Single-nucleotide polymorphisms in one-carbon metabolism genes, Mediterranean diet and the risk of pre- and postmenopausal breast cancer

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Research

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Abstract

Background: One-carbon metabolism pathway could interfere with the carcinogenesis of breast cancer (BC). Dietary pattern plays an important role in one-carbon metabolism linking the combination of dietary micronutrients.

Methods: We assessed associations between single-nucleotide polymorphisms (SNPs) of one-carbon metabolism, adherence to Mediterranean dietary pattern (MDP) and BC risk from a case-control study (818 cases, 935 controls) among Chinese female population. The genotyping of 10 SNPs in seven one-carbon metabolism-related genes (*MTHFD1, MTRR, MAT2B, CDO1, FOLR1, UNG2, ADA*) were performed. Dietary intake measurements were assessed by a validated food-frequency questionnaire. Gene-diet interactions were analyzed.

Results: No evidence demonstrated SNPs of one-carbon metabolism or their haplotypes were associated with BC risk. High adherence to the Mediterranean dietary pattern decreased the risk of breast cancer among post- but not premenopausal women and the association was influenced by the SNPs genotype, with the increasing number of variant allele in *MTHFD1* (rs*11627387), MTHFD1 (rs2281603), MTRR (rs16879334), MTRR (rs2287780), MAT2B (rs4869087), FOLR1 (rs10501409), UNG2 (rs231622) and ADA (rs244072).* The protective effect against BC risk from high adherence to the MDP was gradually weakened and disappeared. For *MTHFD1 (rs8003567)* and *CDO1* (*rs34869)* genotypes, women with homozygous were less affected by adherence to the MDP than to women who with heterozygotes. No significant gene-diet interactions were observed.

Conclusions: SNPs of one-carbon metabolism genes modify the effect of high adherence to MDP against BC risk in Chinese women, as potential effect modifiers. Genetic variants may influence the association between diet and BC risk.

Introduction

The primary risk factors of breast cancer (BC) include overweight or obesity, physical inactivity, exogenous hormone intake (use of oral contraceptive and hormone replacement therapy), reproduction condition (late age at first birth and low parity), family history of cancer and unhealthy diet(Bethea, et al. 2015, Brewer, et al. 2017, Deschasaux, et al. 2017, Ellingjord-Dale, et al. 2017, Neuhouser, et al. 2015, Trinh, et al. 2015). Besides these modifiable risk factors, genetic predisposition also plays an important role in susceptibility to BC. Inherited mutations in *BRCA1 and BRCA2* genes confer a high risk of BC to women of specific families(Torres, et al. 2017), whereas, for a large percentage of cases, BC is more likely attributed to a combination of genetic variations and environmental or lifestyle factors. Elucidating the gene-environment interactions is therefore essential to unveil the molecular mechanisms involved in breast carcinogenesis.

Diet is one of the environmental factors and often related to individuals' health outcomes. A well-balanced dietary pattern, such as the Mediterranean diet, is able to reduce the risks of a variety of diseases. The traditional Mediterranean diet is characterized by the liberal use of olive oil; the high consumption of vegetables, fruits, nuts, legumes and unprocessed cereals; the moderate amounts of fish and wine; the low intake of dairy products (with the exception of cheeses), red meat and meat products(Trichopoulou, et al. 2014). A number of epidemiological studies have suggested that this dietary pattern had substantial health benefits and displayed inverse associations with multiple disease types, including cancer(Soltani, et al. 2019). Our study also showed that high adherence to the Mediterranean dietary pattern significantly reduced post-menopausal BC risk in Chinese women. Since cancer development is generally determined by the interplay between extrinsic and intrinsic factors, the roles of underlying molecular pathways in the association of the Mediterranean diet with BC risk require further investigation.

One-carbon metabolism is a complicated metabolic network composed of cascade reactions based on the transfer of one-carbon units, which is required by nucleotide synthesis, DNA replication, repair and methylation. Folate, a water-soluble vitamin-B found rich in leafy green vegetables and fruits, beans and peas, grains and cereals, is an essential component of this metabolism pathway as the functional donor of one-carbon units. The inadequate intake of nutrients in the daily diet could destroy the normal physiological process of one-carbon metabolism, associated with the disruption of DNA replication and repair as well as aberrant DNA methylation patterns, eventually leading to carcinogenesis(Miranti, et al. 2017). Some epidemiological studies provide some promising results, which focused on *MTHFR* (*rs1801131, rs1801133*) and *MTR* (*rs1805087*), intake of folate and vitamin B6/B12 and BC risk(Ma, et al. 2009, Platek, et al. 2009). Also, genetic polymorphisms in the one-carbon metabolism pathway genes encoding functional enzymes and coenzymes have been suggested to influence individuals' susceptibility to cancer, such as Methylenetetrahydrofolate reductase (MTHFR) and methionine synthase (MTR) (Shao, et al. 2018, Wu, et al. 2016). However, in addition to these most common polymorphisms, the role of genetic variants in other one-carbon metabolism genes and their interactions with diet regarding BC risk remain largely unexplored, especially in the Asian female population.

