.07 (-0.16, 0.02)	Ref	0.12 (0.02, 0.22)
	<i>P</i> value	0.771	0.138	Ref	0.023*
DSB	<i>B</i> (95%CI)	-0.01 (-0.09, 0.09)	-0.03 (-0.12, 0.06)	Ref	0.05 (-0.04, 0.15)
	<i>P</i> value	0.997	0.497	Ref	0.266

MMSE mini-mental state examination, MoCA Montreal Cognitive Assessment, AVLT-IR auditory verbal learning test-immediate recall, AVLT-SR auditory verbal learning test-short recall, AVLT-LR auditory verbal learning test-long recall, SDMT symbol digit modalities test, LMT logical memory test, DSF digit span forwards, DSB digit span backwards

*B*: regression coefficients of linear mixed-effect models

\* $P < 0.05$

## Discussion

In our prospective study including 2533 middle-aged and elderly Chinese persons who initially had normal cognitive performance, we found that dietary vitamin B<sub>12</sub> deficiency was associated with a greater rate of cognitive decline, whereas adequate intakes of folate and vitamin B<sub>6</sub> were correlated with better cognitive reserve, indicating that dietary intakes of B vitamins are important predictors of cognitive changes. In a secondary case-control analysis, MCI patients had a significantly higher level of Hcy, which may result from significantly lower intake and serum levels of folate. Such biomarker patterns were significantly associated with higher levels of oxidative biomarkers and DNA methylation levels of redox-related genes. The interactions of folate and Hcy with DNA methylation could influence cognitive performance. To our knowledge, this is the first study that combined a longitudinal and case-control design to consider the impact on cognitive health of both dietary intakes and biomarker statuses of B vitamins that are involved in DNA methylation and oxidative stress.

Folate, along with vitamins B<sub>12</sub> and B<sub>6</sub>, is essential in one-carbon metabolism, a network of reactions involving the transfer of one-carbon units. In one-carbon metabolism, tetrahydrofolate obtains a carbon unit in a vitamin B<sub>6</sub>-dependent reaction forming 5,10-methylenetetrahydrofolate,

which is then converted to 5-methyltetrahydrofolate. 5-Methyltetrahydrofolate donates its methyl group to homocysteine in a reaction that uses vitamin B<sub>12</sub> as a cofactor. Deficiencies in any of these B vitamins can perturb this complex regulatory network, resulting in hyperhomocysteinemia, which has been demonstrated to be a causal contributor to cognitive decline, MCI, and AD by extensive epidemiological studies in healthy older adults and patient populations [31]. Further research has investigated the relative importance of each of the B vitamins and Hcy for developing MCI and AD. Quadri et al. [32] have reported that participants with the highest Hcy levels (> 14.6  $\mu\text{mol}/\text{L}$ ) and the lowest folate levels (< 13.5  $\text{nmol}/\text{L}$ ) were more than three times likelier to develop AD. In addition, Ramos et al. [33] demonstrated that increased folate concentrations could significantly decrease the risk of developing dementia. To further complicate matters, Hann et al. [34] reported that Hcy is associated with a greater risk of dementia or cognitive impairment without dementia and that higher B<sub>12</sub> concentrations may reduce this risk. It is clear from these findings that Hcy and vitamin B levels are related to MCI and dementia. However, to date, the relative contribution of each of the B vitamins and Hcy is not fully understood and deserves further research.

Extensive longitudinal studies on B vitamins and cognitive function have been conducted in Americans or



**Table 4** Longitudinal associations of dietary intake of vitamin B<sub>12</sub> with global and domain-specific cognitive decline

Cognitive performance		Vitamin B <sub>12</sub> (µg/day)			
		1st quartile	2nd quartile	3rd quartile	4th quartile
MMSE	<i>B</i> (95%CI)	-0.30 (-0.43, -0.16)	-0.21 (-0.34, -0.08)	Ref	0.32 (0.17, 0.46)
	<i>P</i> value	< 0.001*	0.002*	Ref	< 0.001*
MoCA	<i>B</i> (95%CI)	-0.56 (-0.77, -0.36)	-0.49 (-0.69, -0.29)	Ref	0.38 (0.16, 0.60)
	<i>P</i> value	< 0.001*	< 0.001*	Ref	0.001*
AVLT-IR	<i>B</i> (95%CI)	-0.65 (-0.97, -0.33)	-0.60 (-0.92, -0.27)	Ref	-0.01 (-0.35, 0.35)
	<i>P</i> value	< 0.001*	< 0.001*	Ref	0.987
AVLT-SR	<i>B</i> (95%CI)	-0.30 (-0.46, -0.14)	-0.08 (-0.24, 0.09)	Ref	-0.04 (-0.21, 0.14)
	<i>P</i> value	< 0.001*	0.352	Ref	0.687
AVLT-LR	<i>B</i> (95%CI)	-0.32 (-0.49, -0.14)	-0.11 (-0.29, 0.07)	Ref	0.01 (-0.19, 0.20)
	<i>P</i> value	< 0.001*	0.231	Ref	0.957
SDMT	<i>B</i> (95%CI)	-1.78 (-2.47, -1.09)	-1.22 (-1.89, -0.55)	Ref	1.17 (-0.43, 1.92)
	<i>P</i> value	< 0.001*	< 0.001*	Ref	0.272
LMT	<i>B</i> (95%CI)	-0.70 (-1.14, -0.26)	-0.69 (-1.12, -0.27)	Ref	0.17 (-0.30, 0.64)
	<i>P</i> value	0.002*	0.001*	Ref	0.483
DSF	<i>B</i> (95%CI)	-0.13 (-0.22, -0.03)	0.01 (-0.08, 0.09)	Ref	-0.08 (-0.18, 0.02)
	<i>P</i> value	0.007*	0.893	Ref	0.117
DSB	<i>B</i> (95%CI)	-0.11 (-0.20, -0.03)	-0.11 (-0.19, -0.03)	Ref	0.03 (-0.06, 0.12)
	<i>P</i> value	0.007*	0.008*	Ref	0.487

MMSE mini-mental state examination, MoCA Montreal Cognitive Assessment, AVLT-IR auditory verbal learning test-immediate recall, AVLT-SR auditory verbal learning test-short recall, AVLT-LR auditory verbal learning test-long recall, SDMT symbol digit modalities test, LMT logical memory test, DSF digit span forwards, DSB digit span backwards

*B*: regression coefficients of linear mixed-effect models

\**P* < 0.05

Europeans, but limited research has examined the effects in the Chinese population. In a large American cohort entitled the Chicago Health and Aging Project (CHAP), which included 1041 residents aged 65 years or older who were followed for a median of 3.9 years for the development of AD [35], scholars did not find any association between quintile of B vitamin intake and risk of AD. The results were replicated in the Cache County Memory Study (CCMS) [36], another large cohort in the USA with 5092 men and women aged 65 years and older. The folate intake of these two American cohorts showed a similar median or mean intake level to ours, but the vitamin B<sub>6</sub> and B<sub>12</sub> intakes were much higher than ours. In a large French cohort [37], the Three-City Study, including 1321 older persons aged 75.8 years on average, higher folate intake was associated with a decreased risk of dementia, but no association was found for vitamin B<sub>12</sub>. It is noteworthy that compared with our cohort, the French cohort had a relatively low baseline folate status (average intake = 278 µg/day) but relatively adequate intakes of B<sub>12</sub> (average intake = 5.7 µg/day). In a smaller British cohort with 155 participants aged 60–88 years [38], a lower dietary intake of vitamin B<sub>6</sub> (0.9–1.4 mg/day) at baseline predicted a greater-than-expected rate of global cognitive decline (decrease in

MMSE > 0.56 points per year). They concluded that vitamin B<sub>6</sub> may be an important protective factor for maintaining cognitive health on the basis of a mean dietary intake of vitamin B<sub>6</sub> (2.3 mg/day) similar to ours. Morris et al. [13] also observed that a higher intake of folate (median, 742 µg/day) than ours may have been associated with accelerated cognitive decline in 3718 Americans aged 65 years and older. Taken together, the inconsistencies between studies may be attributed to different intakes of B vitamins at baseline. As indicated by Smith and Refsum [15], the associations between the nutrient status and cognitive performance may follow a sigmoidal curve, which illustrates that additional nutrient intake is beneficial to a point but could be harmful at high intake, and it will have no effect at the plateau.

