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RESEARCH ARTICLE

Influence of food groups on plasma total homocysteine for specific *MTHFR C677T* genotypes in Chinese population

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Scope: It has been demonstrated that a mutation of *MTHFR C677T* increases plasma total homocysteine (Hcy) concentration and decreases folate. Natural foods can improve Hcy levels, but the effect of certain foods remains undetermined. The aim of this study was to investigate the association between food groups and Hcy, and to explore the correlations between Hcy and dietary folate/vitamin (Vit) B12 for genotype-specific population.

Methods and results: A total of 4507 adults were enrolled in this study, all of whom underwent physical examinations and genotyping. A dietary recall questionnaire, which assessed the frequency (F) and quantity (Q) of food consumption, was completed by all. For the male CC group, after adjustment for age and BMI, fish (F) was negatively correlated with Hcy; for the male CT group, fish (F) and eggs (F) were negatively associated with Hcy, whereas cereal/wheat (Q) were positively correlated with Hcy; for the male TT group, fish (F), meat (Q), milk (F), and fruits/vegetables (Q) were negatively associated with Hcy, whereas sugar (Q) and salt (Q) were positively associated with Hcy. For the female CC group, fruits/vegetables (Q), eggs (F) and meat (F) were negatively correlated with Hcy, but soy (F) was positively correlated with Hcy; for the female CT group, eggs (F) and meat (Q) were negatively correlated with Hcy, whereas soy (F), fried foods (F) and salt (Q) were positively correlated with Hcy; for the female TT group, fish(F), eggs (F), and fruits/vegetables (F) were negatively associated with Hcy. Furthermore, we found that Hcy was more closely correlated with folate than with Vit B12 for males (CC, CT and TT) and female TT genotype. However, the correlation between Hcy and Vit B12 was stronger for the female CT/CC groups.

Conclusion: Hcy levels were influenced by food groups to varying degrees, which were based on gender and *MTHFR C677T* genotypes. Hcy levels were more closely correlated with folate for males (CC, CT and TT) and the female TT group, but it was more closely correlated with Vit B12 for the female CT/CC groups.

Keywords:
Food group / Folate / Hcy / *MTHFR* / Vitamin B12

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Abbreviations: **ALT**, alanine aminotransferase; **ANOVA**, analysis of variance; **AST**, aspartate transaminase; **BMI**, body mass index; **BUA**, blood uric acid; **CC**, CC homozygote; **CHD**, coronary heart disease; **Cr**, blood creatinine; **CRP**, C-reactive protein; **CT**, CT heterozygote; **CV**, coefficient of variation; **CVD**, cardiovascular disease; **DBP**, diastolic blood pressure; **F**, frequency; **FFQ**, food-frequency questionnaire; **FPG**, fasting plasma glucose; **G2h**, postprandial 2 hours blood glucose; **Hcy**, homocysteine; **HDL**, high-density lipoprotein; **HGB**, hemoglobin; **HPLC**, high performance liquid chromatography; **HPLC-MS/MS**, high performance liquid chromatographytandem mass spectrometry; **LDL**, low-density lipoprotein; **MTHFR**, methylene tetrahydrofolate reductase; **NHANES**, National Health and Nutrition Examination Survey; **NORCCAP**, Norwegian colorectal cancer prevention; **Q**, quantity; **SBP**, systolic blood pressure; **TC**, total cholesterol; **TG**, triglyceride; **TT**, TT homozygote; **Vit**, vitamin; **WC**, waist circumference

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1 Introduction

Hyperhomocysteinemia, defined as a moderately elevated homocysteine (Hcy) concentration, is an established independent risk factor for stroke and vascular disease [1, 2]. A mutation of *MTHFR C677T* decreases the enzyme activity and increases Hcy levels [3–5]. The prevalence of TT homozygosity reaches 25% in China [6], while it only ranges between 5 and 15% in Europe and North America [7,8]. Numerous studies have demonstrated that TT homozygosity was a strong and independent risk factor for stroke in the Chinese population [9–12]. This genetic risk can be influenced by folate, with the highest risk at the lowest levels of folate intake [13, 14]. In China, the average folate intake is far below the recommended level, which means that additional folate supplementation is very necessary [13, 15].