In this study, we evaluated associations between genetic polymorphisms in one-carbon metabolism-related genes and the risk of BC among Chinese women. We further investigated the interaction effects of selected genetic variants with high adherence to the Mediterranean dietary pattern on BC carcinogenesis.

Materials And Methods

Subjects

Subjects were from the Chinese Wuxi Exposure and Breast Cancer Study (2013-2014), a population-based case-control study of the role of biology, diet, lifestyle, and environmental factors in the etiology of BC in Asian women. The subjects were all women who lived in Wuxi city, Jiangsu Province, China, for more than five years. Newly diagnosed breast cancer patients within one year were selected as the case group according to the local cancer registration system. All cases were identified according to the International Classification of Diseases for Oncology (ICD-10, code C50) and were confirmed by pathology, excluding patients with secondary or recurrent BC. For those with multiple incident cancers, only included those with BC as the first original malignancy diagnosed. Controls were matched to the cases in a ratio of 1:1 by the same residence area and age (range of ±5 years), excluding individuals with any cancer

history. The study protocol was approved by the Institutional Review Boards of Jiangsu CDC, and the informed consent were obtained from all subjects. Blood samples were collected from both cases and controls.

Data on diet

The usual diet was assessed by a validated, semi-quantitative food frequency questionnaire (FFQ), which included 149 items along with the recipes commonly used in China(Zhao, et al. 2002). Nutrient and energy intake were calculated through the Chinese Food Composition Database. Dietary intake assessment included whether the food was consumed, consumption frequency (times of per day/week/month/year) and the average amount of food consumption at each time. The 149 food items in the FFQ were classified into 18 predefined food groups based on similarities in nutrient profile and culinary usage.

Mediterranean diet scale

The Alternate Mediterranean Diet Score (aMED) established by Fung et al.,(Fung, et al. 2005) includes nine dietary components and range from 0-9 scores (minimum to maximum conformity). One point is given to each subject when the food intake is equal to or above the median intake of controls for the following seven components regarded as healthy: grains, fruits, vegetables, legumes, nuts, fish, and monounsaturated fat–saturated fat ratio. One point is given when the intake amount of a subject is less than the median intake of unhealthy food such as red meat or processed meat, or alcohol consumption within 5-25 g/day for women as a specified range. The higher the score obtained from the questionnaire, the greater the adherence to the Mediterranean dietary pattern (MDP).

Lifestyle, anthropometric, medical history and reproductive history data

Demographic, lifestyle characteristics, menstrual and reproductive events, dietary intake, disease history and physical activity-related data were collected from a structured questionnaire, through in-person interviews conducted by trained interviewers. Anthropometric measures were obtained by trained personnel following the protocol. Physical activity was measured by referencing the Global Physical Activity Questionnaire. Postmenopause was defined as an absence of menstruation in the past 12 months.

Genotyping assays

A total of 15 single-nucleotide polymorphisms (SNPs) involved in 11 one-carbon metabolism genes including Methylenetetrahydrofolate dehydrogenase 1 (*MTHFD1*), methionine synthase reductase (*MTRR*), methionine adenosyltransferase 1A (*MAT1A*), methionine adenosyltransferase 2B (*MAT2B*), folate receptor 1 (*FOLR1*), cystathionine- -synthase (*CBS*), glutaminase (*GLS*), DNA methyltransferases 3B (*DNMT3B*), uracil N-glycosylase 2 (*UNG2*), adenosine deaminase (*ADA*) and cysteine dioxygenase (*CDO1*), were selected for genotyping analysis. Genomic DNA was extracted from 200 μl of peripheral blood using the QIAamp DNA Blood Mini Kit (QIAGEN, Germany) following the manufacturer's instructions. Purified DNA was evaluated and quantified by agarose gel electrophoresis and spectrophotometer methods. Experimental design and SNP genotyping were carried out by CapitalBio Corporation (Beijing, China) using the Sequenom MassARRAY platform following the manufacturer's instructions. The call rate for each SNP among all the samples was >95%. In addition, five percent of samples were randomly selected and then repeatedly genotyped with a concordant rate of 100%.