In our prospective study, the median intake of vitamin B<sub>6</sub> was higher than the RNI, and folate intake was near the RNI, whereas intake of vitamin B<sub>12</sub> was much less than the RNI. Consequently, significantly adverse effects on cognitive changes were only observed in vitamin B<sub>12</sub> deficiency, whereas beneficial effects on cognitive reserve were observed in all three B vitamins and even harmful effects in the highest quartile of vitamin B<sub>6</sub>. However, in our secondary case-control analysis, MCI patients had significantly lower dietary intake of folate but equivalent

**Table 5** General characteristics, cognitive performance, serum B vitamin biomarkers and oxidative biomarkers of study populations for DNA methylation discovery and validation

	Human Methylation450K BeadChip for discovery group			MassARRAY EpiTYPER for validation group		
	MCI N = 7	Controls N = 5	FDR adjusted P value	MCI N = 102	Controls N = 68	FDR adjusted P value
General characteristics						
Age	60.0 ± 2.4	61.4 ± 2.9	0.481	59.6 ± 3.0	59.6 ± 3.4	1.000
Gender (M/F)	3/4	2/3	1	40/62	40/28	0.041*
Education years	9.1 ± 3.3	10.8 ± 1.6	0.474	9 (9, 12)	11 (9, 15)	0.582
BMI (kg/m <sup>2</sup> )	26.0 ± 2.3	24.1 ± 2.4	0.386	24.2 ± 2.5	25.0 ± 2.7	0.134
Current smoker, n(%)	3 (42.9%)	2 (40.0%)	1	25 (24.5%)	25 (36.8%)	0.226
Current drinker, n(%)	1 (14.3%)	2 (40.0%)	0.572	19 (18.6%)	32 (47.1%)	< 0.001*
Diabetes, n(%)	3 (42.9%)	0 (0.0%)	0.407	20 (19.6%)	8 (11.8%)	0.375
Hypertension, n(%)	3 (42.9%)	2 (40.0%)	1	31 (31.4%)	22 (32.3%)	0.842
CHD, n(%)	2 (28.6%)	0 (0.0%)	0.416	5 (4.9%)	2 (2.9%)	0.691
Cognitive performance						
MMSE	28.0 (24.0, 29.0)	28.0 (29.0, 29.0)	0.411	28.0 (27.0, 29.0)	28.5 (27.0, 30.0)	0.226
MoCA	20.3 ± 2.8	26.6 ± 0.9	0.005*	22.4 ± 3.0	26.1 ± 2.3	< 0.001*
AVLT-IR	13.3 ± 4.1	16.8 ± 2.8	0.323	15.3 ± 4.5	18.4 ± 4.7	< 0.001*
AVLT-SR	4.6 ± 3.6	6.4 ± 1.5	0.474	5.1 ± 2.7	6.4 ± 2.3	0.003*
AVLT-LR	3.9 ± 4.0	3.8 ± 2.7	0.96	4 (2, 7)	6 (3, 8)	0.007*
SDMT	30.4 ± 14.3	41.2 ± 8.6	0.336	33 (25, 40)	37 (31, 46)	0.015*
LMT	8.0 ± 3.6	16.2 ± 3.1	0.010*	10.0 (5.5, 13.0)	14.5 (10.0, 16.5)	< 0.001*
DSF	6.5 ± 1.0	8.8 ± 0.8	0.010*	7.2 ± 1.3	7.9 ± 1.5	0.008*
DSB	4.3 ± 1.0	4.8 ± 0.4	0.474	4 (3, 5)	4 (4, 5)	0.016*
Dietary B vitamin intakes						
Vitamin B <sub>6</sub> (mg/day)	1.9 (1.4, 2.0)	2.6 (1.5, 2.8)	0.596	2.2 (1.8, 2.6)	2.2 (1.7, 2.7)	0.720
Folate (µg/day)	311.6 (203.6, 496.4)	577.2 (239.4, 705.0)	0.596	307.5 (242.6, 442.7)	376.7 (271.8, 532.5)	0.019*
Vitamin B <sub>12</sub> (µg/day)	1.2 (0.3, 2.8)	3.2 (1.8, 3.9)	0.427	2.0 (1.3, 2.9)	2.0 (1.2, 3.1)	0.936
Serum B vitamin biomarkers						
Vitamin B <sub>6</sub> (ng/L)	650.3 ± 125.0	542.3 ± 108.5	0.326	618.6 (531.2, 709.2)	596.5 (514.0, 645.9)	0.193
Folate (µg/L)	15.0 ± 5.6	30.2 ± 3.7	0.003*	17.4 (11.2, 22.8)	32.2 (28.0, 35.6)	< 0.001*
Vitamin B <sub>12</sub> (ng/L)	184.0 (113.0, 305.0)	284.0 (216.0, 333.5)	0.573	211.5 (174.8, 262.9)	198.5 (143.5, 256.3)	0.321
Hcy (µmol/L)	22.5 ± 2.9	9.8 ± 2.8	< 0.001*	21.1 (18.3, 23.4)	9.4 (6.7, 11.9)	< 0.001*
Oxidative biomarkers						
ROS (IU/ml)	1000.8 ± 345.3	779.1 ± 424.8	0.474	1043.8 (780.2, 1209.5)	728.0 (493.8, 964.8)	< 0.001*
MDA (mmol/L)	10.0 ± 1.7	7.0 ± 3.5	0.225	9.7 ± 2.8	8.1 ± 2.6	0.003*
8-OHdG (ng/L)	338.1 ± 112.2	185.2 ± 46.3	0.061	271.0 (201.1, 341.1)	151.2 (125.9, 176.7)	< 0.001*
8-iso-PGF2α (ng/L)	221.6 ± 59.1	118.2 ± 56.22	0.049*	188.0 (152.0, 279.6)	114.0 (90.3, 144.3)	< 0.001*

MMSE mini-mental state examination, MoCA Montreal Cognitive Assessment, AVLT-IR auditory verbal learning test-immediate recall, AVLT-SR auditory verbal learning test-short recall, AVLT-LR auditory verbal learning test-long recall, SDMT symbol digit modalities test, LMT logical memory test, DSF digit span forwards, DSB digit span backwards, BMI body mass index, CHD coronary heart disease, Hcy homocysteine, ROS reactive oxygen species, MDA malondialdehyde, 8-OHdG 8-hydroxy-desoxyguanosine, 8-iso-PGF2α 8-iso-prostaglandin F2α, FDR false discovery rate

Data shown as median (interquartile range) were compared between two groups using the Mann–Whitney U test

Data shown as mean ± standard deviation were compared between two groups using the Student t test

Data shown as n (%) were compared between two groups using the chi-square test or Fisher's exact test

\*P < 0.05

**Table 6** Correlations between serum B vitamin biomarkers and oxidative biomarkers

	Oxidative biomarkers							
	ROS (IU/ml)		MDA (mmol/L)		8-OHdG (ng/L)		8-iso-PGF2α (ng/L)	
	<i>r</i>	FDR adjusted <i>P</i> value	<i>r</i>	FDR adjusted <i>P</i> value	<i>r</i>	FDR adjusted <i>P</i> value	<i>r</i>	FDR adjusted <i>P</i> value
Serum B vitamin biomarkers								
Folate (µg/L)	-0.507	< 0.001*	-0.432	< 0.001*	-0.653	< 0.001*	-0.568	< 0.001*
Vitamin B <sub>6</sub> (ng/L)	0.069	0.742	0.155	0.293	-0.008	0.991	0.194	0.223
Vitamin B <sub>12</sub> (ng/L)	0.114	0.588	0.109	0.588	0.220	0.097	0.177	0.194
Hcy (µmol/L)	0.564	< 0.001*	0.400	< 0.001*	0.666	< 0.001*	0.547	< 0.001*