Folate is mainly provided by two sources: synthetic folic acid and natural foods [16]. Synthetic folic acid can be used as a dietary supplement, but not all of the supplied folic acid is available for the remethylation of Hcy to methionine [17]. Dietary folate supplements, which were derived exclusively from natural sources, improved the plasma folate statuses and Hcy levels for specific *MTHFR C677T* genotypes with different efficiencies [20]. Moreover, dietary folate gains advantages over folic acid fortification, because it also increases the absorption of vitamins, minerals and other necessary nutrients, which are effective in reducing Hcy [18, 19]. Even for those evidence supported that natural foods can improve Hcy levels [21], certain foods are still in controversial [20]. We hypothesized that food items may pose different impacts on Hcy for specific genders and genotypes, and these differences might be correlated with the bioavailability of folate and Vit B12 status. To confirm this hypothesis, we designed a study to investigate the association between Hcy and food groups, and to explore the correlation of plasma folate and Vit B12 with Hcy for each gender and genotype.

2 Subjects and methods

2.1 Subjects

The data were collected from November 2014 to July 2015 at the Health Management Institute of Chinese People's Liberation Army (PLA) General Hospital. The study protocol was approved by the Institutional Ethics Committee of PLA General Hospital. All subjects gave their informed consent to participate in the study. The inclusion criteria were: not taking folic acid supplements or using agents that affect Vit B and folic acid metabolism, such as methotrexate and anticonvulsants, and being free from folic acid supplements for at least 6 months. As a result, a total of 4763 participants aged between 20 and 75 years were enrolled in this study, and completed both a dietary questionnaire and a health examination. Among those, 45 participants were excluded for the inaccuracy of the diet assessment, which was inconsistent with the repetitive test or other questionnaires. In addition, 34 participants with serious renal disease, 54 participants with hepatic disease and 123 participants with hypothyroidism were excluded from the study. In all, a total of 4507 participants were selected for study in this cross-sectional study.

2.2 Outcome measures

2.2.1 Assessment of diet

The participants fulfilled a Chinese food- frequency questionnaire (FFQ), which was developed based on the dietary recall data from the 1992 China Health and Nutrition Survey [22], to assess their food intake over the preceding 12 months. The major food groups for this study were compiled, and some items were modified according to the current Chinese lifestyle. For this study, reported foods were categorized into nine food groups containing more than 130 ordinary foods. These food groups included milk (milk and yogurt), meat (beef, pork, organ meat, and processed meats), fish (fish, seafood, and shellfish), eggs, cereal/wheat (rice, pasta, breads, and noodles), fruits/vegetables, soy (soybean milk, tofu, and other bean products), fried foods, and smoked foods. Subjects were asked to recall the frequency and quantity of food consumption. Food frequencies were ranked from 1 to 4 (1: less than 1 day per week; 2: 1–2 days/week; 3: 3–4 days/week; 4: 5–7 days/week). Daily consumed quantities for each food item was estimated as follows: cereal/wheat (1: $\leq$ 100 g/day; 2: 100–200 g/day; 3: 200–500 g/day; 4: $\geq$ 500 g/day); meat (1: $\leq$100 g/day; 2: 100–200 g/day; 3: $\geq$ 200 g/day); fruits/vegetables (1: $\leq$ 200 g/day: 2: 200–500 g/day; 3: $\geq$ 500 g/day); sugar(1: $\leq$30 g/day; 2: 30–40 g/day; 3: 40–50 g/day; 4: $\geq$ 50 g/day), and salt (1: $\leq$ 6 g/day; 2: 6–8 g/day; 3: $\geq$ 8 g/day). The reliability of the Chinese FFQ was testified by other questionnaires that are also widely accepted as reliable, such as the estimated-diet record, which was based on a checklist and a set of photographs for the foods [23].

2.2.2 Assessment of MTHFR C677T genotype

Genetic polymorphisms *MTHFR* 677 C→T were detected using gene chip hybrid analysis. Genomic DNA was extracted from the whole blood of the participants using the QIAamp® DNA Mini Kit (CAT No. 51304, Germany). The PCR, hybridization, gene array detection and analysis were conducted strictly according to the manuals of the BaiO genotype detecting gene array kit and equipment (BaiO Technology Corp., Shanghai).

