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Gender-specific association of SLC19A1 and MTHFR genetic polymorphism with oxidative stress biomarkers and plasma folate levels in older adults

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ABSTRACT

Background: Plasma folate levels are closely related to antioxidant capacity and are regulated by folate pathway gene polymorphism. However, few studies have explored the gender-specific association of folate pathway gene polymorphism with oxidative stress biomarkers. The present study was designed to explore the gender-specific independent and combined impacts of solute carrier family 19 member 1 (*SLC19A1*) and methylenetetrahydrofolate reductase (*MTHFR*) genetic polymorphisms on oxidative stress biomarkers in older adults.
Methods: A total of 401 subjects were recruited, including 145 males and 256 females. Demographic characteristics of the participants were collected by using a self-administered questionnaire. Fasting venous blood samples were taken for folate pathway gene genotyping, circulating lipids parameters and erythrocyte oxidative stress biomarkers measurement. The difference of genotype distribution and the Hardy-Weinberg equilibrium was calculated by the Chi-square test. The general linear model was applied to compare the plasma folate levels and erythrocyte oxidative stress biomarkers. Multiple linear regression was used to explore the correlation between genetic risk scores and oxidative stress biomarkers. Logistic regression was used to explore the association of genetic risk scores of folate pathway gene with folate deficiency.
Results: The male subjects have lower plasma folate and HDL-C levels than the female ones, and the male carrying *MTHFR* rs1801133 (CC) or *MTHFR* rs2274976 (GA) genotypes have higher erythrocyte SOD activity. The plasma folate levels, erythrocyte SOD and GSH-PX activities were negatively correlated with genetic risk scores in the male subjects. A positive correlation between the genetic risk scores and folate deficiency was observed in the male subjects.
Conclusions: There was association between folate pathway gene polymorphism of Solute Carrier Family 19 Member 1 (*SLC19A1*) and Methylenetetrahydrofolate Reductase (*MTHFR*) with erythrocyte SOD and GSH-PX activities, and folate levels in male but not in female aging subjects. Genetic variant of genes involved in folate metabolism has strong impact on plasma folate levels in the male aging subjects. Our data demonstrated that there was a potential interaction of gender and its genetic background in affecting the body's antioxidant capacity and the risk of folate deficiency in aging subjects.

1. Introduction

Folate, also known as vitamin B9, has important physiological functions as a carrier of one-carbon units involved in nucleotide synthesis, cell division and proliferation, and methylation. Recently, a study had shown that folate is closely related to oxidative stress *in vivo* (Asbaghi et al., 2021), which may affect the body's antioxidant capacity by affecting the level of homocysteine (Dose-dependent effects of folic acid on blood concentrations of homocysteine: a meta-analysis of the randomized trials, 2005) and the activity of antioxidant enzymes. Moreover, gender difference of circulating folate level was also reported by published documents. Results from Kreusler and coworkers' study in assessing serum folate status demonstrated age-and sex-dependent folate status in children. Similarly, cross-sectional studies conducted in

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Chinese and Americans found that men had lower serum folate concentrations than women (Hao et al., 2003; Ford and Bowman, 1999), and the risk of folate deficiency in the males was twice that of the females (Cohen et al., 2021). In contrast, Selhub et al. found similar blood folate concentrations in American women and men, despite much higher folate intake in women (Selhub et al., 1993). The inconsistent data from different studies may be attributed to the physiological metabolism, dietary structure, and genetic factors. Given the relation between plasma folate level and body oxidative stress, it is worthy to explore the gender differences of plasma folate levels and the oxidative stress capacity, especially in aging subjects who were in high risk to have folate malnutrition (Kado et al., 2005).

Folate deficiency is associated with a variety of diseases, including cardiovascular disease, neurodegenerative disease, and cancer. The occurrence of folate deficiency may be affected by the polymorphism of folate pathway genes, the impaired expression of folate metabolism-related genes and the individual's daily dietary folate intake (Carr et al., 2009). The one-carbon folate pathway genes include solute carrier family 19 member 1 (*SLC19A1*) and methylenetetrahydrofolate reductase (*MTHFR*). The *SLC19A1* gene is located on chromosome 21, commonly expressed in all human cells, especially in the liver. As a membrane protein involved in folate transport but not thiamine, *SLC19A1* is responsible for regulating intracellular folate levels (Zhao and Goldman, 2013). The *MTHFR* gene is located on chromosome 1 and encodes MTHFR protein that helps the process folate by catalysis the conversion of 5, 10-methylene tetrahydrofolate to 5-methylene tetrahydrofolate (Goyette et al., 1994), which is involved in the synthesis of methionine. Mutations in the *MTHFR* gene have been found to be associated with MTHFR protein activity. Mutations in *MTHFR* A1298C and *MTHFR* C677T have been reported to result in reduced MTHFR protein activity (Botto and Yang, 2000; Raghubeer and Matsha, 2021; Moll and Varga, 2015), increased homocysteine level and decreased plasma folate levels (Xuan et al., 2011). These results suggest that folate pathway gene polymorphisms are closely associated with circulating folate levels and further affect the risk of folate deficiency-related diseases.

Additionally, most diseases are polygenic genetic architecture (Lewis and Vassos, 2020), combined effect of genetic polymorphisms on the risk of chronic diseases has been indicated by constructing genetic risk scores (Xu et al., 2018), suggesting the joint impacts of genetic polymorphism on the risk of disease. Therefore, a cross-sectional study was conducted on older adults' population to explore the gender-specific association of folate pathway genes (*SLC19A1* and *MTHFR*) polymorphisms with plasma folate levels and erythrocyte oxidative stress biomarkers in the population. Besides, we also constructed genetic risk scores based on these single nucleotide polymorphisms (SNPs) to predict the combined impact of gene polymorphism on the risk of folate deficiency in older adults. Our study will provide basic evidence for formulating precise folate supplementation strategies according to gender and individual's genetic background.

2. Material and methods

2.1. Study population

Totally, 401 participants (145 males and 256 females)were recruited from both Nanyuan and Wulituo communities (Beijing, China). They were all Han Chinese without ethnic differences and did not take folate supplements history. Folate deficiency was defined when the plasma folate level was <10 nmol/L (4 ng/mL) (de Benoist, 2008). The study protocol was approved by the Ethics Committee of Capital Medical University (No. 2012SY23), and the study procedures strictly complied with the ethical standards of the Helsinki Declaration of 1975. All participants provided written informed consent for their participation.

2.2. Demographic characteristics and anthropometric measures

Participants underwent a general information survey, medical history survey and physical examination. Information on demographic characteristics (age, gender), lifestyle factors [smoking (yes or no), alcohol drinking (yes or no) and physical activity (never, 1–3 times/week, 4–5 times/week, everyday)], and medical history of chronic disease [hyperlipidemia (yes or no) and hypertension (yes or no)] were collected by using a self-administered questionnaire. Their height and body weight were measured by the nurses of the community medical service center. Body mass index (BMI) was calculated as weight (kg) / height (m^2).