Statistical analysis

Chi-square tests were carried out to examine Hardy–Weinberg equilibrium (HWE) in the control group. Linkage disequilibrium between SNPs was calculated as D' and R^2 values. The SNPs with strong linkage disequilibrium will be constructed as haplotypes for further analysis.

Unconditional logistic regression methods were used to assess: (1) associations between SNPs polymorphisms and BC risk (statistical significance should be after Benjamini & Yekutieli correction for multiple testing, $p<0.05$). (2) associations between adherence to the MDP and BC risk. (3) associations between adherence to the MDP and BC risk stratified by SNPs genotype.

The potential confounders included are age at diagnosis for cases or enrollment for controls (by years), area (urban, rural), education (ordered as illiterate and primary, middle and high school, university and above), tobacco smoking (no, or yes: including smoking and second-hand smoking 3day/week), moderate physical activity (minutes/day), oral contraceptives use (no, or yes: current use or ever use), hormone replacement therapy (no, or yes: current use or ever use), family history of breast cancer (no, or yes: in a first-degree relative), history of benign breast disease (no, or yes: including lactation mastitis, plasma cell mastitis, cyclomastopathy, fibroadenoma of breast, galactocele), age at menarche (by years), parity (0, 1, 2, 3), age at first full-term delivery (by years), breastfeeding (no, or yes), body mass index (BMI; in kg/m^2).

A priori causal model of the causal relationship between the one-carbon metabolism pathway, Mediterranean dietary pattern (MDP) and breast cancer was constructed to determine which confounders need to be adjusted, presenting as a directed acyclic graph (DAG)(Greenland, et al. 1999) (**Supplemental Figure 1)**.

The test of interaction between each SNP genotype and adherence to the MDP were based on both multiplicative model and addictive model. Multiplicative model based on likelihood ratio test and addictive model based on Bootstrap estimation.

All subjects were included in the present analysis, with the missing covariates data imputed by using the fully conditional specification Multivariate Imputation by Chained Equations method(Azur, et al. 2011). All analyses were performed with R version 4.0.0 (The R Project for Statistical Computing, USA; http://www.r-project. org/).

Results

From Nov 2013 to Nov 2014, a total of 1410 newly diagnosed breast cancer cases were identified in Wuxi City, 1072 cases meeting the inclusion criteria and 818 of them were recruited in this study. 1072 controls were screened and 935 of them were recruited. Of the 818 cases and 935 controls, the demographic characteristics and anthropometric measures of the subjects stratified by menopausal status are presented in **Supplemental Table 1**. The priori causal model (using the DAG) that reflects the current knowledge about the relationship between the one-carbon metabolism pathway, Mediterranean dietary pattern (MDP) and breast cancer is shown in **Supplemental Figure 1**. The minimal sufficient adjustment sets included age, area, education, tobacco smoking, moderate physical activity, oral contraceptives use, hormone replacement therapy, family history of breast cancer, history of benign breast disease, age at menarche, parity, age at first full-term delivery, breastfeeding and body mass index.

Characteristics and frequencies of one-carbon metabolism genes

Genotype and allele frequencies of the 15 SNPs in eleven one-carbon metabolism pathway-related genes *(MTHFD1, MTRR, MAT1A, MAT2B, CDO1, FOLR1, CBS, GLS, DNMT3B, UNG2, ADA)* among cases and controls are shown in **Table 1**. Because there is no genetic variation (minor allele frequency less than 0.05 was found in the *MTHFD1* T>C (*rs2230491*), *MAT1A* T>C (*rs10887718*), *CBS* T>C (*rs11701048*), *GLS* T>C (*rs12185688*) and *DNMT3B* G>A (*rs13045669*), they will not be introduced in the following analyses for the association of BC risk and the interaction of diet. The genotype frequencies of the SNPs included in the control group did not deviate from Hardy–Weinberg equilibrium (HWE).

Associations between single-nucleotide polymorphisms (SNPs) and breast cancer risk

In both adjusted and unadjusted model, women homozygous for the variant of the 10 SNPs: *MTHFD1* G>A (*rs11627387*), *MTHFD1* T>C (*rs2281603*), *MTHFD1* G>A (*rs8003567*) , *MTRR* G>C (*rs16879334*), *MTRR* T>C (*rs2287780*), *MAT2B* C>A (*rs4869087*), *CDO1* G>C (*rs34869*), *FOLR1* T>G (*rs10501409*), *UNG2* G>A (*rs231622*), *ADA* G>A（*rs244072*）demonstrated no statistically significant (p<0.005) increased pre- and post-menopausal BC risk, when compared to the wild-type carriers, as shown in **Supplemental Table 2**.