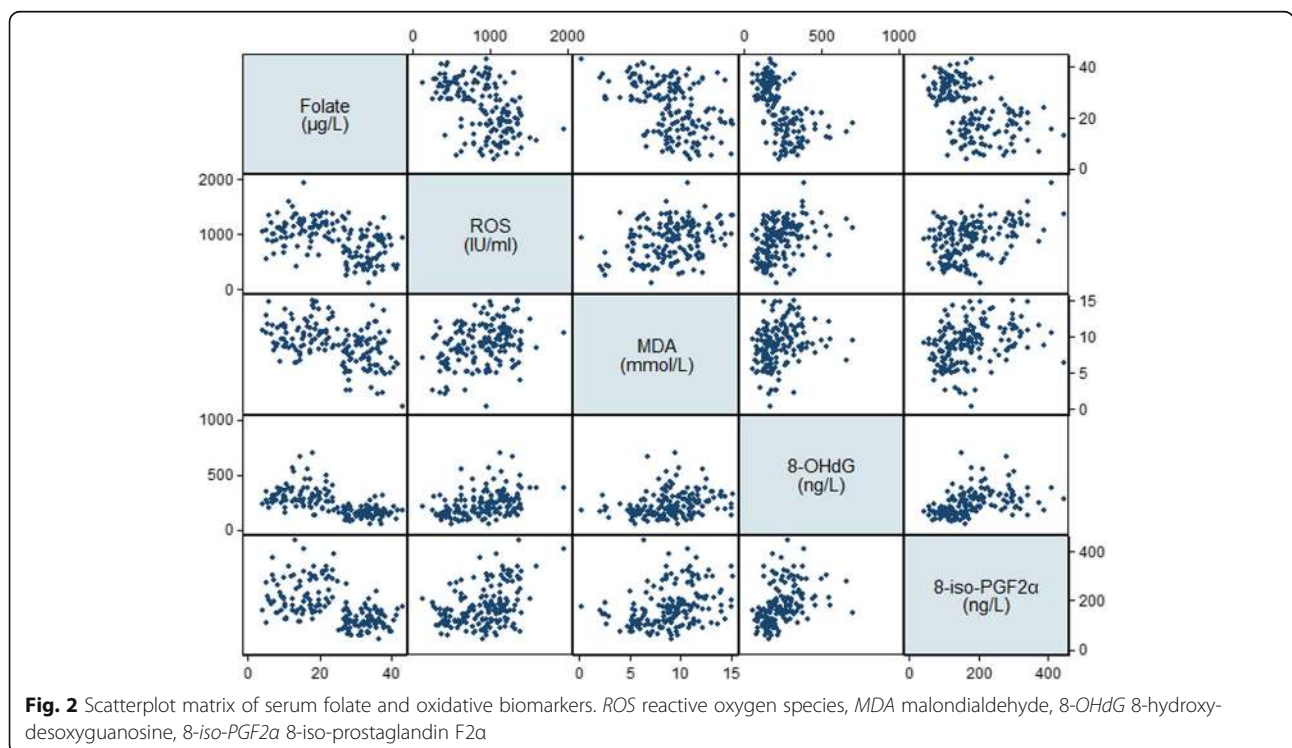
Hcy homocysteine, ROS reactive oxygen species, MDA malondialdehyde, 8-OHdG 8-hydroxy-desoxyguanosine, 8-iso-PGF2α 8-iso-prostaglandin F2α, FDR false discovery rate

\**P* < 0.05

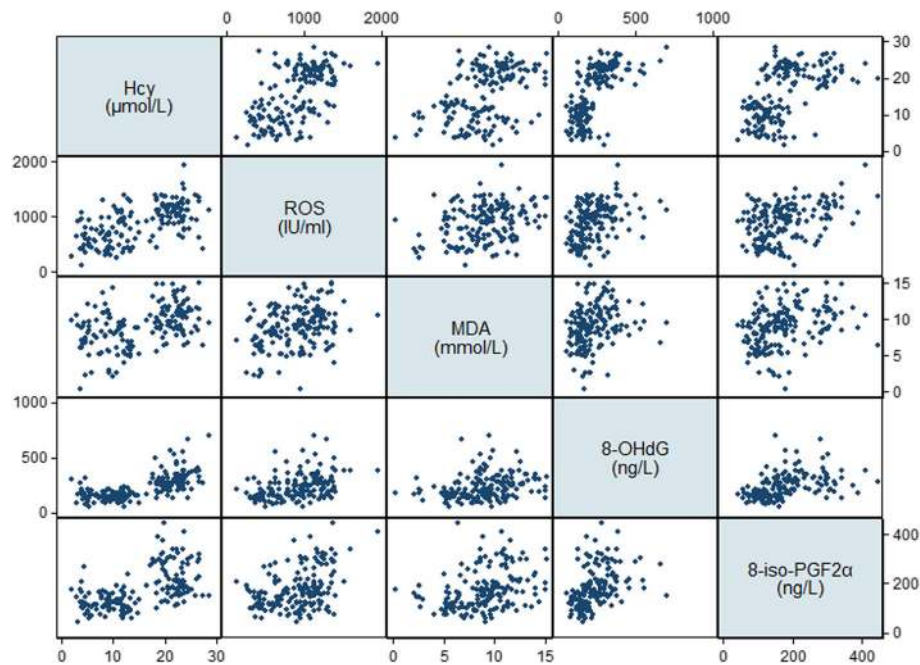
intakes of vitamin B<sub>6</sub> and B<sub>12</sub> compared with controls, which was in line with their serum profiles and thus contributed to increased Hcy level in the MCI group. With respect to the serum level of Hcy, a systemic review and meta-analysis [39] revealed a positive trend between cognitive impairment and increased Hcy concentration. Meanwhile, the vast majority of case-control studies also observed significantly decreased blood folate levels in MCI and AD patients [40–44]. In contrast to folate, there are some discrepancies with regard to the blood level of vitamin B<sub>12</sub>. Despite the same trend for vitamin B<sub>12</sub> as with folate in the above studies, many studies have found no significant differences between MCI/AD patients and healthy controls [32, 45–49], which was consistent with our results. The discrepant

results indicate that the nutrient status of vitamin B<sub>12</sub> in the subgroup of patients with cognitive impairment will not always be identical for the general population.

It is well documented that folate in one-carbon metabolism plays a central role in the synthesis, repair, and methylation of DNA, where it acts as a methyl donor. Folate homeostasis disruption could affect methylation potential through DNA gene hyper- or hypomethylation reactions and lead to gene transcription alterations (overexpression and/or gene silencing) [50, 51]. In our discovery group, genome-wide DNA methylation analysis identified that folate was significantly associated with DNA methylation of antioxidant genes NUDT15 and TXNRD1, both of which were hypermethylated in the MCI group. In addition to altered DNA methylation patterns, we found



**Fig. 2** Scatterplot matrix of serum folate and oxidative biomarkers. ROS reactive oxygen species, MDA malondialdehyde, 8-OHdG 8-hydroxy-desoxyguanosine, 8-iso-PGF2α 8-iso-prostaglandin F2α



**Fig. 3** Scatterplot matrix of serum Hcy and oxidative biomarkers. *Hcy* homocysteine, *ROS* reactive oxygen species, *MDA* malondialdehyde, *8-OHdG* 8-hydroxy-desoxyguanosine, *8-iso-PGF2α* 8-iso-prostaglandin F2α

that MCI patients also had imbalanced redox status, as indicated by significantly increased levels of ROS, damage markers of DNA oxidation (8-OHdG), and lipid peroxidation (MDA and 8-iso-PGF2α), suggesting that the production of oxidant species overwhelmed the endogenous antioxidant ability to destroy them [52], which corresponded to the hypermethylation and low/no transcription or gene silencing of *NUDT15* and *TXNRD1*.

*NUDT15*, also known as *MTH2*, is a member of the phosphatase protein family, which metabolizes a wide range of nucleotide substrates by hydrolyzing nucleoside triphosphates to their monophosphates and preventing the formation of 8-OHdG, the integration of the damaged purine nucleotides into DNA, and avoiding subsequent mismatch repair [53]. Lin et al. revealed that chronic hepatitis B virus X protein (HBx) could result in the accumulation of 8-OHdG in hepatocytes by inhibiting the expression of *NUDT15* [54]. We also observed that the DNA methylation levels of CpG<sub>19</sub> located in TSS1500 (promoter region) in *NUDT15* were positively correlated with ROS and 8-OHdG, indicating that the low/no transcription or gene silencing of *NUDT15* was associated with increased DNA damage and decreased genome stability, which was implicated in the initiation and progression of neurodegenerative diseases [55]. The cytosolic selenoprotein thioredoxin reductase 1 (TrxR1, encoded by *TXNRD1*) is a member of the thioredoxin system that is indispensable for redox homeostasis. With the support of several antioxidant systems, its

physiologic functions may protect normal cells from oxidative stress [56]. We also found that the hypermethylated CpG<sub>11</sub> of *TXNRD1* was positively correlated with 8-iso-PGF2α, an oxidative damage marker of lipid peroxidation in cell membranes, suggesting a dysregulation of TrxR1 owing to the downregulation of *TXNRD1*.