2.2.3 Assessment of covariate

The patients were subjected to a health examination, including height and weight measurements and blood

pressure. Blood was drawn from fasting subjects, and tubes with plasma containing EDTA were stored at –80°C for biochemical analyses. Plasma Hcy was analyzed by HPLC (LC-9A,Shimadzu Corp., Kyoto, Japan) with fluorescence detection (F-1080, Hitachi Ltd., Tokyo, Japan) [24]. Serum folate concentration was measured using a dual count Solid Phase Boil Radioassay (Diagnostic Products, Los Angeles, CA). Serum Vit B12 was detected by liquid chromatography–tandem mass spectrometry (HPLC-MS/MS, Agilent Model 6410) [25]. The blood glucose levels, including FPG(fasting plasma glucose) and G2h (postprandial 2 hours blood glucose) were measured using the hexokinase method; the total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDH), triglyceride (TG), hemoglobin (HGB), blood uric acid (BUA), alanine aminotransferase (ALT), aspartate transaminase (AST), and blood creatinine (Cr) were measured with an autoanalyzer (Cobas c 501 autoanalyzer, Roche Diagnostics, Germany). C-reactive protein (CRP) was measured using commercially available enzyme-linked immunosorbent assay kits (ELISA kits, Biosource, Int. CA). Standard quality control procedures were performed each day with standard samples (CV < 10%).

2.3 Statistical analysis

A Hardy–Weinberg equilibrium analysis was performed to determine if the allelic frequency was representative of the general population. Comparisons of continuous variables between the groups were assessed using a *t*-test or ANOVA. Chi-square tests were used for nominal variables. Multiple linear analyses were performed between Hcy and the food groups with stepwise regression for each gender and genotype, using the standardized coefficients to estimate the strength of the associations. The correlation between Hcy and folate/Vit B12 was conducted by Spearman's rank order correlation analysis. Missing data for each variable were replaced with the average values of the analytical variable [26]. All tests were two-tailed, and *P*-values less than 0.05 were considered to indicate significant differences. The statistical analyses were carried out using SPSS version 17.0 and SAS version 8.02 or 9.1.

3 Results

3.1 Clinical characteristics

In total, 4507 individuals participated in this study, including 3146 males (CC = 715, CT = 1523 and TT = 908) and 1361 females (CC = 300, CT = 689 and TT = 372). The average ages for males and females were 49.07 ± 7.44y and 49.00 ± 8.68y, respectively. In males, comparisons between the genotype groups showed significant differences in Hcy, folate, Vit B12, HDL, TG and height ($P < 0.05$). In females, significant differences only existed in Hcy, G2h, and HDL between the genotype groups ($P < 0.05$) (see Table 1).

3.2 Test of Hardy-Weinberg equilibrium

The results showed that the genotypes conformed to the Hardy-Weinberg equilibrium in males, females, and the entire population. The respective allelic frequencies of C and T were 46.93 and 53.07% in males ($\chi^2 = 2.49$, $P > 0.05$), 47.35 and 52.65% in females ($\chi^2 = 0.32$, $P > 0.05$), and 47.06% and 52.94% in the general population ($\chi^2 = 1.02$, $P > 0.05$) (see Table 2).

3.3 Association of food groups and Hcy for each gender and *MTHFR C677T* genotype

The models were all adjusted for age and BMI. For the male CC group, only fish (F) (β = –0.497, $P < 0.001$) was negatively correlated with Hcy. For the male CT group, fish (β = –0.555, $P < 0.001$) and eggs (F) (β = –0.290, $P = 0.050$) were negatively associated with Hcy, whereas cereal/wheat (Q) (β = 0.461, $P = 0.024$) was positively correlated with Hcy. For the male TT group, meat (Q) (β = –1.423, $P = 0.008$), fish (F) (β = –1.265, $P < 0.001$), milk (F) (β = –0.901, $P = 0.002$), and fruits/vegetables (Q) (β = –1.619, $P = 0.001$) were negatively associated with Hcy, but sugar (Q) (β = 0.946, $P = 0.006$) and salt (Q) (β = 0.734, $P = 0.052$) were positively associated with Hcy (see Table 3).