For linkage disequilibrium analysis, two considerable degree of linkage disequilibrium were observed between the *MTRR* T>C (*rs227780*) and *MTRR* G>C (*rs16879334*) (D'=0.99, r^2=0.99) and *MTHFD1* G>A (*rs8003567*) and *MTHFD1* G>A (*rs11627387*) (D'=0.96, r^2=0.35), as shown in **Figure 1**. Haplotypes with a frequency greater than 0.03 were constructed in the case group and control group, but no significant difference was found in the distribution of haplotypes between cases and the controls, as shown in **Supplemental Table 3.**

Associations between adherence to the Mediterranean dietary pattern and breast cancer risk, stratified by the SNPs genotypes

A higher aMED score indicated a lower postmenopausal BC risk with a p-trend value<0.0000, the OR value is 0.54 (95% CI: 0.38-0.78) for comparing top with bottom quartiles, and OR value for per 1 point increase of the aMED is 0.80 (95% CI: 0.70-0.90). However, no significant association was found among premenopausal women (**Table 2**).

Among postmenopausal women, we found that the association between MDP and BC risk was affected by the SNPs genotype. Stratified by the genotypes of a dominant model (e.g., AA+AB versus BB, A is the risk allele) and an additive model (e.g., AA versus AB versus BB, A is the risk allele), with the increasing number of variant allele, the protective effect against BC risk from high adherence to the MDP was gradually weakened and disappeared. While for *MTHFD1 (rs8003567)* and *CDO1* (*rs34869)* genotypes, women with homozygous were less affected by high adherence to the MDP than to women who with heterozygotes. The results were shown in **Table 3** and **Figure 2**, and the alternate Mediterranean Dietary Pattern score calculated as a continuous variable and a quartile variable, separately.

Interaction analyses between high adherence to the Mediterranean dietary pattern, One-carbon metabolism genes and breast cancer risk

In the interaction analyses, the wild-type genotype of the SNPs at the lowest quartile values of aMED was used as the reference group. We found a nominal statistical significance of the relevant interaction between *MTHFD1* G>A (*rs8003567*) polymorphisms with postmenopausal breast cancer risk based on the additive and dominant genotypic effects (P-interaction=0.0260 for additive genotypic effects and P-interaction=0.0465 for dominant genotypic effects, based on multiplication model), **Table 3**. However, this interaction is no longer significant under the Benjamini & Yekutieli correction for multiple comparisons. The detail was shown in **Supplemental Table 4 and Table 5**.

Discussion

To our knowledge, this is the first study performed in China that investigated the associations between genetic variants in one-carbon metabolism genes and BC risk as well as their interactions with MDP on BC risk. Among 10 SNPs involved in the present study, none of them were statistically significantly associated with the risk of BC despite the menopausal status. However, some meaningful results were found when analyzing the associations between high adherence to MDP and BC risk based on the stratification of genotypes of the SNPs studied. The association between high adherence to MDP and risk

reduction of BC was affected by the SNPs genotype associated with the one-carbon metabolism pathway. Our study suggests that the one carbon-metabolism genes may act as modifiers between diet and BC risk.

The numerous health benefits of the Mediterranean dietary pattern are widely recognized. Since this dietary pattern is composed of many foods rich in fiber and antioxidants, such as flavonoids, vitamins and carotenoids, it has been linked to the prevention of various diseases, including several common cancers. The underlying mechanisms by which the Mediterranean dietary pattern modulated BC risk were previously identified as the decrease of endogenous estrogens,(Carruba, et al. 2006) neutralization of free radicals to prevent DNA damage(Visioli, et al. 2004) as well as reduction of oxidative stress(Mitjavila, et al. 2013). Although an inverse association of adherence Mediterranean dietary pattern with BC risk has been unveiled by many studies(van den Brandt and Schulpen 2017), such observation failed to be confirmed by others,(Couto, et al. 2013, Demetriou, et al. 2012) or was confined to specific subject subgroups defined by menopausal status(Buckland, et al. 2013, Trichopoulou, et al. 2010). The inconsistent results across different studies may be attributed to differences in study design, racial background, sample size and dietary assessment method. Meanwhile, the influences of unknown genetic variations on BC risk as potential effect modifiers cannot be ignored. Our findings demonstrated that compliance with the Mediterranean diet pattern decrease the risk of breast cancer among post- but not pre-menopausal women. Furthermore, SNPs of one carbon-metabolism gene could modulate the association found above.