2.3. Dietary survey

Participants' daily food intake was obtained using a food frequency questionnaire (FFQ). Details of the investigation, such as, consumption frequency (daily and weekly) and consumption quantity, were described in previous reports (Zhen et al., 2018). The food items included fruits, vegetables, legumes, whole grains, meat, nut, milk, egg, and fish. The dietary survey was conducted by specially trained nurses from the community health center.

2.4. Biochemical measurements

The fasting venous blood (5 mL) was collected from each subject in the morning. After centrifuging at 480*g* for 20 min, the plasma was separated and used for measurement of folate, erythrocyte oxidative stress biomarkers and lipids profile. Plasma folate concentration was measured by an assay kit purchased from R-Biopharm AG (Darmstadt, Germany) according to the manufacturer's instruction. Total cholesterol (TC) and triglyceride (TG) were measured with ILAB600 clinical chemistry analyzer (Instrumentation Laboratory, Lexington, WI, USA). High-density lipoprotein cholesterol (HDL—C) was determined by a commercially available assay from the Instrumentation Laboratory (Lexington, WI, USA), and low-density lipoprotein cholesterol (LDL-C) was calculated according to the Friedewald formula (Nierenberg and Nann, 1992). Plasma total antioxidant capacity (T-AOC), erythrocyte antioxidant enzymes activity including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-PX), glutathione S-transferase (GST) and glutathione reductase (GR) measurements were all used the commercial assay kits (Nanjing Jiancheng Biotechnology Institute Co., Ltd., Nanjing, China) according to the manufacturer's instruction. Serum VE (α-tocopherol, α-TOH) and VA (retinol) levels were measured by using the HPLC method according to a previously published study (Nierenberg and Nann, 1992). All samples for each participant were analyzed within a single batch, and the inter-assay coefficients of variation (CV) was <5 %.

2.5. Genotyping

DNA from the whole blood sample was extracted using the Wizard genomic DNA purification kit (Promega, Madison, WI, USA). The genetic polymorphisms of *SLC19A1* G80A (rs1051266), *MTHFR* A1298C (rs1801131), *MTHFR* C677T (rs1801133) and *MTHFR* G1793A (rs2274976) were detected by SNPscan genotyping assay according to the method described by a previous study (Du et al., 2014).

2.6. Genetic risk scores and groups

According to the published literature on folate pathway genes and the plasma folate levels in this population, the genotypes of different SNPs were assigned. The homozygote of risk gene was defined as '2', the heterozygote of risk gene was defined as '1', and the homozygote of control gene was defined as '0' [*SLC19A1* rs1051266 (A/A = 2, G/A = 1, G/G = 0) (Eklöf et al., 2008), *MTHFR* rs1801131(A/A = 2, C/A + C/C =

1) (Lu et al., 2022), *MTHFR* rs1801133 (T/T = 2, C/T = 1, C/C = 0) (Fredriksen et al., 2007), and *MTHFR* rs2274976 (G/G = 2, G/A = 1) (Lu et al., 2022)]. Among them, *MTHFR* rs1801131 control gene homozygote (C/C) was combined with risk gene heterozygote (C/A) because of the small sample size in subjects with C/C genotype ($n = 3$). In the present study, we did not detect the control gene homozygous (A/A) of *MTHFR* rs2274976 in this population. Therefore, no score value was assigned to this genotype. According to the genetic risk scores, the participants were divided into low risk (2–5), middle risk (6) and high risk (7–8) groups, respectively.

2.7. Statistical analysis

The data were statistically analyzed by SPSS 26.0 software (Chicago, IL, USA). The figures were drawn with R 4.2.1 language. Continuous variables were represented as mean ± standard deviation (SD). The allele and genotype frequency were calculated from the observed genotypic counts. The Chi-square test was used to compare the differences in percentages and assess the Hardy-Weinberg equilibrium. General linear model (GLM) was used to compare the plasma folate levels and erythrocyte oxidative stress biomarkers between the groups. Potential confounding factors, including age, smoking, alcohol drinking, plasma VA and α-VE levels, were adjusted during data analysis. Multiple linear regression was applied to ascertain the correlation between plasma folate and erythrocyte oxidative stress biomarkers with genetic risk scores. The association of genetic risk scores with the risk of folate deficiency according to gender was analyzed by using logistic regression. The power was analyzed by using G*power 3.1 software. $P < 0.05$ was considered as significant difference.

3. Results

3.1. Demographic characteristics of the participants

Gender differences in demographic characteristics are shown in Table 1. The participants ($n = 401$) included 145 males (36.3 %) and 256 females (63.7 %). The male group had a higher percentage of subjects with smoking and alcohol drinking habits than the female group ($P < 0.001$). The male subjects had significantly lower plasma folate and α-VE levels than the female ones, and the levels of VA in the male group were significantly higher than that in the female group ($P < 0.005$). The level of erythrocyte CAT activity in the male group was higher than that in the female group ($P < 0.05$), but the activity of GSH-PX in the male group was lower than that in the female group ($P < 0.05$). The male subjects have lower plasma HDL-C levels than the female ones ($P = 0.005$).

3.2. Association between folate levels with dietary intake

As shown in Table 2, the male subjects have higher daily meat but fewer fruit intakes than the female subjects ($P < 0.05$). The gender-specific correlation between daily dietary intakes and plasma folate levels is shown in Fig. 1. In the total population, the intake of vegetables and the total intake of fruits and vegetables was positively correlated with the plasma folate levels ($r_{vegetables} = 0.119$, $P = 0.017$; $r_{fruits\ and\ vegetables} = 0.114$, $P = 0.023$). In the male group, the plasma folate levels were positively correlated with daily intake of fruits, fruits and vegetables, whole grains, fish, and milk. In the female group, we did not find the correlation of daily dietary intake with plasma folate levels ($P > 0.05$).

3.3. Distribution of alleles and genotypes according to gender

As shown in Table 3, the SNPs of *SLC19A1* rs1051266, *MTHFR* rs1801131, *MTHFR* rs1801133, *MTHFR* rs2274976 followed Hardy-Weinberg equilibrium (HWE) ($P > 0.05$). The *SLC19A1* rs1051266

Table 1
Demographic characteristics and parameters of the participants.