For the female CC group, fruits/vegetables (Q) (β = –1.089, $P = 0.001$), eggs (β = –0.776, $P = 0.001$), and meat (β = –0.830, $P < 0.001$) were negatively correlated with Hcy, but soy (β = 0.436, $P = 0.059$) was positively correlated with Hcy. For the female CT group, eggs (β = –0.430, $P = 0.006$) and meat (Q) (β = –0.660, $P = 0.011$) were negatively correlated with Hcy, but soy (β = 0.391, $P = 0.016$), fried foods (β = 0.383, $P = 0.040$), and salt (β = 0.467, $P = 0.004$) were positively correlated with Hcy. For the female TT group, fish (β = –1.045, $P < 0.001$), eggs (β = –0.649, $P = 0.035$), and fruits/vegetables (F) (β = –1.167, $P = 0.004$) were negatively associated with Hcy (see Table 3).

3.4 Correlation of serum folate and Vit B12 with Hcy for each gender and genotype

Specifically, we found a negative correlation between Log (folate) and Log (Hcy) in all the groups, which was more significant in the TT genotype (male: $r = –0.610$, $P_{trend} < 0.001$; female: β = –0.429, $P_{trend} < 0.001$). The correlation of Log (Vit B12) and Log (Hcy) was weaker than that of Log (folate) and Log (Hcy), except in the female CC and CT groups (female-CC: $r = –0.380$, $P_{trend} < 0.001$; female-CT: $r = –0.413$, $P_{trend} < 0.001$) (see Table 4).

Table 1. Clinical characteristics of the participants for each gender and genotype