The nutrients associated with one-carbon metabolism could not naturally be manufactured by the human body, which means they need to be obtained from foods. The disruption on the one-carbon metabolism pathway could interfere with DNA-repair, DNA-replication, and gene expression regulation, which could be highly carcinogenetic(Kim 2004, Locasale 2013). That has motivated lots of studies focusing on the potential link between the nutrients associated with one-carbon metabolism pathway and carcinogenesis(Milne, et al. 2017, Weinstein, et al. 2006). A previous study showed that SNPs of one-carbon metabolism gene have interactions with folate intake to affect the BC risk(Kakkoura, et al. 2017). However, most previous studies focus on the effect of individual nutrients rather than a combination of foods(Lissowska, et al. 2007), which have been limited in interpreting the high degree of intercorrelation among various nutrients. These associations were always weak because it is hard to attribute effects to single independent component foods(Kim 2004). In this context, we choose a ‘posterior’ dietary pattern instead of individual nutrients, which could better capture specific diet characteristics and cumulative effects of nutrients.

The Mediterranean dietary pattern is characterized by high consumption of vegetables, fruit, legumes, and fish, rich in folate, choline, vitamins and methionine. Sufficient levels of micronutrients play an important role in the one-carbon metabolism(Park, et al. 2012, Woodside, et al. 2005), because specific enzymes and co-enzymes in one-carbon metabolism require ample quantities of dietary micronutrients (e.g., folate, methionine and other specific amino acids and B_2, B_6 and B_{12} and other vitamins), as substrates to achieve their biological functions(Lucock 2000, Stevens, et al. 2007).

One-carbon metabolism is interconnected to the biological processes of DNA methylation and DNA synthesis(Xu and Chen 2009). Both processes are thought to play key roles in carcinogenesis(Lewis, et al. 2006, Maruti, et al. 2009). DNA methylation is an epigenetic mechanism by which cells regulate gene expression, which involves the addition of a methyl (-CH3) to the 5-carbocytosine residue, predominantly in the cytosine guanine dinucleotide (CPGs). Dietary micronutrients involved in one-carbon metabolism play an essential role in DNA methylation, such as folic acid, choline, betaine, riboflavin, vitamins B_6 and B_{12}, and the amino acid methionine (**Figure 3**)(Crider, et al. 2012, Locasale 2013). Especially folic acid (or called vitamin B_9), whose role is crucial in the DNA methylation process, producing the methyl group donor, S-adenosylmethionine.

A nominally significant interaction result of *MTHFD1* G>A (*rs8003567*) also implies that one-carbon metabolism genes may be related to diet and BC risk by affecting DNA methylation. The *MTHFD1* gene product is a multifunctional enzyme possessing the activities of methylene-THF dehydrogenase, methenyl-THF cyclohydrolase and formyl-THF synthetase in one-carbon metabolism pathway(MacFarlane, et al. 2011). It usually catalyzes sequential and reversible reactions in multiple conversion of tetrahydrofolate (THF), the active form of folate, into 5,10 methylene-THF, which is essential for the *de novo* purine and thymidylate synthesis as well as the supply of one-carbon units for subsequent DNA methylation. The deficiency or dysregulation of the *MTHFD1* enzyme may influence cell division and global methylation pattern, eventually contributing to tumorigenesis(Ding, et al. 2018, MacFarlane, et al. 2011). Since *rs8003567* located in the intronic region of the *MTHFD1* gene and no disease-related studies on SNPs have been reported before, another possible explanation cannot be excluded that there are additional functional genetic variants in linkage disequilibrium with these two SNPs that modify BC risk in Chinese female population. However, the interpretation of a nominally significant interaction should be cautious, because the corrected *P*-values for multiple comparisons is no longer significant, the gene-diet interaction obtained may be a false positive result. Thus, replication of the findings in other independent studies is needed before the firm conclusions can be drawn.

However, the relationship between nutrients and DNA methylation is complicated, and there is no unified conclusion now. A recent study in rural African women support that one-carbon nutrient may affect methylation levels, dietary intake of one-carbon metabolites and cofactors in diet fluctuates with seasons. The concentration of biomarkers of maternal carbon metabolism nutrients during pregnancy was associated with the methylation of metastable epi-alleles in DNA from birth infant’s lymphocytes and hair follicles. Specifically, plasma concentrations of riboflavin and vitamin B_6 indicate this association(Dominguez-Salas, et al. 2014), and previous studies(Dominguez-Salas, et al. 2013) had shown a positive correlation between the two biomarkers and carefully measured dietary intake. However, a recent big cross-sectional study included 5186 adults does not found any log-linear association between the intake of one-carbon metabolic nutrients and individual CpG methylation.