Variable	Male ($n = 145$)	Female ($n = 256$)	Total ($n = 401$)	*P*-value[a]
Age	67.12 ± 5.22	65.60 ± 5.14	66.15 ± 5.21	0.005
BMI (kg/m^2)	24.98 ± 3.11	25.29 ± 3.16	25.18 ± 3.15	0.349
Lifestyle				
Smoking				0.000
Yes	55 (37.9)	12 (4.7)	67 (16.7)	
No	90 (62.1)	244 (95.3)	334 (83.3)	
Alcohol drinking				0.000
Yes	71 (49.0)	39 (15.2)	110 (27.4)	
No	74 (51.0)	217 (84.8)	291 (72.6)	
Physical activity				0.363
No	19 (13.1)	28 (10.9)	47 (11.7)	
1–3 d/w	12 (8.3)	36 (14.1)	48 (12.0)	
4–6 d/w	20 (13.8)	36 (14.1)	56 (14.0)	
Everyday	94 (64.8)	156 (60.9)	250 (62.3)	
Diseases				
Hyperlipidemia				0.211
Yes	47 (32.4)	99 (38.7)	146 (36.4)	
No	98 (67.6)	157 (61.3)	255 (63.6)	
Hypertension				0.392
Yes	78 (53.8)	149 (58.2)	227 (56.6)	
No	67 (46.2)	107 (41.8)	174 (43.4)	
Parameters				
Folate (μg/L)	5.23 ± 3.93	6.87 ± 4.40	6.27 ± 4.30	0.000
VA (μg/mL)	0.75 ± 0.15	0.71 ± 0.14	0.72 ± 0.14	0.004
α-VE (μg/mL)	9.34 ± 2.28	11.12 ± 3.14	10.47 ± 2.99	0.000
TC (mmol/L)	4.60 ± 0.88	4.96 ± 0.96	4.83 ± 0.95	0.318
TG (mmol/L)	1.44 ± 0.77	1.59 ± 0.76	1.54 ± 0.77	0.877
HDL-C (mmol/L)	1.26 ± 0.41	1.38 ± 0.32	1.34 ± 0.36	0.005
LDL-C (mmol/L)	3.07 ± 0.85	3.35 ± 0.90	3.25 ± 0.89	0.542
T-AOC (U/mL)	11.91 ± 3.93	11.43 ± 3.75	11.61 ± 3.82	0.182
SOD (U/g Hb)	31.27 ± 4.48	31.35 ± 4.25	31.32 ± 4.33	0.366
CAT (U/g Hb)	2.38 ± 0.36	2.33 ± 0.38	2.35 ± 0.37	0.044
GSH-PX (U/g Hb)	24.47 ± 6.94	26.50 ± 7.45	25.77 ± 7.33	0.013
GST (U/g Hb)	0.33 ± 0.13	0.34 ± 0.15	0.33 ± 0.14	0.814
GR (U/g Hb)	0.57 ± 0.19	0.54 ± 0.18	0.55 ± 0.18	0.177

Data were expressed as means ± SD or n (%). Demographic characteristics and plasma vitamin levels, including age, BMI, folate, VA and α-VE were compared by using *t*-tests. Lifestyle and medical history of chronic disease were compared by using Chi-square tests. Parameters including erythrocyte oxidative stress biomarkers and lipids were compared by general linear model (GLM). Confounding factors, including age, smoking, alcohol drinking, VA and α-VE, were adjusted during data analysis. $P < 0.05$ was considered as significant difference. BMI, body mass index; VA, vitamin A; α-VE, α-tocopherols; TC, total cholesterol; TG, triglyceride; HDL—C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; T-AOC, total antioxidant capacity; SOD, superoxide dismutase; CAT, catalase; GSH-PX, glutathione peroxidase; GST, glutathione s-transferase; GR, glutathione reductase.

[a] Bold numbers represent statistically significant *P*-values ($P < 0.05$).

Table 2
Daily dietary intakes of the participants.

Dietary (g/d)	Male ($n = 145$)	Female ($n = 256$)	*P*-value[a]
Fruit	106.90 ± 91.66	131.72 ± 94.89	0.011
Vegetable	342.07 ± 138.15	334.18 ± 143.40	0.592
Fruit + vegetable	448.97 ± 175.17	465.90 ± 189.64	0.378
Legume	27.25 ± 20.04	25.40 ± 20.46	0.382
Whole grain	21.44 ± 13.06	20.36 ± 13.07	0.427
Meat	45.27 ± 23.09	34.57 ± 22.99	0.000
Nut	13.72 ± 14.94	13.16 ± 12.86	0.695
Milk	113.83 ± 89.26	116.17 ± 92.82	0.806
Egg	28.65 ± 13.67	26.12 ± 15.29	0.099
Fish	21.02 ± 13.08	19.48 ± 12.66	0.250

Data are expressed as the means ± SD. Difference between groups was compared by using *t*-tests.

[a] Bold numbers represent statistically significant *P*-values ($P < 0.05$).

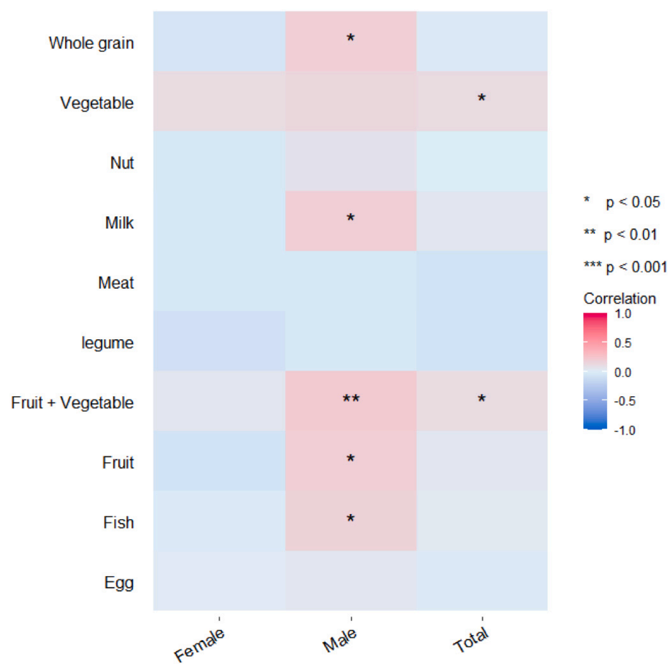

Fig. 1. Correlation between daily dietary intake and plasma folate level by gender. The correlation was analyzed by Pearson correlation analysis.

allele and genotype distribution in the participants were gender specific. The male subjects carry more 'A' alleles than the female subjects, and the number of female subjects with AA genotype was lower than the male ones ($P < 0.05$).

3.4. Folate levels according to gene polymorphism and gender

The difference of plasma folate levels according to gene polymorphism and gender is shown in Table 4. We found that the male carriers with the *MTHFR* rs1801131 (AA), *MTHFR* rs1801133 (TT) and *MTHFR* rs2274976 (GG) genotypes had lower plasma folate levels than the subjects with other genotypes ($P < 0.05$). Female subjects with *MTHFR* rs1801131 (AA), *MTHFR* rs1801133 (TT) and *MTHFR* rs2274976 (GG) genotypes had significantly higher plasma folate levels than the male ones ($P < 0.05$). There is no *SLC19A1* rs1051266 genotype and gender differences in plasma folate levels in the participants ($P > 0.05$).