Variables	Male				Female				P‡
	CC (*n*=715)	CT (*n*=1523)	TT (*n*=908)	P†	CC (*n*=300)	CT (*n*=689)	TT(n=372)	P†	
Stroke (*n*)	6	13	10	0.80	3	3	2	–	0.254
CHD (*n*)	27	78	55	0.11	15	20	16	0.22	0.051
HBP (*n*)	245	468	318	0.06	51	98	72	0.09	< 0.001*
Age (years)	48.83 ± 7.76	49.09 ± 7.23	49.22 ± 7.50	0.58	48.95 ± 8.64	48.29 ± 8.73	50.19 ± 8.49	0.99	< 0.001*
BMI	26.17 ± 3.23	26.23 ± 3.09	26.38 ± 3.05	0.36	23.72 ± 3.39	23.56 ± 3.15	23.92 ± 3.14	0.21	0.143
Height (cm)	172.71 ± 6.04	173.20 ± 5.66	173.44 ± 5.72	0.04	161.05 ± 5.58	161.61 ± 5.25	161.21 ± 5.15	0.09	< 0.001*
Weight (kg)	78.21 ± 11.51	78.75 ± 10.55	79.40 ± 10.25	0.08	61.42 ± 8.49	61.55 ± 8.85	62.21 ± 8.84	0.80	< 0.001*
WC (cm)	92.34 ± 9.14	92.69 ± 8.61	93.01 ± 8.45	0.31	79.97 ± 9.21	79.92 ± 9.01	81.50 ± 9.09	0.96	0.005*
SBP (mmHg)	120.88 ± 17.40	121.90 ± 16.72	121.46 ± 16.77	0.42	111.40 ± 18.69	109.50 ± 19.18	111.65 ± 19.47	0.55	<0.001*
DBP (mmHg)	79.41 ± 11.52	79.72 ± 11.47	79.76 ± 11.48	0.80	74.42 ± 10.21	73.443 ± 10.52	74.96 ± 11.03	0.57	0.004*
Hcy (μmol/L)	12.02 ± 3.43	12.94 ± 4.66	19.37 ± 9.49	0.00*	8.78 ± 3.53	9.22 ± 3.38	11.40 ± 5.06	0.00*	<0.001*
Folate (ng/mL)	10.22 ± 3.60	9.37 ± 3.46	8.20 ± 3.62	0.00*	11.74 ± 3.73	11.30 ± 3.89	10.13 ± 3.69	0.82	0.026*
Vit B12 (pg/ml)	585.42 ± 246.87	569.36 ± 236.36	525.85 ± 228.85	0.00*	627.93 ± 283.83	616.20 ± 284.77	604.77 ± 282.19	0.57	<0.001*
FPG (mmol/L)	5.88 ± 1.62	5.89 ± 1.53	5.93 ± 1.70	0.79	5.24 ± 0.76	5.29 ± 0.94	5.33 ± 0.81	0.85	<0.001*
G2h (mmol/L)	7.55 ± 2.78	7.63 ± 2.73	7.70 ± 2.82	0.57	7.38 ± 2.16	7.19 ± 2.02	7.54 ± 1.90	0.03*	<0.001*
TC (mmol/L)	4.77 ± 0.92	4.75 ± 0.89	4.73 ± 0.90	0.71	4.76 ± 0.93	4.72 ± 0.93	4.77 ± 0.92	0.93	0.360
LDL (mmol/L)	3.11 ± 0.81	3.11 ± 0.78	3.11 ± 0.82	0.99	3.07 ± 0.83	3.08 ± 0.84	3.13 ± 0.82	0.59	0.267
HDL (mmol/L)	1.14 ± 0.30	1.11 ± 0.28	1.11 ± 0.26	0.05*	1.46 ± 0.36	1.39 ± 0.34	1.41 ± 0.34	0.02*	<0.001*
TG (mmol/L)	2.12 ± 1.95	2.13 ± 1.86	2.15 ± 1.98	0.04*	1.30 ± 0.87	1.33 ± 0.98	1.27 ± 0.70	0.06	<0.001*
CRP (mg/L)	0.20 ± 0.27	0.22 ± 0.44	0.24 ± 0.56	0.17	0.17 ± 0.17	0.21 ± 0.39	0.18 ± 0.16	0.08	<0.001*
HGB (g/L)	154.03 ± 10.61	154.19 ± 10.32	154.35 ± 9.85	0.81	130.93 ± 12.39	130.60 ± 11.91	130.96 ± 11.81	0.57	<0.001*
BUA (μmol/L)	382.52 ± 78.47	378.79 ± 74.02	375.24 ± 75.88	0.15	263.94 ± 57.81	268.41 ± 56.63	264.69 ± 54.15	0.42	<0.001*
ALT (U/L)	28.34 ± 20.74	28.55 ± 22.86	27.65 ± 16.64	0.58	16.40 ± 9.83	17.78 ± 11.45	18.14 ± 12.62	0.23	<0.001*
AST (U/L)	22.35 ± 12.07	22.44 ± 21.51	21.51 ± 8.89	0.38	19.94 ± 5.37	18.53 ± 6.31	18.78 ± 6.92	0.06	<0.001*
Cr (μmol/L)	74.20 ± 11.48	74.15 ± 12.81	73.68 ± 14.69	0.65	54.58 ± 8.59	54.85 ± 8.17	54.59 ± 8.57	0.85	<0.001*

Note: HBP: hypertension; CHD: coronary heart disease; BMI: body mass index; WC: waist circumference; SBP: systolic blood pressure; DBP: diastolic blood pressure; Hcy: homocysteine; FPG: fasting plasma glucose; G2h: postprandial 2 h blood glucose; TC: total cholesterol; LDL: low-density lipoprotein; HDL: high-density lipoprotein; TG: triglyceride; CRP: C-reactive protein; HGB: hemoglobin; BUA: Blood uric acid; ALT: alanine aminotransferase; AST: aspartate transaminase; Cr: blood creatinine. *P*†: comparisions between genotypes. *P*‡: comparisions between male and female.
*$P < 0.05$.