The strength of our study is that we focus on the genetic variants in the one-carbon metabolism pathway associated with BC risk linking a specific dietary pattern. Most of the previous studies historically focused on individual nutrients(Kim, et al. 2016, Matejcic, et al. 2017) could not capture the complicated interrelationships among nutrients and their cumulative effects(Turati, et al. 2018). The dietary pattern is a combination of food groups rather than isolated nutrients. A ‘posterior’ Mediterranean dietary pattern contains adequate levels of micronutrients associated with one-carbon metabolisms, such as Vitamins B_2, B_6, B_{12}, folate and choline, which are all involved in DNA methylation and synthesis(Mas, et al. 2007). Thus, it may be more predictive for vivo situation(Brennan, et al. 2010, Gerber 2003) and interpretative for disease risk and biological mechanism.

Several limitations should also be taken into account in our study. First, we did not obtain adequate information on various subtypes of breast cancer, such as hormone receptor status. The BC risk based on the stratification analysis of breast cancer subtypes is not able to be further evaluated. Second, although we examined several SNPs in multiple key genes thought to be important in the one-carbon metabolism pathway, some other potential polymorphic sites may not be involved in the present study. Third, data were collected from a case-control study, which might be partially influenced by the biases inherent in case-control designs, we analyzed the effect of selection bias in **Supplemental Figure 3**. Finally, since the number of cases and controls enrolled in this project is relatively small, the associations we founded requires replication in other larger sample independent studies. Further work should assess associations of BC risk and the concentrations of these nutrients in plasma associated with one-carbon metabolism and DNA methylation.

Conclusion

In conclusion, our results support that the SNPs in one-carbon metabolism genes modify the effect of high adherence to Mediterranean dietary pattern against BC risk in Chinese women. Genetic variants may as potential effect modifiers influence the association between diet and BC risk.

Declarations

Ethical approval

This study was approved by the ethical committee of the Jiangsu Center for Disease Control and Prevention (Jiangsu, China).

Informed consent

Informed consent was obtained from all individual participants included in the study.

Consent for publication

Not applicable

Availability of data and materials

The datasets generated and analyzed during the current study are not publicly available due to the racial genetic characteristics but are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Author's Contributions

All authors contributed to the preparation of the manuscript. PMW and MW (guarantors) had the original idea for the study and carried out the study design, formed the investigator group that obtained the funding, as well as oversaw the study implementation and data collection. JYZ, WL, YQD and YQ assisted data collection and interpreted the data. SC and ZZ carried out the analysis reported in this paper, under the supervision of PMW and MW, and also prepared the initial draft of the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1.

Genotype and minor allele frequencies for the single-nucleotide polymorphisms (SNPs) in one-carbon metabolism pathway related genes

Gene	dbSNP ID[a]	Risk(variant) allele	Cases/Controls[b]			MAF[c]	Hardy–Weinberg[d]
			Wild-type	Heterozygote	Homozygote	Cases/Controls	(*P*-value)
MTHFD1	rs11627387	A	276/334	390/424	128/160	0.41/0.41	0.45
	rs2230491	C	801/922	0/2	0/0	0/0	-
	rs2281603	C	297/360	393/438	104/119	0.38/0.37	0.73
	rs8003567	A	503/585	263/291	33/46	0.20/0.21	0.46
MTRR	rs16879334	C	551/633	229/267	16/19	0.16/0.17	0.32
	rs2287780	C	552/635	231/269	16/20	0.16/0.17	0.39
MAT1A	rs10887718	C	767/890	33/35	0/0	0.02/0.02	-
MAT2B	rs4869087	A	607/718	177/189	15/15	0.13/0.12	0.82
CDO1	rs34869	C	356/402	342/427	100/99	0.34/0.34	0.66
FOLR1	rs10501409	G	414/496	325/376	59/59	0.28/0.27	0.48
CBS	rs11701048	C	782/907	17/15	0/0	0.01/0.01	-
GLS	rs12185688	C	800/924	800/924	0/0	0/0	-
DNMT3B	rs13045669	A	800/922	800/922	0/0	0/0	-
UNG2	rs231622	A	264/299	388/450	141/171	0.43/0.43	0.98
ADA	rs244072	A	542/641	231/252	24/26	0.18/0.17	0.9

[a] SNP identifier based on NCBI dbSNP

[b]The number of cases and controls may differ from those of study-subjects due to missing genotype values

[c] MAF minor allele frequency

[d] *P* value from Chi-square test performed for Hardy–Weinberg equilibrium (HWE) evaluation, using only controls

Table 2.