3.5. Relationship between oxidative stress biomarkers with gene polymorphisms

As shown in Table 5, for the *MTHFR* rs1801133 gene polymorphism, male subjects with CC genotype have higher erythrocyte SOD activity than subjects with other genotypes ($P < 0.05$). The male carriers with *MTHFR* rs2274976 GA genotype have higher erythrocyte SOD activity than carriers with other genotypes ($P < 0.05$). We did not observe the difference in oxidative stress biomarkers according to *SLC19A1* rs1051266 and *MTHFR* rs1801131 genotypes ($P > 0.05$) (Supplement Tables 1, 2).

3.6. Relationship between oxidative stress biomarkers and genetic risk scores

The genetic risk scores range from 2 to 8 (Fig. 2). In the total population, the percentage of subjects with genetic risk score from 2 to 8 was 1.2 %, 6.0 %, 12.0 %, 18.2 %, 30.4 %, 23.7 % and 8.5 %, respectively. For male subjects, the respective percentage was 6.2 %, 9.0 %, 17.9 %, 28.3 %, 25.5 % and 13.1 %; while for female subjects, it was 2.0 %, 5.9 %, 13.7 %, 18.4 %, 31.6 %, 22.7 % and 5.9 %, respectively.

The multiple linear regression was run to analyze the relationship between oxidative stress biomarkers with genetic risk scores. The plasma folate levels and erythrocyte SOD and GSH-PX activities were negatively correlated with genetic risk scores in the male subjects ($P < 0.05$) but not in female subjects ($P > 0.05$) (Table 6).

3.7. Oxidative stress biomarkers according to gender and genetic risk scores

The participants were regrouped according to the genetic risk score, their statistic difference in oxidative stress biomarkers among groups according to gender was compared (Table 7). Although no statistical significance was found, a decreased trend in plasma folate levels and erythrocyte GSH-PX activity was displayed following the increase of genetic risk scores in the male subjects. Comparing with the female subjects, the male subjects with high genetic risk showed lower plasma folate levels and erythrocyte GSH-PX activity, and there was statistically significant difference ($P < 0.05$). The gender difference of other antioxidant biomarkers according to genetic risk was not observed ($P > 0.05$).

Table 3
The allele and genotype distribution of candidate genes according to gender.

SNPs	Gender	Allele [n (%)]		*P*-value	Genotype [n (%)]			*P*-value	HWE
		Major	Minor		Major type	Heterozygote	Minor type		
SLC19A1 (RFC-1)									
rs1051266		G	A	0.025	GG	GA	AA	0.047	0.086
	Male	145(50.0)	145(50.0)		42(29.0)	61(42.1)	42(29.0)		
	Female	298(58.2)	214(41.8)		89(34.8)	120(46.9)	47(18.2)		
MTHFR									
rs1801131		A	C	0.170	AA	CA + CC		0.212	0.123
	Male	258(89.0)	32(11.0)		113(77.9)	32(22.1)			
	Female	438(85.5)	74(14.5)		185(72.3)	71(27.7)			
rs1801133		T	C	0.238	TT	CT	CC	0.252	1.000
	Male	186(64.1)	104(35.9)		57(39.3)	72(49.7)	16(11.0)		
	Female	308(59.9)	206(40.1)		94(36.7)	118(46.1)	44(17.2)		
rs2274976		G	A	0.348	GG	GA	–	0.333	0.621
	Male	277(95.5)	13(4.5)		132(91.0)	13(9.0)	–		
	Female	481(94.0)	31(6.0)		225(87.9)	31(12.1)	–		

Data were expressed as n (%). Allele frequency, genotype frequency, and HWE were compared by using Chi-square tests. $P < 0.05$ was considered as significant difference. SNP, single nucleotide polymorphism; SLC19A1, solute carrier family member 1; MTHFR, 5,10-methylenetetrahydrofolate reductase; HWE, Hardy-Weinberg equilibrium.

Table 4
Plasma folate levels according to gender and folate pathway gene polymorphism.

SNPs	Gender	Genotypes			P-value
		Major type	Heterozygote	Minor type	
SLC19A1		GG	GA	AA	
rs1051266	Male	5.04 ± 4.02	5.18 ± 4.32	5.49 ± 3.27	0.951
	Female	7.20 ± 4.51	6.56 ± 3.85	7.10 ± 5.02	0.524
	P-value	0.104	0.852	0.285	
MTHFR		AA	CA + CC		
rs1801131	Male	4.86 ± 3.56	6.55 ± 4.87		0.028
	Female	7.14 ± 4.46	6.14 ± 4.16		0.137
	P-value	0.025	0.531		
MTHFR		TT	CT	CC	
rs1801133	Male	4.09 ± 2.52	6.19 ± 4.74	5.00 ± 2.95	0.014
	Female	6.95 ± 4.47	6.37 ± 3.63	7.99 ± 5.79	0.124
	P-value	0.011	0.471	0.357	
MTHFR		GG	GA	–	
rs2274976	Male	4.96 ± 3.60	7.93 ± 5.96	–	0.006
	Female	6.78 ± 4.21	7.49 ± 5.59	–	0.409
	P-value	0.047	0.559	–	

Data were expressed as means ± SD. Folate levels was compared using general linear model (GLM). Confounding factors, including age, smoking, alcohol drinking, VA and α-VE, were adjusted during data analysis. $P < 0.05$ was considered as significant difference. SLC19A1, solute carrier family member 1; MTHFR, 5,10-methylenetetrahydrofolate reductase.

Table 5
Differences in oxidative stress biomarkers between SNPs in male.

SNPs	Biomarkers (U/g Hb)	Genotypes			P-value[a]
		Major type	Heterozygote	Minor type	
MTHFR rs1801133	SOD	TT 30.91 ± 3.82	CT 30.87 ± 4.14	CC 34.34 ± 6.75	0.024
MTHFR rs2274976	SOD	GG 31.03 ± 4.19	GA 33.64 ± 6.55	– –	0.028

Data were expressed as means ± SD. Oxidative stress biomarkers were compared by general linear model (GLM). Confounding factors, including age, smoking, alcohol drinking, VA and α-VE, were adjusted during data analysis. $P < 0.05$ was considered as significant difference. SOD, superoxide dismutase; MTHFR, 5,10-methylenetetrahydrofolate reductase.

[a] Bold numbers represent statistically significant *P*-values ($P < 0.05$).