Table 2. Hardy–Weinberg equilibrium testing for *MTHFR C677T* genotypes

	Genotype			Allelic frequency		Hardy–Weinberg equilibrium	
	CC	CT	TT	*C*(%)	*T*(%)	χ^2	*P*
Male (3146)	715	1523	908	46.93	53.07	2.49	0.11
Female (1361)	300	689	372	47.35	52.65	0.32	0.57
Total (4507)	1015	2212	1280	47.06	52.94	1.02	0.31

4 Discussion

4.1 Influence of food groups on Hcy for specific gender and MTHFR C677T genotypes

The Hordaland Homocysteine Study [27] and the National Health and Nutrition Examination Survey (NHANES) [28] have demonstrated that Hcy concentration could be improved by foods. However, the improvement effects of certain foods (e.g. milk, eggs, meat, and soy) were still in disagreement [28–30]. In addition, folate from natural food sources could improve plasma folate statuses and Hcy levels with different efficiencies for specific *MTHFR C677T* genotypes [20]. Therefore, it was necessary to identify which foods would lower Hcy levels, and their efficiencies in improving Hcy for a specific genotype.

Table 3. Correlations between food groups and Hcy for each gender and *MTHFR C677T* genotype

	Variables	*B*	Standard error	Standardized coefficient	*P* value
Male-CC					
	Fish (F)	–0.497	0.136	–0.145	0.000
ANOVA: F=1.907, P=0.035; R^2=0.29, adjusted R^2=0.144					
Male-CT					
	Cereal/wheat (Q)	0.461	0.205	0.063	0.024
	Eggs (F)	–0.290	0.149	–0.059	0.050
	Fish (F)	–0.555	0.136	–0.113	0.000
ANOVA: F=3.442, $P < 0.001$; R^2=0.335, adjusted R^2=0.270					
Male-TT					
	Fruits/vegetables (Q)	–1.619	0.474	–0.120	0.001
	Milk (F)	–0.901	0.286	–0.108	0.002
	Fish (F)	–1.265	0.345	–0.125	0.000
	Meat (Q)	–1.423	0.534	–0.100	0.008
	Sugar (Q)	0.946	0.346	0.101	0.006
	Salt (Q)	0.734	0.377	0.066	0.052*
ANOVA: F=5.509, $P < 0.001$; R^2=0.492, adjusted R^2=0.394					
Female-CC					
	Fruits/vegetables (Q)	–1.089	0.311	–0.209	0.001
	Eggs (F)	–0.776	0.226	–0.213	0.001
	Meat (F)	–0.830	0.217	–0.242	0.000
	Soy (F)	0.436	0.230	0.114	0.059*
ANOVA: F=4.373, $P < 0.001$, R^2=0.177, adjusted R^2=0.136					
Female-CT					
	Eggs (F)	–0.430	0.157	–0.122	0.006
	Meat (Q)	–0.660	0.259	–0.111	0.011
	Soy (F)	0.391	0.161	0.107	0.016
	Fried foods (F)	0.383	0.186	0.107	0.040
	Salt (Q)	0.467	0.161	0.119	0.004
ANOVA: F=3.190, $P < 0.001$; R^2=0.68, adjusted R^2=0.47					
Female-TT					p
	Fruits/vegetables (F)	–1.167	0.399	–0.191	0.004
	Eggs (F)	–0.649	0.307	–0.124	0.035
	Fish (F)	–1.045	0.266	–0.214	0.000
ANOVA: F=3.814, $P < 0.001$; R^2=0.724, adjusted R^2=0.62					

Notes: *F*: frequency; *Q*: quantity (daily amount); *P : *P*-value approaches 0.05

Table 4. Correlations between folate/vitamin B12 and Hcy for each gender and *MTHFR C677T* genotype

	Log(Hcy) and Log (folate)		Log(Hcy) and Log(Vit B12)	
	*r**	*P*	*r**	*P*
Male				
CC	–0.342	0.000	–0.316	0.000
CT	–0.409	0.000	–0.297	0.000
TT	–0.610	0.000	–0.456	0.000
Female				
CC	–0.241	0.000	–0.380	0.000
CT	–0.269	0.000	–0.413	0.000
TT	–0.429	0.000	–0.367	0.000

Note: *r**: Spearman's coefficient.