Multivariate-adjusted odd risks (ORs) of breast cancer risk in relation to the alternate Mediterranean Diet Score (aMED), stratified by menopausal status

aMed	Premenopausal women (n=600)		Postmenopausal women (n=1153)	
	Case/Control	Adjusted OR[a] (95 % CI)	Case/Control	Adjusted OR[a,b] (95 % CI)
Quartile 1	52/106	1.00	190/146	1.00
Quartile 2	63/95	1.34 (0.83,2.20)	170/133	0.97 (0.69,1.35)
Quartile 3	58/83	1.55 (0.94,2.57)	135/128	0.77 (0.54,1.09)
Quartile 4	41/102	0.90 (0.53,1.53)	109/142	0.54 (0.38,0.78)
P-trend		0.8241		0.0005
Per SD increase		0.98 (0.82,1.17)		0.80 (0.70,0.90)

[a] Adjusted for age at diagnosis for cases or enrollment for controls, area, education, tobacco smoking, moderate physical activity, oral contraceptives use, hormone replacement therapy, family history of breast cancer, history of benign breast disease, age at menarche, number of full term births, age at first full term delivery, breastfeeding and body mass index.

[b] Adjusted for the menopausal age.

Table 3.

Associations between breast cancer risk and adherence to Mediterranean dietary pattern (MDP), stratified by genotypes

Gene	SNP	Alleles	Cases/Controls[a]	Adjusted OR (95 % CI)[b,c]	*P*-interaction[d]	Gene	SNP	Alleles	Cases/Controls[a]	Adjusted OR (95 % CI)[b,c]	*P* in
MTHFD1	rs11627387	GG	202/201	0.80 (0.68, 0.94)	0.7837	MAT2B	rs4869087	CC	446/417	0.85 (0.76, 0.94)	0
		GA	288/239	0.85 (0.74, 0.98)				CA	129/119	0.90 (0.74, 1.09)	
		AA	93/101	0.82 (0.65, 1.02)				AA	12/9	1.09 (0.57, 2.09)	
		GG	202/201	0.80 (0.68, 0.94)	0.8567			CC	446/417	0.85 (0.76, 0.94)	0
		GA+AA	381/340	0.86 (0.77, 0.96)				CA+AA	141/128	0.91 (0.75, 1.09)	
	rs2281603	TT	215/212	0.79 (0.68, 0.92)	0.1345	CDO1	rs34869	GG	261/237	0.93 (0.82, 1.06)	0
		TC	291/254	0.87 (0.75, 0.99)				GC	253/263	0.82 (0.71, 0.94)	
		CC	77/75	1.00 (0.79, 1.26)				CC	75/47	0.77 (0.54, 1.09)	
		TT	215/212	0.79 (0.68, 0.92)	0.1685			GG	261/237	0.93 (0.82, 1.06)	0
		TC+CC	368/329	0.89 (0.80, 1.00)				GC+CC	328/310	0.80 (0.71, 0.91)	
	rs8003567	GG	373/348	0.89 (0.80, 1.00)	0.0260	FOLR1	rs10501409	TT	301/293	0.84 (0.75, 0.96)	0
		GA	190/169	0.81 (0.68, 0.95)				TG	250/213	0.88 (0.77, 1.01)	
		AA	24/27	0.60 (0.38, 0.95)				GG	36/37	1.11 (0.62, 2.04)	
		GG	373/348	0.89 (0.80, 1.00)	0.0465			TT	301/293	0.84 (0.75, 0.96)	0
		GA+AA	214/196	0.79 (0.68, 0.92)				TG+GG	286/250	0.88 (0.77, 1.00)	
MTRR	rs16879334	GG	415/376	0.83 (0.75, 0.93)	0.3800	UNG2	rs231622	GG	200/175	0.91 (0.77, 1.06)	0
		GC	159/152	0.91 (0.77, 1.07)				GA	272/282	0.81 (0.71, 0.92)	
		CC	11/13	0.78 (0.37, 1.67)				AA	112/86	0.83 (0.64, 1.06)	
		GG	415/376	0.83 (0.75, 0.93)	0.3267			GG	200/175	0.91 (0.77, 1.06)	0
		GC+CC	170/165	0.90 (0.77, 1.06)				GA+AA	384/368	0.82 (0.73, 0.91)	
	rs2287780	TT	416/377	0.83 (0.74, 0.92)	0.2499	ADA	rs244072	GG	405/370	0.85 (0.76, 0.95)	0