3.8. Gender-specific effects of genetic risk and folate deficiency

The longitudinal association of genetic risk groups with folate deficiency according to gender is shown in Table 8. In crude model 1, compared with the male subjects in the low genetic risk group, the subjects in the high genetic risk group showed significantly increased risk of folate deficiency (*OR* = 5.13, *95%CI:* 2.22–11.85, *P* = 0.000, *P for trend* = 0.000). After adjusting potential confounding factors, similar trend was observed in Model 2 (*OR* = 5.14, *95%CI:* 2.15–12.28, *P* = 0.000). For the genetic risk scores, the result implied that higher genetic risk scores were associated with higher risk of folate deficiency in male subjects (*OR* = 1.59, *95 % CI:* 1.21–2.08, *P* = 0.001). There was no statistical significance between each group of the genetic risk scores and the risk of its folate deficiency in female subjects (all $P > 0.05$).

4. Discussion

Gender differences in plasma folate levels have been reported previously, reporting that the females had higher plasma folate levels than the males (Cohen et al., 2019; Kreusler et al., 2021; Xie et al., 2015). Results from a cross-sectional study conducted by Tomioka et al. also demonstrated that a gender-dependent cut-off value was needed to define the plasma folate levels in schizophrenia subjects (Tomioka et al., 2020). These data indicated the important role of gender in affecting the relationship between circulating folate level and the risk of disease. Consistently, results in the present study found that plasma folate levels were significantly lower in the males than in the females. Except gender different impact on the plasma folate levels, lifestyles, dietary food intake and pattern *etc.* also have impacts on it. Studies have found that smoking and alcohol drinking reduces plasma folate levels (Okumura and Tsukamoto, 2011; Halsted et al., 2002). In line with this result, results in this study showed that the male group has a higher proportion of subjects with smoking (*37.9 % vs 4.7 %*) and alcohol drinking (*49.0 % vs 15.2 %*) habits than the female group. These data suggest that differences in lifestyle between the gender may contribute to the gender difference in circulating folate levels, and the male subjects with smoking and alcohol drinking habits might have increased demand for daily dietary folate intake in response to the decreased folate levels associated with poor lifestyle.

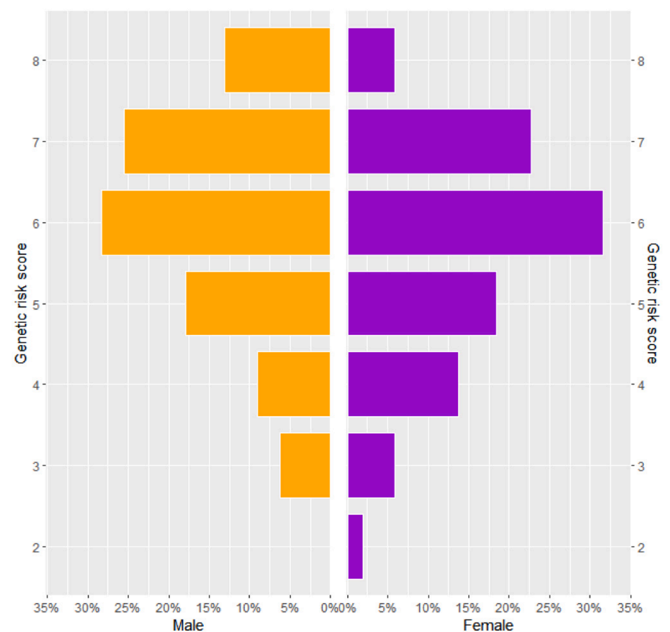

Fig. 2. The distribution of percentage of participants with genetic risk scores according to gender. Data are expressed as percentages.

Additionally, the subjects from the male group have higher plasma VA and lower VE levels than those from the female group. As fat-soluble vitamins, animal-derived foods are good resources for VA and VE (Gannon et al., 2021). The male subjects have higher daily meat intakes than those in the females. We, therefore, speculated that the gender difference in plasma VA and VE levels might be due to the discrepancy in dietary patterns. Given the potential role of VA and VE in the body's antioxidant defense system (Blaner et al., 2021), we adjusted individuals' plasma VA and VE levels as covariates in the subsequent correlation analysis of the relationship between plasma folate levels and oxidative stress biomarkers in older adults. Results further indicated that the male subjects had lower erythrocyte GSH-PX but higher CAT activities than the female ones. Previous studies have reported the impact of VA on CAT activity (Navigatore-Fonzo et al., 2014), suggesting that increased erythrocyte CAT activity in males might be associated with higher circulating VA levels.

Moreover, we also observed that the male subjects have lower HDL-C levels than the females. Epidemiological studies have found that, due to the lower blood HDL-C and folate levels, males have a higher risk of

Table 6
Correlation of genetic risk scores and oxidative stress biomarker levels according to gender.

Biomarkers	Genetic risk score (male)					Genetic risk score (female)				
	Unstandardized coefficient	Std. error	Standardized coefficient	t	*P*-value	Unstandardized coefficient	Std. error	Standardized coefficient	t	*P*-value
Folate (μg/L)	−0.564	0.236	−0.197	−2.393	0.018	−0.062	0.198	−0.020	−0.315	0.753
T-AOC (U/mL)	0.257	0.237	0.090	1.083	0.281	0.048	0.173	0.018	0.279	0.780
SOD (U/g Hb)	−0.734	0.267	−0.225	−2.752	0.007	−0.276	0.193	−0.090	−1.427	0.155
CAT (U/g Hb)	−0.042	0.022	−0.161	−1.931	0.056	0.003	0.017	0.010	0.162	0.871
GSH-PX (U/g Hb)	−0.911	0.417	−0.180	−2.185	0.031	0.200	0.339	0.037	0.590	0.555
GST (U/g Hb)	0.002	0.008	0.021	0.250	0.803	−0.008	0.007	−0.073	−1.189	0.236
GR (U/g Hb)	0.001	0.011	0.006	0.076	0.940	0.005	0.008	0.038	0.599	0.550

Multiple linear regression was used to ascertain the correlation between genetic risk scores (SLC19A1: GG = 0, GA = 1, AA = 2; MTHFR rs1801131: CA + CC = 1, AA = 2; MTHFR rs1801131: CC = 0, CT = 1, TT = 2; MTHFR rs2274976: GA = 1, GG = 2) and the oxidative stress biomarkers in the different gender. Age, smoking, alcohol drinking, VA and α-VE were adjusted for the statistical analysis. $P < 0.05$ was considered as significant difference. T-AOC, total antioxidant capacity; SOD, superoxide dismutase; CAT, catalase; GSH-PX, glutathione peroxidase; GST, glutathione s-transferase; GR, glutathione reductase.

Table 7
Oxidative stress biomarkers in genders with different genetic risk.