The methylation of CpG sites in *NUDT15* and *TXNRD1* was significantly correlated with folate and Hcy. Previous studies also investigated the relationship between folate and DNA methylation profile in redox-related genes in animal models and cells [57, 58]. They found that folate may suppress oxidative stress by inducing hypomethylation of *BNIP3* and *VPO1* but hypermethylation of *EC-SOD*, which probably indicated two important components of changes in DNA methylation. On the one hand, global hypomethylation, i.e., at most genes, demonstrated the role of folate as a methyl group donor and a reduction in the methylation of cytosine in DNA due to folate deficiency; on the other hand, focal hypermethylation might be affected by elevated Hcy levels. Such a phenomenon has been observed in carcinogenesis [59, 60].

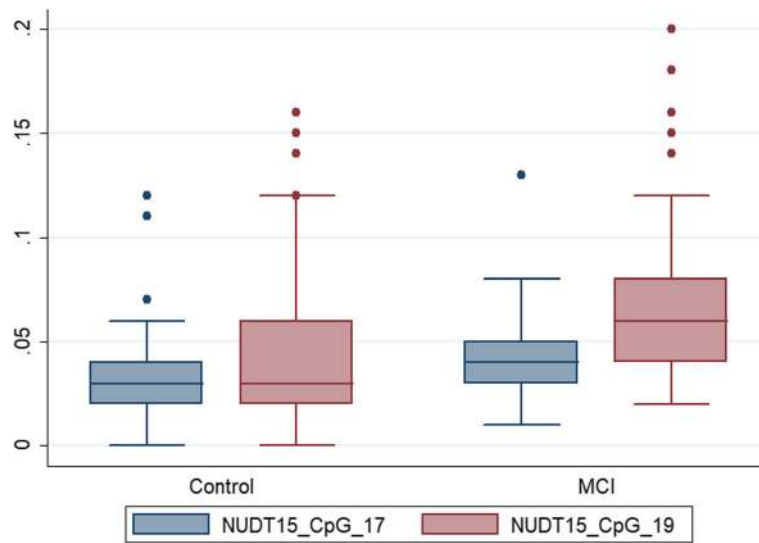
The interaction of Hcy with *NUDT15* and *TXNRD1* was inversely associated with the cognitive performance of global cognition, processing speed, and attention. In contrast, the interaction between folate and *TXNRD1* was positively associated with global cognition and immediate verbal memory. In line with our results, Fioravanti et al. [61] found significant improvement of memory and attention in folic acid supplementation

**Table 7** Variation of the methylation patterns of CpG sites located in NUDT15 and TNXR1 from validation

Gene	Gene feature	CpG	Sites	Fold change in methylation (MCI vs. control)	FDR adjusted P value
NUDT15	TSS1500	cg01596986	CpG_1	0.751	0.586
			CpG_2	0.955	0.714
			CpG_3	0.955	0.714
			CpG_4	0.795	0.089
			CpG_5	1.180	0.122
			CpG_6	0.649	0.842
			CpG_7	1.078	0.973
			CpG_8	0.992	0.975
			CpG_9	1.105	0.910
			CpG_10	2.351	0.582
			CpG_11	1.048	0.476
			CpG_12	1.062	0.661
			CpG_13	0.919	0.582
			CpG_14	1.026	0.810
			CpG_15	0.959	0.935
			CpG_16	1.088	0.447
			CpG_17	1.157	0.030*
			CpG_18	1.210	0.525
			CpG_19	1.288	0.024*
			CpG_20	0.952	0.529
			CpG_21	1.210	0.525
			CpG_22	1.245	0.276
			CpG_23	1.022	0.935
			CpG_24	1.092	0.594
			CpG_25	1.119	0.529
TNXR1	5'UTR	cg12166806	CpG_1	0.917	0.714
			CpG_2	0.787	0.615
			CpG_3	1.495	0.375
			CpG_4	1.092	0.375
			CpG_5	0.941	0.890
			CpG_6	1.092	0.375
			CpG_7	1.156	0.447
			CpG_8	1.217	0.386
			CpG_9	1.015	0.029*
			CpG_10	0.987	0.910
			CpG_11	1.191	0.018*
			CpG_12	1.120	0.910
			CpG_13	0.982	0.975
			CpG_14	0.940	0.475
			CpG_15	1.139	0.241
			CpG_16	1.054	0.626
			CpG_17	1.310	0.475

FDR false discovery rate, *NUDT15* nudix hydrolase 15, *TNXR1* thioredoxin reductase 1, *TSS1500* 1500 base pairs around the transcription start site, *5'UTR* 5'untranslated region

\* $P < 0.05$

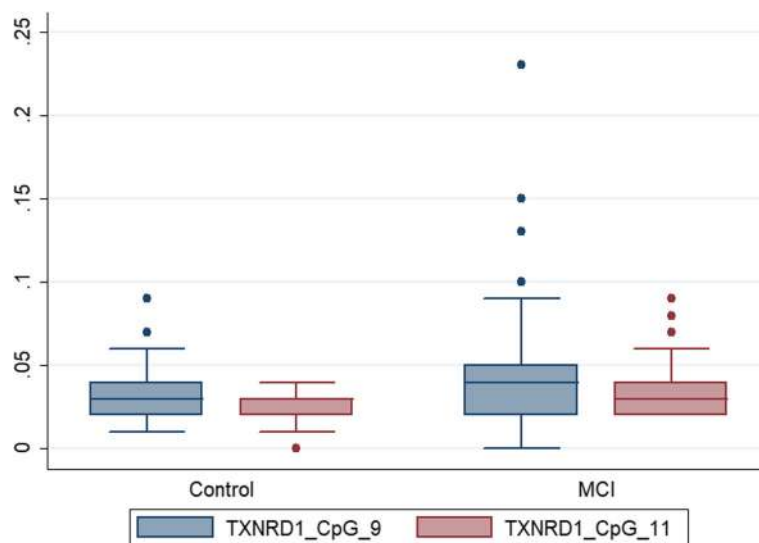


**Fig. 4** Comparisons in DNA methylation for CpG<sub>17</sub> and CpG<sub>19</sub> located within *NUDT15* between MCI patients and controls. The box lines represent median ± interquartile ranges of DNA methylation, which is expressed as a beta value (0-1). *NUDT15* nudix hydrolase 15, *TXNRD1* thioredoxin reductase 1, *MCI* mild cognitive impairment

among older participants selected for low initial folate in a small pilot trial. Durga et al. [62] conducted a randomized, double-blind, placebo-controlled study that included participants aged 50 years or above with high levels of Hcy and found significant beneficial effects of folic acid supplementation on memory and processing speed. Taken together, the results seem to demonstrate that disruption of the homeostasis of one-carbon metabolism induces altered DNA methylation patterns of *NUDT15* and *TXNRD1* and thus leads to oxidative

stress overload and increased susceptibility to cognitive impairment.