European studies have shown that Hcy levels are positively correlated with meat consumption, but negatively correlated with cereal/wheat consumption [27, 31]. This suggests that moderate supplementation of cereal/wheat for the western diet would lower Hcy levels, but increased meat consumption would increase Hcy levels [28]. In this study, we found that cereal/wheat consumption was positively correlated with Hcy for male CT and female TT groups. Our results coincide with a previous study performed in China, which suggested that higher intakes of refined cereals would decrease plasma folate levels and increase Hcy levels [30]. Further analysis showed that the background of the dietary pattern in China, which contains more cereal/wheat but less meat, was significantly different from that in the West [28]. Moreover, we found that meat consumption was negatively correlated with Hcy for male TT and female CC/CT groups. This was in accordance with the Hordaland Homocysteine Study, which identified that non-processed meat consumption were inversely associated with Hcy [27]. However, our study further revealed that this correlation was only supportive for specific genders and genotypes.

In addition, the study also identified that eggs, fish, and milk were negatively correlated with Hcy, which further corroborated the findings of the Hordaland Homocysteine study [32]. However, we found that these food groups were correlated with Hcy concentrations with different strength for each gender and genotype, Furthermore, fruits and vegetables have been demonstrated to increase the serum folate levels and decrease the Hcy levels [33, 34]. Similarly, we identified an inverse relationship between the fruits/vegetables intake and the Hcy levels, but this association was more significant in male TT group and female CC/TT groups.

We found that soy, sugar, salt, and fried foods were positively correlated with Hcy in the Chinese population. Previous studies demonstrated that soy foods did not reduce Hcy levels in women, even though they were associated with a reduced risk of coronary heart disease (CHD) [35, 36]. However, after stratified by genotypes, we found soy consumption increased Hcy levels for female CC and CT groups, but not for other groups. Furthermore, we also found that sugar and salt were both risk factors for high levels of Hcy, which was also consistent with previous studies [27]. The consumption of fried foods increases Hcy levels, which might be result from the increased oil intake in fried foods and altered nutrients in processed foods.

4.2 Correlations of folate/vit B12 status with Hcy for each gender and genotype

In the Norwegian Colorectal Cancer Prevention (NORCCAP) cohort study, the network between *MTHFR* and B vitamins showed that the effect of B vitamins (riboflavin, cobalamin, or vitamin B6) on Hcy was strongest in the TT group [37]. Moreover, the threshold level of folate to keep homocysteine levels in a normal range was higher in the TT genotype than in the CC or CT genotypes [38]. In this study, we observed a negative correlation between folate and Hcy for each gender and genotype, and this correlation was more significant in the TT genotype. However, what we need to mention was that serum folate only reflected recent intakes but not the 12 months covered by their FFQ. Similarly, a negative correlation also existed between Hcy and Vit B12, but this correlation was weaker than that between Hcy and folate, except in the female CC and CT groups. This suggested that Hcy might be improved by dietary folate more efficiently in males (CC, CT and TT) and female TT group, but by Vit B12 in females (CC and CT) more efficiently.

4.3 Limitations

Limitations still existed in this study. First, the study lacked exact nutrient divisions and detailed cooking methods to conclusively determine the effects of the foods. Second, ideal cut-off values for the frequency or quantity of each food should be explored, in order to build a general guideline for a specific population group. Third, this cross-sectional study was based on retrospective data, with substantial bias for the available data. Finally, it is still unclear whether reducing Hcy using

dietary intakes is a valid method to lower the risk of CVD. It was necessary to perform a prospective study to verify this hypothesis.

4.4 Conclusion

Plasma Hcy levels were correlated with food groups to varying degrees, based on individual's gender and *MTHFR C677T* genotype. Hcy levels were closely correlated with folate in males (CC, CT and TT) and female TT group, but it was closely correlated with Vit B12 for female CT/CC groups. These results might be useful for personalized nutritional recommendations in the future.

Q.Z. and H.X. had full access to all of data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Q.Z.—obtained funding, design the study and critical revision of the manuscript; Q.Z., H.X., F.L. and T.Y.X.—analyze and interpret the data, drafting of the manuscript; W.M.W., C.M.—data collection; H.X.C., C.Y.—administrative, technical, or material support. All authors read and approved the final manuscript.

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