Parameters	Gender	Genetic risk			*P*-value
		Low	Middle	High	
Folate (μg/L)	Male	6.11 ± 4.28	5.33 ± 3.65	4.41 ± 3.71	0.148
	Female	7.11 ± 4.73	6.02 ± 4.03	7.46 ± 4.22	0.105
	P-value	0.627	0.248	0.002	
T-AOC (U/mL)	Male	11.69 ± 3.83	11.71 ± 3.84	12.25 ± 4.11	0.577
	Female	11.12 ± 3.97	11.55 ± 3.88	11.75 ± 3.26	0.559
	P-value	0.662	0.377	0.760	
SOD (U/g Hb)	Male	32.35 ± 5.06	30.78 ± 4.26	30.69 ± 3.99	0.091
	Female	31.90 ± 5.03	31.46 ± 3.52	30.45 ± 3.65	0.066
	P-value	0.439	0.527	0.524	
CAT (U/g Hb)	Male	2.46 ± 0.38	2.37 ± 0.31	2.34 ± 0.37	0.235
	Female	2.33 ± 0.43	2.33 ± 0.40	2.33 ± 0.29	0.999
	P-value	0.106	0.152	0.564	
GSH-PX (U/g Hb)	Male	25.62 ± 7.33	24.19 ± 6.72	23.70 ± 6.75	0.339
	Female	26.57 ± 7.73	25.67 ± 7.43	27.32 ± 7.09	0.397
	P-value	0.183	0.681	0.024	
GST (U/g Hb)	Male	0.32 ± 0.13	0.35 ± 0.12	0.33 ± 0.14	0.503
	Female	0.34 ± 0.14	0.34 ± 0.13	0.32 ± 0.19	0.670
	P-value	0.804	0.699	0.825	
GR (U/g Hb)	Male	0.55 ± 0.17	0.61 ± 0.20	0.55 ± 0.18	0.285
	Female	0.54 ± 0.19	0.54 ± 0.17	0.55 ± 0.17	0.834
	P-value	0.888	0.150	0.471	

Data were expressed as means ± SD. Genetic risk grouping was based on genetic risk score (2–5: low risk, 6: middle risk, 7–8: high risk). Folate and oxidative stress biomarkers were compared by general linear model (GLM). Confounding factors, including age, smoking, alcohol drinking, VA and α-VE, were adjusted during data analysis. $P < 0.05$ was considered as significant difference. T-AOC, total antioxidant capacity; SOD, superoxide dismutase; CAT, catalase; GSH-PX, glutathione peroxidase; GST, glutathione s-transferase; GR, glutathione reductase.

cardiovascular disease than females (Kannel et al., 1976). High daily intake of animal-derived foods, especially the consumption of red meat, might increase the intake of both cholesterol and saturated fatty acids. In our study, compared with the females, higher meat intake and lower fruit and vegetable intake were found in the male subjects, which might partially explain the lower plasma HDL-C level in the male subjects.

It was well known that folate could not be synthesized directly in the body and could only be obtained from diets (mainly vegetables and fruits) (Duthie et al., 2018). In the current study, we found that although the females consumed more fruit and less meat than the males, the total daily intake of vegetables and fruit did not differ between groups. Additionally, a significant positive correlation between plasma folate levels and fruit and vegetables was detected in the male group, but not in the female group, suggesting that the gender differences in plasma folate levels could not be completely attributed to the different dietary patterns in the participants. Therefore, the influence of individual's genetic background, especially the genetic polymorphism of the folate pathway gene, should be identified while analyzing the relationship between circulating folate levels and the oxidative stress biomarkers in aging subjects.

It was reported that the genetic polymorphism of *SLC19A1* rs1051266 caused the change of guanine to adenine (G80A) at position 80 in exon 2 of *SLC19A1*, which results in the substitution of arginine to histidine in the functional carrier protein (Yates and Lucock, 2005). Moreover, the 'A' allele of *SLC19A1* rs1051266 was associated with decreased plasma folate levels (Eklöf et al., 2008). In our study, we found that the distribution of alleles and genotypes of *SLC19A1* rs1051266 was gender-dependent, which was demonstrated by the higher percentage of male subjects with AA genotypes than that of the females (*29.0 % vs. 18.2 %*, Table 3). Although we failed to detect the impact of *SLC19A1* rs1051266 polymorphism on plasma folate levels according to gender, our data indicated a much lower plasma folate levels in male subjects with AA genotype as compared with the females (Table 4). These data further indicated that the genetic polymorphism of *SLC19A1* rs1051266 seems to have a stronger impact on plasma folate levels in males. Therefore, in the present study, we defined this gene's 'A' allele as the risk gene for folate deficiency and included it in the subsequent construction of the genetic risk score model.

A significant association between *MTHFR* rs1801131, rs1801133 and rs2274976 genetic polymorphism with plasma folate levels was found in male subjects. However, the absence of such genotypic differences among females indicates that variations in these SNPs of this gene have little effect on plasma folate in females, further suggesting that circulating folate levels in the male subjects was susceptible to polymorphism of *MTHFR* gene. Male subjects with *MTHFR* rs1801131 AA genotype, rs1801133 TT genotype, and rs2274976 GG genotype displayed lower plasma folate levels than subjects with other genotypes. A population-based study found that the 'C' allele was associated with decreased

Table 8
Longitudinal association between folate deficiency and genetic risk groups according to gender.

Genetic risk	Deficiency/control (total number)	Male				Deficiency/control (total number)	Female			
		Model 1		Model 2			Model 1		Model 2	
		OR 95%CI	*P*-value	OR 95%CI	*P*-value		OR 95%CI	*P*-value	OR 95%CI	*P*-value
Low	14/34 (48)	1.00				27/75 (102)	1.00			
Middle	18/23 (41)	1.90 0.79–4.57	0.151	1.90 0.76–4.74	0.170	30/51 (81)	1.63 0.87–3.07	0.127	1.62 0.85–3.10	0.143
High	38/18 (56)	5.13 2.22–11.85	0.000	5.14 2.15–12.28	0.000	18/55 (73)	0.91 0.46–1.81	0.787	0.90 0.44–1.83	0.777
P for trend		2.28 1.50–3.47	0.000	2.28 1.48–3.53	0.000		0.99 0.71–1.37	0.933	0.98 0.70–1.38	0.911
Genetic risk scores		1.59 1.21–2.08	0.001	1.63 1.24–2.15	0.001		1.04 0.85–1.26	0.724	1.04 0.85–1.28	0.687

The longitudinal association between folate deficiency and genetic risk groups was determined by logistic regression. Model 1 was a crude model; model 2 adjusted for age, smoking, alcohol drinking, VA and α-VE. $P < 0.05$ was considered as significant difference.

circulating folate in *MTHFR* rs1801131 and that TT had lower folate levels than CC in *MTHFR* rs1801133 (Fredriksen et al., 2007). In our study, we consistently found that subjects with *MTHFR* rs1801133 TT genotype had lower plasma folate. However, *MTHFR* rs1801131 AA male subjects showed higher plasma folate levels than subjects with CC genotype. The difference between studies might be due to racial differences. Bi et al. reported that the 'T' allele of *MTHFR* rs1801133 combined with *ApoE4* increased the risk of Alzheimer's disease (Bi et al., 2009). Lu and coworkers' study also found that mutations of *MTHFR* rs1801131 (CC) and *MTHFR* rs2274976 (AA) were associated with a lower risk of type 2 diabetic mellitus (Lu et al., 2022). Therefore, in the genetic risk model, we defined the 'A' of *MTHFR* rs1801131, 'T' of *MTHFR* rs1801133, and 'G' of *MTHFR* rs2274976 as risk alleles.