Our findings indicate that the harmful role of vitamin B<sub>12</sub> deficiency and beneficial effects of adequate folate intake in populations with relatively low basal vitamin B<sub>12</sub> and folate status, such as middle-aged and elderly persons from China, a country with no B vitamin fortification and relatively low average intake levels of vitamin B<sub>12</sub> and folate, may be worth exploring in future dementia prevention trials that implement food vitamin



**Fig. 5** Comparisons in DNA methylation for CpG<sub>9</sub> and CpG<sub>11</sub> located within *TXNRD1* between MCI patients and controls. The box lines represent median ± interquartile ranges of DNA methylation, which is expressed as a beta value (0-1). *NUDT15* nudix hydrolase 15, *TXNRD1* thioredoxin reductase 1, *MCI* mild cognitive impairment

**Table 8** Correlations for serum B vitamin biomarkers, oxidative biomarkers, and DNA methylation of NUDT15 and TXNRD1 genes

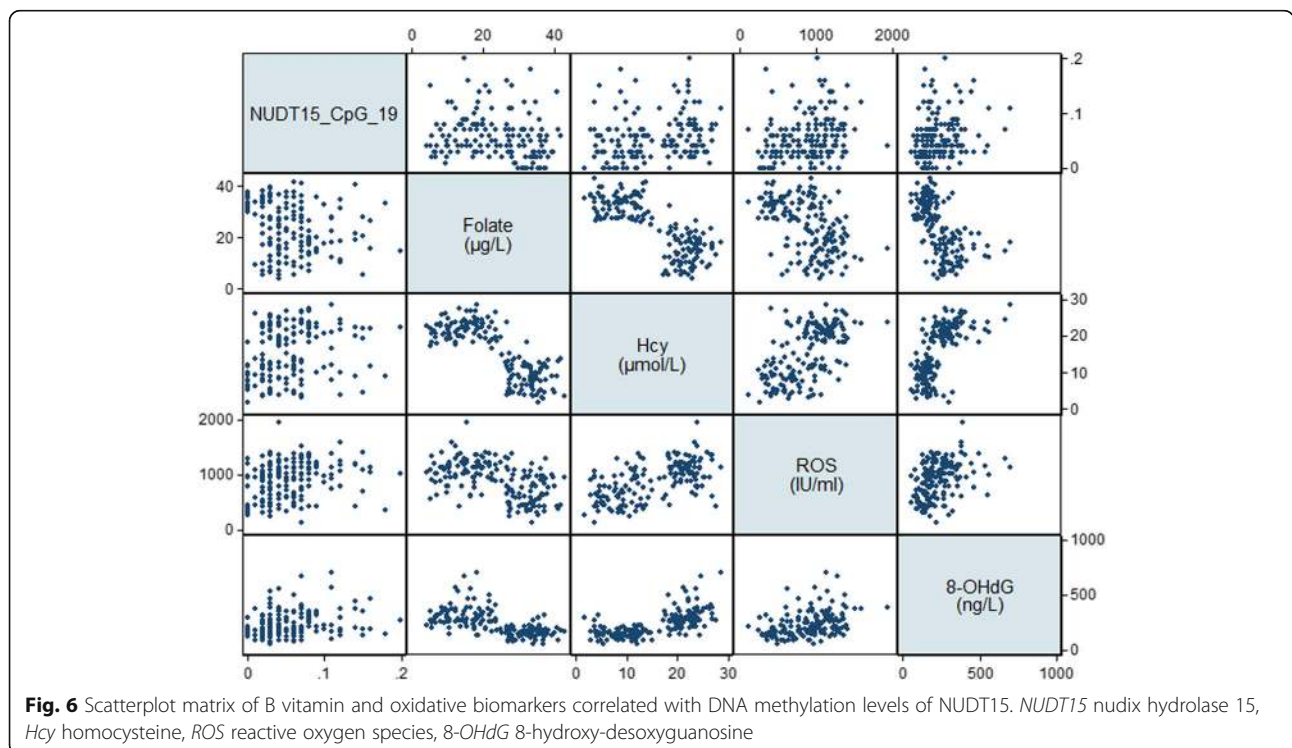
	NUDT15_CpG_17		NUDT15_CpG_19		TXNRD1_CpG_9		TXNRD1_CpG_11	
	<i>r</i>	FDR adjusted <i>P</i> value	<i>r</i>	FDR adjusted <i>P</i> value	<i>r</i>	FDR adjusted <i>P</i> value	<i>r</i>	FDR adjusted <i>P</i> value
B vitamin and metabolites								
Folate (µg/L)	-0.101	0.417	-0.219	0.028*	-0.201	0.133	-0.192	0.041*
Vitamin B <sub>6</sub> (ng/L)	-0.030	0.815	-0.074	0.559	0.009	0.949	0.029	0.930
Vitamin B <sub>12</sub> (ng/L)	0.022	0.962	0.041	0.930	0.086	0.738	0.079	0.738
Hcy (µmol/L)	0.098	0.434	0.251	0.010*	0.198	0.133	0.225	0.045*
Oxidative biomarkers								
ROS (IU/ml)	0.069	0.762	0.230	0.020*	0.164	0.169	0.157	0.176
MDA (mmol/L)	0.068	0.561	0.125	0.268	0.143	0.236	0.123	0.296
8-OHdG (ng/L)	0.132	0.248	0.259	0.008*	0.180	0.145	0.171	0.121
8-iso-PGF2α (ng/L)	0.130	0.248	0.183	0.074	0.154	0.210	0.192	0.042*

*Hcy* homocysteine, *ROS* reactive oxygen species, *MDA* malondialdehyde, *8-OHdG* 8-hydroxy-desoxyguanosine, *8-iso-PGF2α* 8-iso-prostaglandin F2α, *FDR* false discovery rate, *NUDT15* nudix hydrolase 15, *TXNRD1* thioredoxin reductase 1  
 \**P* < 0.05

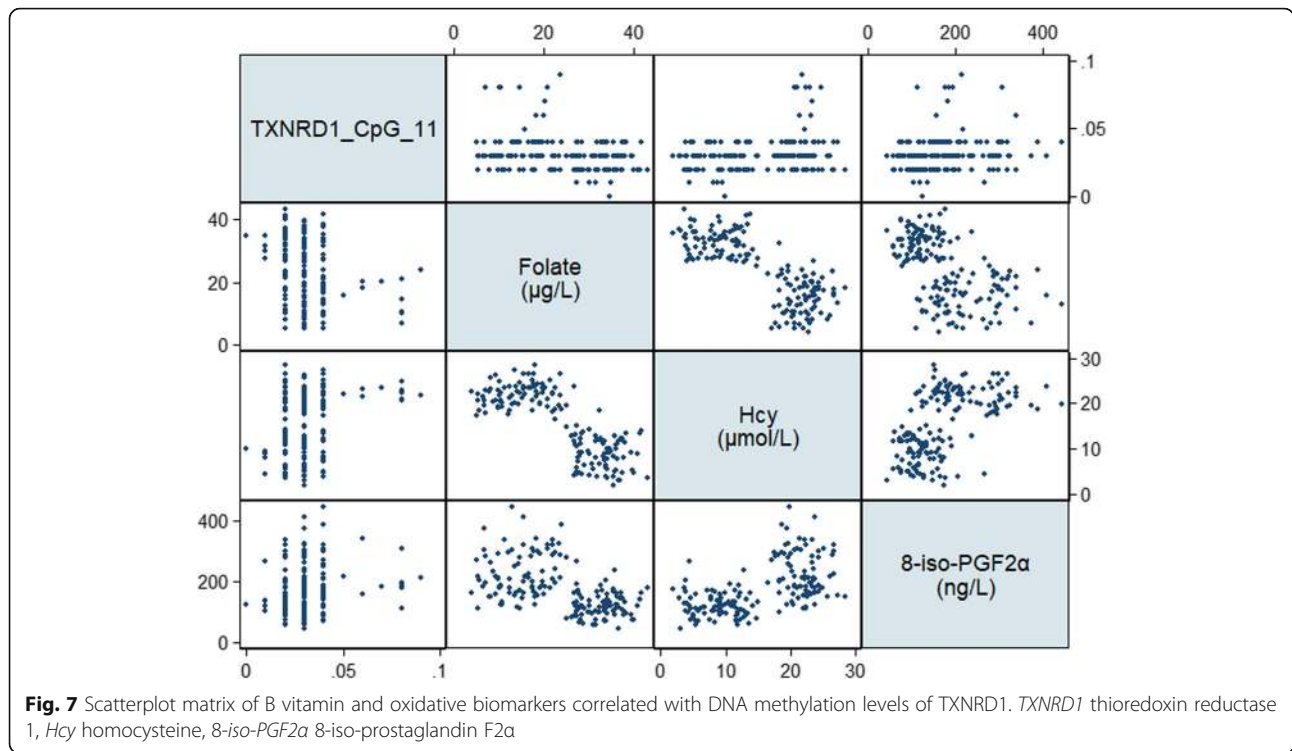
B<sub>12</sub> and folate fortification programs to cover the requirements of the target population. Sanchez et al. [50] have reported that several countries, including Chile, started a policy of food folate and vitamin B<sub>12</sub> fortification for older adults which led to elevated serum levels and decreased prevalence of deficiency. Cui et al. [57] demonstrated in vivo that folic acid supplementation may reduce oxidative stress and ROC levels by changing the DNA methylation of the oxidative stress-related gene VPO1. The validated genes in our study could also serve

as potential novel targets to prevent dementia in MCI patients.