In the relationship between folate pathway genes and oxidative stress biomarkers, we found that male subjects carrying the *MTHFR* rs1801133 CC genotype had higher erythrocyte SOD activity. In contrast, male carriers of the *MTHFR* rs2274976 GG genotype had lower erythrocyte SOD activity (Table 5). As for plasma folate levels (Table 4), we found that male carrying the MTHFR rs2274976 GA genotype showed higher folate levels and erythrocyte SOD activity than those with the GG genotype, and male carrying the MTHFR rs1801133 TT genotype showed lower folate levels and erythrocyte SOD activity than those with the CC genotype. These data demonstrated a consistent changing trend of erythrocyte SOD activity with plasma folate levels in males with different *MTHFR* genotypes. Studies have shown that folate supplementation can improve body antioxidant capacity (Asbaghi et al., 2021). Data from *in vitro* experiments also demonstrated that folate could significantly enhance the reactive oxygen species scavenging ability of CAT and SOD (Lee and Murthy, 2007). These data suggested that plasma folate levels could affect the antioxidative stress ability by affecting erythrocyte SOD activity, especially in males.

The total potency of *SLC19A1* rs1051266, *MTHFR* rs1801131, *MTHFR* rs1801133, and *MTHFR* rs2274976 on oxidative stress ability was assessed by genetic risk scores. We found that the females generally had lower genetic risk scores than the males (Fig. 2), suggesting that males were more sensitive to folate deficiency than the females in the aging population. Additionally, we found that genetic risk score was negatively correlated with plasma folate levels and erythrocyte SOD and GSH-PX activities in the males, further demonstrating the correlation between circulating folate levels with the antioxidant capacity in the aging male subjects.

After dividing the genetic risk score into low-risk, middle-risk, and high-risk groups, we found that both the male and the female subjects in the low-risk group have higher erythrocyte SOD activity than the subjects from other risk groups, demonstrating that the relation between plasma folate levels and erythrocyte SOD activity was gender-independent. We also found that the risk of folate deficiency was 5.14 (*95%CI:* 2.15–12.28, $P = 0.000$) in the high-risk group compared with the low-risk group. There was no association of folate deficiency with any of the genetic risk groups in females. Our data indicate the gender-dependent plasma folate levels in aging subjects. We, therefore, speculate that, except for different daily folate intakes, dietary patterns, and lifestyles, the genetic background-based discrepancy of folate metabolism between males and females might contribute to this gender-specific risk for folate deficiency. Among them, *MTHFR* is the rate-limiting enzyme in folate cycle metabolism, and the mutation of *MTHFR* C677T can affect the activity and heat resistance of the enzyme (Frosst et al., 1995), which is specifically manifested as the CC genotype as the reference, CT activity is 65 %, TT activity is 30 %. Other studies also demonstrated that *SLC19A1*, as a folate transporter, the presence of its 'A' allele can reduce the uptake efficiency of cells, thereby reducing the utilization of folate by cells (Eklöf et al., 2008; Yates and Lucock, 2005). In our study, we found that the male subjects in the high genetic-risk group had lower plasma folate levels and increased risk for folate deficiency. These data further indicated that the males were more sensitive to the genetic variants of genes involved in folate metabolism. Additionally, the gender difference as demonstrated by lower plasma folate levels in the males as comparing with the females might correlate with unhealthy lifestyle, such as smoking and alcohol drinking in the male aging subjects (Okumura and Tsukamoto, 2011; Halsted et al., 2002). Studies have also shown that folate is closely related to the body's antioxidant capacity as previously reported (Asbaghi et al., 2021). We therefore speculate that the unhealthy lifestyles combined with genetic variant-related change of plasma folate levels might further contribute to the gender-dependent body's antioxidant capacity in aging subjects. These data demonstrated the potential interaction of gender, individual's genetic background, and lifestyle (including dietary intakes) in affecting the body's antioxidant capacity and the risk of folate deficiency in aging subjects.

The study has several limitations. Firstly, the relatively small sample size is one of the limitations of this cross-sectional study. In order to make the sample size of each group comparable, the participants were grouped according to the number of people instead of using the equal score segment. Secondly, the dietary part can only be evaluated by the intake of vegetables and fruits without knowing the specific food types and corresponding folate intake, which cannot accurately reflect the influence of dietary folate intake on plasma folate levels. Thirdly, as the population of this study was focused on the elder population, there are limitations in the generalization of conclusions, which may not be applicable to different age groups and regions. Future validation of the findings in large populations is needed.

5. Conclusion

In conclusion, our data reveal that genetic polymorphism of *SLC19A1* and *MTHFR* was associated with plasma folate levels and

erythrocyte oxidative stress biomarkers in aging subjects. The genetic variants of the folate pathway gene predisposed the aging male subjects with lower circulating folate levels and a high risk for folate deficiency. The correlation between erythrocyte SOD and GSH-PX activities and plasma folate levels were regulated by folate pathway gene polymorphism and gender. Large-scale population-based cohort studies are needed to reveal the gender-specific interaction between folate pathway gene polymorphism circulating folate levels and the body's antioxidant capacity in aging subjects.

Author's contributions

Linhong Yuan designed the study. Xixiang Wang, Jingjing Xu, Xiaojun Ma, Yujie Guo, Lu Liu, Yu Liu and Lifang Gao conducted the investigation and blood sample collection. Xixiang Wang performed data statistical analysis. Xixiang Wang, Linhong Yuan, Shaobo Zhou and Ying Wang drafted the manuscript.

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CRediT authorship contribution statement

Xixiang Wang: Methodology, Formal analysis, Writing – original draft, Visualization, Investigation. **Ying Wang:** Writing – review & editing. **Xiaojun Ma:** Investigation. **Shaobo Zhou:** Writing – review & editing. **Jingjing Xu:** Investigation. **Yujie Guo:** Investigation. **Lu Liu:** Investigation. **Yu Liu:** Investigation. **Lifang Gao:** Investigation. **Linhong Yuan:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that there is no potential conflict of interest.

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Appendix A. Supplementary data

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References

Dose-dependent effects of folic acid on blood concentrations of homocysteine: a meta-analysis of the randomized trials. Am. J. Clin. Nutr. 82 (4), 2005, 806–812.

Asbaghi, O., et al., 2021. Effects of folic acid supplementation on oxidative stress markers: a systematic review and meta-analysis of randomized controlled trials. Antioxidants (Basel, Switzerland) 10 (6).

de Benoist, B., 2008. Conclusions of a WHO technical consultation on folate and vitamin B12 deficiencies. Food Nutr. Bull. 29 (2 Suppl), S238–S244.

Bi, X.-H., et al., 2009. Association of RFC1 A80G and MTHFR C677T polymorphisms with Alzheimer's disease. Neurobiol. Aging 30 (10), 1601–1607.

Blaner, W.S., Shmarakov, I.O., Traber, M.G., 2021. Vitamin a and vitamin E: will the real antioxidant please stand up? Annu. Rev. Nutr. 41, 105–131.

Botto, L.D., Yang, Q., 2000. 5,10-methylenetetrahydrofolate reductase gene variants and congenital anomalies: a HuGE review. Am. J. Epidemiol. 151 (9), 862–877.

Carr, D.F., et al., 2009. Investigation of inter-individual variability of the one-carbon folate pathway: a bioinformatic and genetic review. Pharmacogenomics J. 9 (5), 291–305.

Cohen, E., et al., 2019. Gender differences in homocysteine concentrations, a population-based cross-sectional study. Nutr Metab Cardiovasc Dis 29 (1).

Cohen, E., et al., 2021. Sex differences in folate levels: a cross sectional study of a large cohort from Israel. Isr. Med. Assoc. J. 23 (1), 17–22.

Du, W., et al., 2014. A rapid method for simultaneous multi-gene mutation screening in children with nonsyndromic hearing loss. Genomics 104 (4), 264–270.

Duthie, S.J., et al., 2018. Effect of increasing fruit and vegetable intake by dietary intervention on nutritional biomarkers and attitudes to dietary change: a randomised trial. Eur. J. Nutr. 57 (5), 1855–1872.

Eklöf, V., et al., 2008. The reduced folate carrier (RFC1) 80G > A and folate hydrolase 1 (FOLH1) 1561C > T polymorphisms and the risk of colorectal cancer: a nested case-referent study. Scand. J. Clin. Lab. Invest. 68 (5), 393–401.

Ford, E.S., Bowman, B.A., 1999. Serum and red blood cell folate concentrations, race, and education: findings from the third National Health and Nutrition Examination Survey. Am. J. Clin. Nutr. 69 (3), 476–481.

Fredriksen, A., et al., 2007. Large-scale population-based metabolic phenotyping of thirteen genetic polymorphisms related to one-carbon metabolism. Hum. Mutat. 28 (9), 856–865.

Frosst, P., et al., 1995. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat. Genet. 10 (1), 111–113.

Gannon, B.M., Rogers, L.M., Tanumihardjo, S.A., 2021. Metabolism of neonatal vitamin a supplementation: a systematic review. Adv. Nutr. (Bethesda, Md.) 12 (3), 942–958.

Goyette, P., et al., 1994. Human methylenetetrahydrofolate reductase: isolation of cDNA, mapping and mutation identification. Nat. Genet. 7 (2), 195–200.

Halsted, C.H., et al., 2002. Metabolic interactions of alcohol and folate. J. Nutr. 132 (8 Suppl), 2367S–2372S.

Hao, L., et al., 2003. Geographical, seasonal and gender differences in folate status among Chinese adults. J. Nutr. 133 (11), 3630–3635.

Kado, D.M., et al., 2005. Homocysteine versus the vitamins folate, B6, and B12 as predictors of cognitive function and decline in older high-functioning adults: MacArthur studies of successful aging. Am. J. Med. 118 (2), 161–167.

Kannel, W.B., et al., 1976. Menopause and risk of cardiovascular disease: the Framingham study. Ann. Intern. Med. 85 (4), 447–452.

Kreusler, P., et al., 2021. Folate and cobalamin serum levels in healthy children and adolescents and their association with age, sex, BMI and socioeconomic status. Nutrients 13 (2).

Lee, S., Murthy, N., 2007. Targeted delivery of catalase and superoxide dismutase to macrophages using folate. Biochem. Biophys. Res. Commun. 360 (1), 275–279.

Lewis, C.M., Vassos, E., 2020. Polygenic risk scores: from research tools to clinical instruments. Genome Med. 12 (1), 44.

Lu, X., et al., 2022. Associations between serum betaine, methyl-metabolizing genetic polymorphisms and risk of incident type 2 diabetes: a prospective cohort study in community-dwelling Chinese adults. Nutrients 14 (2).

Moll, S., Varga, E.A., 2015. Homocysteine and MTHFR mutations. Circulation 132 (1), e6–e9.

Navigatore-Fonzo, L.S., et al., 2014. Daily rhythms of catalase and glutathione peroxidase expression and activity are endogenously driven in the hippocampus and are modified by a vitamin A-free diet. Nutr. Neurosci. 17 (1), 21–30.

Nierenberg, D.W., Nann, S.L., 1992. A method for determining concentrations of retinol, tocopherol, and five carotenoids in human plasma and tissue samples. Am. J. Clin. Nutr. 56 (2), 417–426.

Okumura, K., Tsukamoto, H., 2011. Folate in smokers. Clin. Chim. Acta 412 (7–8), 521–526.

Raghubeer, S., Matsha, T.E., 2021. Methylenetetrahydrofolate (MTHFR), the one-carbon cycle, and cardiovascular risks. Nutrients 13 (12).

Selhub, J., et al., 1993. Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. JAMA 270 (22), 2693–2698.

Tomioka, Y., et al., 2020. Association between serum folate levels and schizophrenia based on sex. Psychiatry Clin. Neurosci. 74 (9), 466–471.

Xie, D., et al., 2015. Hyperhomocysteinemia predicts renal function decline: a prospective study in hypertensive adults. Sci. Rep. 5, 16268.

Xu, Z., et al., 2018. Association between a genetic risk score based on single nucleotide polymorphisms of coronary artery disease-related genes and left Main coronary artery disease. Biomed. Res. Int. 2018, 8610368.

Xuan, C., et al., 2011. Association between polymorphism of methylenetetrahydrofolate reductase (MTHFR) C677T and risk of myocardial infarction: a meta-analysis for 8,140 cases and 10,522 controls. Arch. Med. Res. 42 (8), 677–685.

Yates, Z., Lucock, M., 2005. G80A reduced folate carrier SNP modulates cellular uptake of folate and affords protection against thrombosis via a non homocysteine related mechanism. Life Sci. 77 (22), 2735–2742.

Zhao, R., Goldman, I.D., 2013. Folate and thiamine transporters mediated by facilitative carriers (SLC19A1-3 and SLC46A1) and folate receptors. Mol. Asp. Med. 34 (2–3), 373–385.

Zhen, J., et al., 2018. Association of ApoE genetic polymorphism and type 2 diabetes with cognition in non-demented aging Chinese adults: a community based cross-sectional study. Aging Dis. 9 (3), 346–357.