Given the interactive nature of nutrient metabolism and action and their different dietary sources, e.g., the vitamin B complex group, nutrient patterns (NPs) are advantageous since they capture the interactive effect of nutrients in combination [63]. Our previous cross-sectional study used exploratory factor analysis to generate three dietary nutrient intake combination patterns [9]. The first pattern was characterized as a “vitamin and mineral pattern” that



**Fig. 6** Scatterplot matrix of B vitamin and oxidative biomarkers correlated with DNA methylation levels of NUDT15. *NUDT15* nudix hydrolase 15, *Hcy* homocysteine, *ROS* reactive oxygen species, *8-OHdG* 8-hydroxy-desoxyguanosine



**Fig. 7** Scatterplot matrix of B vitamin and oxidative biomarkers correlated with DNA methylation levels of TXNRD1. TXNRD1 thioredoxin reductase 1, Hcy homocysteine, 8-iso-PGF2α 8-iso-prostaglandin F2α

**Table 9** Effects of DNA methylation and the interaction with B vitamin biomarkers on global and domain-specific cognitive performance

Cognitive performance		NUDT15	NUDT15xFolate	NUDT15xHcy	TXNRD1	TXNRD1xFolate	TXNRD1xHcy
MMSE	<i>B</i>	-0.018	0.044	-0.082	-0.005	0.147	-0.103
	<i>P</i> value	0.809	0.559	0.287	0.947	0.048*	0.178
MoCA	<i>B</i>	-0.127	0.096	-0.325	-0.124	0.268	-0.345
	<i>P</i> value	0.077	0.183	< 0.001*	0.082	< 0.001*	< 0.001*
AVLT-IR	<i>B</i>	-0.026	0.102	-0.125	-0.123	0.163	-0.259
	<i>P</i> value	0.752	0.208	0.130	0.126	0.042*	0.001*
AVLT-SR	<i>B</i>	0.013	0.066	-0.055	-0.082	0.022	-0.151
	<i>P</i> value	0.870	0.39	0.489	0.283	0.775	0.056
AVLT-LR	<i>B</i>	-0.004	0.063	-0.062	-0.054	0.090	-0.151
	<i>P</i> value	0.960	0.421	0.442	0.487	0.251	0.060
SDMT	<i>B</i>	-0.086	-0.002	-0.132	-0.090	0.051	-0.137
	<i>P</i> value	0.213	0.980	0.059	0.185	0.456	0.049*
LMT	<i>B</i>	-0.046	0.098	-0.187	-0.129	0.091	-0.269
	<i>P</i> value	0.549	0.194	0.015*	0.186	0.231	< 0.001*
DSF	<i>B</i>	-0.087	0.034	-0.221	-0.095	0.106	-0.252
	<i>P</i> value	0.262	0.664	0.005*	0.223	0.174	0.001*
DSB	<i>B</i>	-0.064	-0.003	-0.079	0.020	0.118	-0.052
	<i>P</i> value	0.410	0.969	0.314	0.797	0.127	0.509

MMSE mini-mental state examination, MoCA Montreal Cognitive Assessment, AVLT-IR auditory verbal learning test-immediate recall, AVLT-SR auditory verbal learning test-short recall, AVLT-LR auditory verbal learning test-long recall, SDMT symbol digit modalities test, LMT logical memory test, DSF digit span forwards, DSB digit span backwards, Hcy homocysteine, NUDT15 nudix hydrolase 15, TXNRD1 thioredoxin reductase 1

*B*: standardized regression coefficients of multiple linear regression

\**P* < 0.05



included eight vitamins and six minerals, such as vitamin B<sub>1</sub>, vitamin B<sub>2</sub>, vitamin B<sub>3</sub>, vitamin B<sub>6</sub>, and folate. This pattern was identified as the most protective combination, with an adjusted odds ratio (OR) of 0.77 (0.71–0.83) for developing MCI. Our longitudinal studies with follow-ups will also generate and use NPs to explore and better define the cause-effect relationships between dietary nutrient intakes and cognitive decline as well as the distribution of benefits and harmful effects of the vitamin B complex group in this cohort.

A highlight of this study is the combination of prospective and case-control studies to investigate the effects of dietary and serum biomarker patterns of B vitamins in the general population and MCI patients, accompanied by mechanistic exploration. One limitation of the present study is the lack of RNA isolation to evaluate redox-related gene expression and its association with DNA methylation levels. However, information on oxidative damage markers suggests that hypermethylated redox-related genes were less expressed. The discovery group included a relatively smaller sample size, which may limit the candidate genes correlated with B vitamins. The lack of additional serum biomarkers of vitamin B<sub>12</sub> (e.g., methylmalonic acid or holotranscobalamin) could have decreased the precision of status assessment. Finally, the method used to determine methylation status could have influenced the obtained results.

## Conclusion

Inadequate intake of vitamin B<sub>12</sub> contributes to global and domain-specific cognitive decline, but adequate folate, vitamin B<sub>6</sub>, and vitamin B<sub>12</sub> have beneficial effects on cognitive reserve in cognitively healthy persons. Decreased levels of circulating folate and increased Hcy were associated with hypermethylated redox-related genes and oxidative damage in MCI patients. The interaction between biomarker patterns of B vitamins and hypermethylated genes has significant effects on cognitive performance. These findings may provide unique leads for the combination of oxidative stress and DNA methylation when unraveling the mechanisms underlying the deleterious effects of B vitamin deficiency and hyperhomocysteinemia on cognition.

## Abbreviations

8-iso-PGF<sub>2</sub>α: 8-Iso-prostaglandin F<sub>2</sub>α; 8-OHdG: 8-Hydroxy-desoxyguanosine; AD: Alzheimer's disease; AVLT: Auditory Verbal Learning Test; BMI: Body mass index; CHD: Coronary heart disease; DSB: Digit span backward; DSF: Digit span forward; EMCOA: Effects and Mechanism Investigation of Cholesterol and Oxysterol on Alzheimer's disease; FDR: False discovery rate; FFQ: Food Frequency Questionnaire; Hcy: Homocysteine; LMT: Logical Memory Test; MCI: Mild cognitive impairment; MDA: Malondialdehyde; MMSE: Mini-Mental State Examination; MoCA: Montreal Cognitive Assessment; NUDT15: Nudix hydrolase 15; RCT: Randomized controlled trials; ROS: Reactive oxygen species; SAM: S-adenosyl-methionine; SDMT: Symbol Digit Modalities Test; TXNRD1: Thioredoxin reductase 1; WMS-RC: Wechsler Memory Scale-Revised, Chinese version

## Acknowledgements

Not applicable.

## Authors' contribution

RX conceived and designed the study, YA performed the analyses and wrote the manuscript. LF, XZ, YW, YW, LT, and ZQ helped collect and analyze the data. All authors read and approved the final manuscript.

## Funding

This work was supported by the State Key Program of the National Natural Science Foundation of China (Grant No. 81330065) and National Natural Science Foundation of China (Grant No. 81673149 & 81973021).

## Availability of data and materials

The datasets during and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Ethics approval and consent to participate

The study design was ethically approved by the Ethics Committee of Capital Medical University (2013SY35). All participants were provided written informed consent at the beginning of the study.

## Consent for publication

All the co-authors and participants have given their consent for publication in *Clinical Epigenetics*.

## Competing interests

The authors declare that they have no competing interests.

## Author details

<sup>1</sup>School of Public Health, Capital Medical University, No.10 Xitoutiao, You An Men Wai, Beijing 100069, China. <sup>2</sup>Peking University First Hospital, Beijing, China. <sup>3</sup>Jincheng People's Hospital, Jincheng, China.

Received: 7 April 2019 Accepted: 8 September 2019

Published online: 11 October 2019

## References

- Jutten RJ, Harrison JE, Lee MKP, Ingala S, Vreeswijk R, van Deelen R, de Jong FJ, Opmeer EM, Aleman A, Ritchie CW, et al. Assessing cognition and daily function in early dementia using the cognitive-functional composite: findings from the Catch-Cog study cohort. *Alzheimers Res Ther*. 2019;11(1):45.
- Garre-Olmo J, Vilalta-Franch J, Calvo-Pexas L, Lopez-Pousa S. A path analysis of dependence and quality of life in Alzheimer's disease. *Am J Alzheimers Dis Other Demen*. 2017;32(2):108–15.
- Moore K, Hughes CF, Ward M, Hoey L, McNulty H. Diet, nutrition and the ageing brain: current evidence and new directions. *Proc Nutr Soc*. 2018; 77(2):152–63.
- Martyr A, Nelis SM, Quinn C, Rusted JM, Morris RG, Clare L. The relationship between perceived functional difficulties and the ability to live well with mild-to-moderate dementia: Findings from the IDEAL programme. *Int J Geriatr Psychiatry*. 2019.
- Stites SD, Harkins K, Rubright JD, Karlawish J. Relationships between cognitive complaints and quality of life in older adults with mild cognitive impairment, mild Alzheimer disease dementia, and normal cognition. *Alzheimer Dis Assoc Disord*. 2018;32(4):276–83.
- Babulal GM, Quiroz YT, Albenis BC, Arenaza-Urquijo E, Astell AJ, Babiloni C, Bahar-Fuchs A, Bell J, Bowman GL, Brickman AM, et al. Perspectives on ethnic and racial disparities in Alzheimer's disease and related dementias: Update and areas of immediate need. *Alzheimers Dement*. 2018.
- Forlenza OV, Diniz BS, Stella F, Teixeira AL, Gattaz WF. Mild cognitive impairment. Part 1: clinical characteristics and predictors of dementia. *Rev Bras Psiquiatr*. 2013;35(2):178–85.
- Reay JL, Smith MA, Riby LM. B vitamins and cognitive performance in older adults: review. *ISRN Nutr*. 2013;2013:650983.
- Lu Y, An Y, Guo J, Zhang X, Wang H, Rong H, Xiao R. Dietary intake of nutrients and lifestyle affect the risk of mild cognitive impairment in the Chinese elderly population: a cross-sectional study. *Front Behav Neurosci*. 2016;10:229.

10. Beydoun MA, Beydoun HA, Gamaldo AA, Teel A, Zonderman AB, Wang Y. Epidemiologic studies of modifiable factors associated with cognition and dementia: systematic review and meta-analysis. *BMC Public Health*. 2014;14:643.
11. Porter K, Hoey L, Hughes CF, Ward M, McNulty H. Causes, consequences and public health implications of low B-vitamin status in ageing. *Nutrients*. 2016;8(11).
12. Kim H, Kim G, Jang W, Kim SY, Chang N. Association between intake of B vitamins and cognitive function in elderly Koreans with cognitive impairment. *Nutr J*. 2014;13(1):118.
13. Morris MC, Evans DA, Bienias JL, Tangney CC, Hebert LE, Scherr PA, Schneider JA. Dietary folate and vitamin B12 intake and cognitive decline among community-dwelling older persons. *Arch Neurol*. 2005;62(4):641–5.
14. Li MM, Yu JT, Wang HF, Jiang T, Wang J, Meng XF, Tan CC, Wang C, Tan L. Efficacy of vitamins B supplementation on mild cognitive impairment and Alzheimer's disease: a systematic review and meta-analysis. *Curr Alzheimer Res*. 2014;11(9):844–52.
15. Smith AD, Refsum H. Homocysteine, B vitamins, and cognitive impairment. *Annu Rev Nutr*. 2016;36:211–39.
16. Bonetti F, Brombo G, Magon S, Zuliani G. Cognitive status according to homocysteine and B-group vitamins in elderly adults. *J Am Geriatr Soc*. 2015;63(6):1158–63.
17. Obeid R, Herrmann W. Mechanisms of homocysteine neurotoxicity in neurodegenerative diseases with special reference to dementia. *Febs Lett*. 2006;580(13):2994–3005.
18. Athanasiopoulos D, Karagiannis G, Tsolaki M. Recent findings in Alzheimer disease and nutrition focusing on epigenetics. *Adv Nutr*. 2016;7(5):917–27.
19. Sanchez-Espinosa MP, Atienza M, Cantero JL. Sleep mediates the association between homocysteine and oxidative status in mild cognitive impairment. *Sci Rep*. 2017;7(1):7719.
20. An Y, Feng L, Zhang X, Wang Y, Wang Y, Tao L, Lu Y, Qin Z, Xiao R. Patterns of cognitive function in middle-aged and elderly Chinese adults—findings from the EMCOA study. *Alzheimers Res Ther*. 2018;10(1):93.
21. 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.
22. Price KL, DeSantis SM, Simpson AN, Tolliver BK, McRae-Clark AL, Saladin ME, Baker NL, Wagner MT, Brady KT. The impact of clinical and demographic variables on cognitive performance in methamphetamine-dependent individuals in rural South Carolina. *Am J Addict*. 2011;20(5):447–55.
23. Ma J, Zhang Y, Guo Q. Comparison of vascular cognitive impairment—no dementia by multiple classification methods. *Int J Neurosci*. 2015;125(11):823–30.
24. Wang C, An Y, Yu H, Feng L, Liu Q, Lu Y, Wang H, Xiao R. Association between exposure to the Chinese famine in different stages of early life and decline in cognitive functioning in adulthood. *Front Behav Neurosci*. 2016;10:146.
25. Miu J, Negin J, Salinas-Rodriguez A, Manrique-Espinoza B, Sosa-Ortiz AL, Cumming R, Kowal P. Factors associated with cognitive function in older adults in Mexico. *Glob Health Action*. 2016;9:30747.
26. Lam B, Middleton LE, Masellis M, Stuss DT, Harry RD, Kiss A, Black SE. Criterion and convergent validity of the Montreal cognitive assessment with screening and standardized neuropsychological testing. *J Am Geriatr Soc*. 2013;61(12):2181–5.
27. Lu J, Li D, Li F, Zhou A, Wang F, Zuo X, Jia XF, Song H, Jia J. Montreal cognitive assessment in detecting cognitive impairment in Chinese elderly individuals: a population-based study. *J Geriatr Psychiatry Neurol*. 2011;24(4):184–90.
28. Zhang CX, Ho SC. Validity and reproducibility of a food frequency Questionnaire among Chinese women in Guangdong province. *Asia Pac J Clin Nutr*. 2009;18(2):240–50.
29. Zhang CX, Pan MX, Li B, Wang L, Mo XF, Chen YM, Lin FY, Ho SC. Choline and betaine intake is inversely associated with breast cancer risk: a two-stage case-control study in China. *Cancer Sci*. 2013;104(2):250–8.
30. Zeng FF, Liu YT, Lin XL, Fan YY, Zhang XL, Xu CH, Chen YM. Folate, vitamin B6, vitamin B12 and methionine intakes and risk for nasopharyngeal carcinoma in Chinese adults: a matched case-control study. *Br J Nutr*. 2016;115(1):121–8.
31. Kennedy DO. B Vitamins and the Brain: Mechanisms, dose and efficacy—a review. *Nutrients*. 2016;8(2):68.
32. Quadri P, Fragiaco C, Pezzati R, Zanda E, Forloni G, Tettamanti M, Lucca U. Homocysteine, folate, and vitamin B-12 in mild cognitive impairment, Alzheimer disease, and vascular dementia. *Am J Clin Nutr*. 2004;80(1):14–22.
33. Ramos MI, Allen LH, Mungas DM, Jagust WJ, Haan MN, Green R, Miller JW. Low folate status is associated with impaired cognitive function and dementia in the Sacramento Area Latino Study on Aging. *Am J Clin Nutr*. 2005;82(6):1346–52.
34. Haan MN, Miller JW, Aiello AE, Whitmer RA, Jagust WJ, Mungas DM, Allen LH, Green R. Homocysteine, B vitamins, and the incidence of dementia and cognitive impairment: results from the Sacramento Area Latino Study on Aging. *Am J Clin Nutr*. 2007;85(2):511–7.
35. Morris MC, Evans DA, Schneider JA, Tangney CC, Bienias JL, Aggarwal NT. Dietary folate and vitamins B-12 and B-6 not associated with incident Alzheimer's disease. *J Alzheimers Dis*. 2006;9(4):435–43.
36. Nelson C, Wengreen HJ, Munger RG, Corcoran CD. Dietary folate, vitamin B-12, vitamin B-6 and incident Alzheimer's disease: the cache county memory, health and aging study. *J Nutr Health Aging*. 2009;13(10):899–905.
37. Lefevre-Arbogast S, Fear C, Dartigues JF, Helmer C, Letenneur L, Samieri C. Dietary B vitamins and a 10-year risk of dementia in older persons. *Nutrients*. 2016;8(12).
38. Hughes CF, Ward M, Tracey F, Hoey L, Molloy AM, Pentieva K, McNulty H. B-vitamin intake and biomarker status in relation to cognitive decline in healthy older adults in a 4-year follow-up study. *Nutrients*. 2017;9(1).
39. Setien-Suero E, Suarez-Pinilla M, Suarez-Pinilla P, Crespo-Facorro B, Ayesa-Arriola R. Homocysteine and cognition: a systematic review of 111 studies. *Neurosci Biobehav Rev*. 2016;69:280–98.
40. Chen H, Liu S, Ji L, Wu T, Ma F, Ji Y, Zhou Y, Zheng M, Zhang M, Huang G. Associations between Alzheimer's disease and blood homocysteine, vitamin B12, and folate: a case-control study. *Curr Alzheimer Res*. 2015;12(1):88–94.
41. Ma F, Wu T, Zhao J, Ji L, Song A, Zhang M, Huang G. Plasma homocysteine and serum folate and vitamin B12 levels in mild cognitive impairment and Alzheimer's disease: a case-control study. *Nutrients*. 2017;9(7).
42. Malaguarnera M, Ferri R, Bella R, Alagona G, Carnemolla A, Pennisi G. Homocysteine, vitamin B12 and folate in vascular dementia and in Alzheimer disease. *Clin Chem Lab Med*. 2004;42(9):1032–5.
43. Dimopoulos N, Piperi C, Salonicioti A, Psarra V, Gazi F, Nounopoulos C, Lea RW, Kalofoutis A. Association of cognitive impairment with plasma levels of folate, vitamin B12 and homocysteine in the elderly. *In Vivo*. 2006;20(6):895–9.
44. Koseoglu E, Karaman Y. Relations between homocysteine, folate and vitamin B12 in vascular dementia and in Alzheimer disease. *Clin Biochem*. 2007;40(12):859–63.
45. Kim G, Kim H, Kim KN, Son JI, Kim SY, Tamura T, Chang N. Relationship of cognitive function with B vitamin status, homocysteine, and tissue factor pathway inhibitor in cognitively impaired elderly: a cross-sectional survey. *J Alzheimers Dis*. 2013;33(3):853–62.
46. Faux NG, Ellis KA, Porter L, Fowler CJ, Laws SM, Martins RN, Pertile KK, Rembach A, Rowe CC, Rumble RL, et al. Homocysteine, vitamin B12, and folic acid levels in Alzheimer's disease, mild cognitive impairment, and healthy elderly: baseline characteristics in subjects of the Australian Imaging Biomarker Lifestyle study. *J Alzheimers Dis*. 2011;27(4):909–22.
47. Kim S, Choi BY, Nam JH, Kim MK, Oh DH, Yang YJ. Cognitive impairment is associated with elevated serum homocysteine levels among older adults. *Eur J Nutr*. 2018.
48. Irizarry MC, Guro ME, Raju S, Diaz-Arrastia R, Locascio JJ, Tennis M, Hyman BT, Growdon JH, Greenberg SM, Bottiglieri T. Association of homocysteine with plasma amyloid beta protein in aging and neurodegenerative disease. *Neurology*. 2005;65(9):1402–8.
49. Gorgone G, Ursini F, Altamura C, Bressi F, Tombini M, Curcio G, Chioyenda P, Squitti R, Silvestrini M, Ientile R, et al. Hyperhomocysteinemia, intima-media thickness and C677T MTHFR gene polymorphism: a correlation study in patients with cognitive impairment. *Atherosclerosis*. 2009;206(1):309–13.
50. Sanchez H, Hossain MB, Lera L, Hirsch S, Albal C, Uauy R, Broberg K, Ronco AM. High levels of circulating folate concentrations are associated with DNA methylation of tumor suppressor and repair genes p16, MLH1, and MGMT in elderly Chileans. *Clin Epigenetics*. 2017;9:74.
51. de Oliveira Y, Lima R, Luna R, Monteiro M, Da SC, Do NR, de Farias LK, Andrade ESA, de Lima FF, de Toledo VR, et al. Decrease of the DNA methylation levels of the ADRB3 gene in leukocytes is related with serum folate in eutrophic adults. *J Transl Med*. 2018;16(1):152.
52. Pratico D. Oxidative stress hypothesis in Alzheimer's disease: a reappraisal. *Trends Pharmacol Sci*. 2008;29(12):609–15.
53. Hashiguchi K, Hayashi M, Sekiguchi M, Umezaki K. The roles of human MTH1, MTH2 and MTH3 proteins in maintaining genome stability under oxidative stress. *Mutat Res*. 2018;808:10–9.

54. Lin YT, Liu W, He Y, Wu YL, Chen WN, Lin XJ, Lin X. Hepatitis B Virus X Protein Increases 8-Oxo-7,8-Dihydro-2'-Deoxyguanosine (8-Oxodg) Level via Repressing MTH1/ MTH2 Expression in Hepatocytes. *Cell Physiol Biochem*. 2018;51(1):80–96.
55. Wojsiat J, Zoltowska KM, Laskowska-Kaszub K, Wojda U: Oxidant/antioxidant imbalance in Alzheimer's disease: therapeutic and diagnostic prospects. *Oxid Med Cell Longev* 2018, 2018:6435861.
56. Amer E. Targeting the selenoprotein thioredoxin reductase 1 for anticancer therapy. *Adv Cancer Res*. 2017;136:139–51.
57. Cui S, Lv X, Li W, Li Z, Liu H, Gao Y, Huang G. Folic acid modulates VPO1 DNA methylation levels and alleviates oxidative stress-induced apoptosis in vivo and in vitro. *Redox Biol*. 2018;19:81–91.
58. Lakshmi SV, Naushad SM, Reddy CA, Saumya K, Rao DS, Kotamraju S, Kutala VK. Oxidative stress in coronary artery disease: epigenetic perspective. *Mol Cell Biochem*. 2013;374(1-2):203–11.
59. Paz MF, Avila S, Fraga MF, Pollan M, Capella G, Peinado MA, Sanchez-Cespedes M, Herman JG, Esteller M. Germ-line variants in methyl-group metabolism genes and susceptibility to DNA methylation in normal tissues and human primary tumors. *Cancer Res*. 2002;62(15):4519–24.
60. Novakovic P, Stempak JM, Sohn KJ, Kim YI. Effects of folate deficiency on gene expression in the apoptosis and cancer pathways in colon cancer cells. *Carcinogenesis*. 2006;27(5):916–24.
61. Fioravanti M, Ferrario E, Massaia M, Cappa G, Rivolta G, Grossi E, Buckley AE. Low folate levels in the cognitive decline of elderly patients and the efficacy of folate as a treatment for improving memory deficits. *Arch Gerontol Geriatr*. 1998;26(1):1–13.
62. Durga J, van Boxtel MP, Schouten EG, Kok FJ, Jolles J, Katan MB, Verhoef P. Effect of 3-year folic acid supplementation on cognitive function in older adults in the FACIT trial: a randomised, double blind, controlled trial. *Lancet*. 2007;369(9557):208–16.
63. Berti V, Murray J, Davies M, Spector N, Tsui WH, Li Y, Williams S, Pirraglia E, Vallabhajosula S, McHugh P, et al. Nutrient patterns and brain biomarkers of Alzheimer's disease in cognitively normal individuals. *J Nutr Health Aging*. 2015;19(4):413–23.

## Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Ready to submit your research? Choose BMC and benefit from:**

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

**At BMC, research is always in progress.**

Learn more [biomedcentral.com/submissions](https://www.biomedcentral.com/submissions)