	TC	161/154	0.92 (0.78, 1.08)		GA	163/157	0.89 (0.74, 1.06)	
	CC	11/14	0.81 (0.38, 1.73)		AA	18/15	0.56 (0.34, 0.95)	
	TT	416/377	0.83 (0.74, 0.92)	0.2212	GG	405/370	0.85 (0.76, 0.95)	0
	TC+CC	172/168	0.92 (0.78, 1.07)		GA+AA	181/172	0.85 (0.71, 1.00)	

[a] The number of cases and controls may differ from those of study-subjects due to missing genotype values

[b] Adjusted for age at diagnosis for cases or enrollment for controls, area, education, tobacco smoking, moderate physical activity, oral contraceptives use, hormone replacement therapy, family history of breast cancer, history of benign breast disease, age at menarche, number of full term births, age at first full term delivery, breastfeeding and body mass index

[c] Adjusted for the menopausal age

[d] *P*-interaction from Multiplication model result, SNP genotype were as 2 categories or 3categories to calculate the interaction with adherence to MDP on BC risk, respectively.

Figures

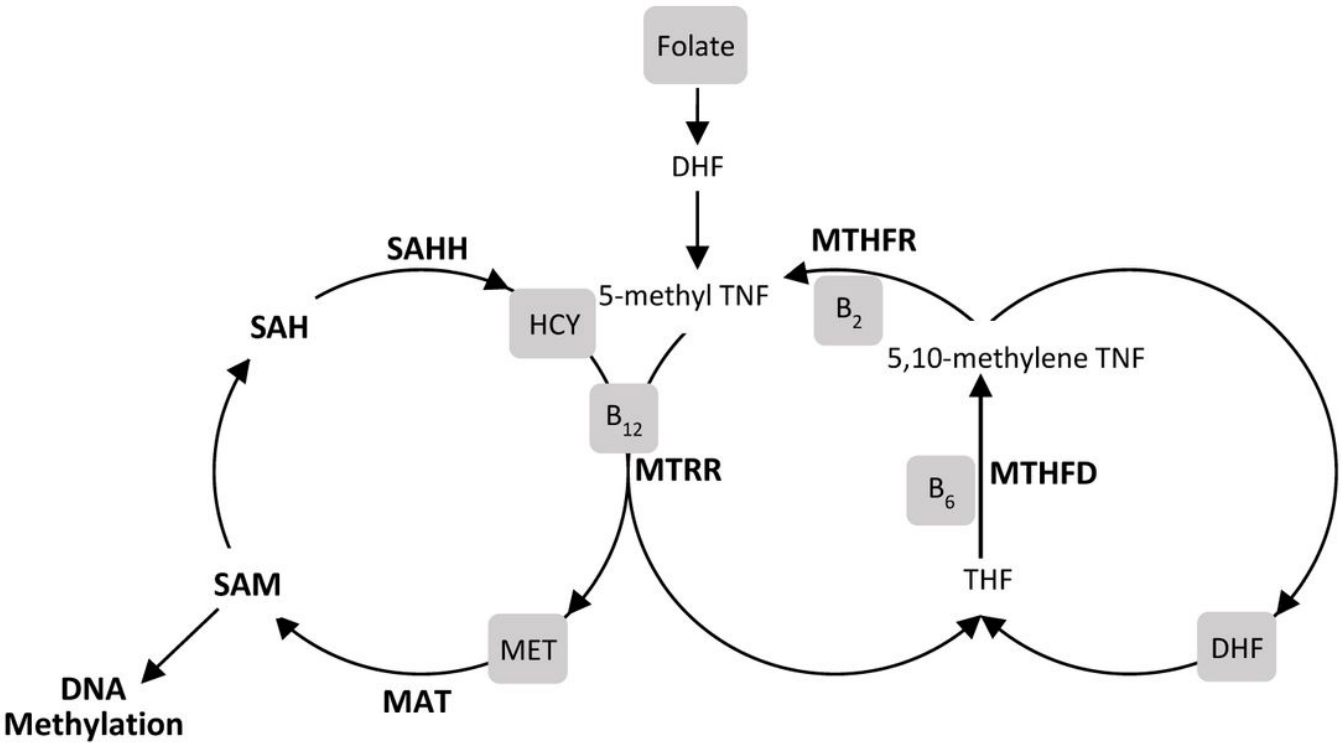

Figure 1

One-carbon metabolism, shading indicates the substrate is obtained via the diet. DHF, dihydrofolate; DMG, dimethyl glycine; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; THF, tetrahydrofolate; DHF, dihydrofolate; MET, methionine; HCY, Homocysteine.

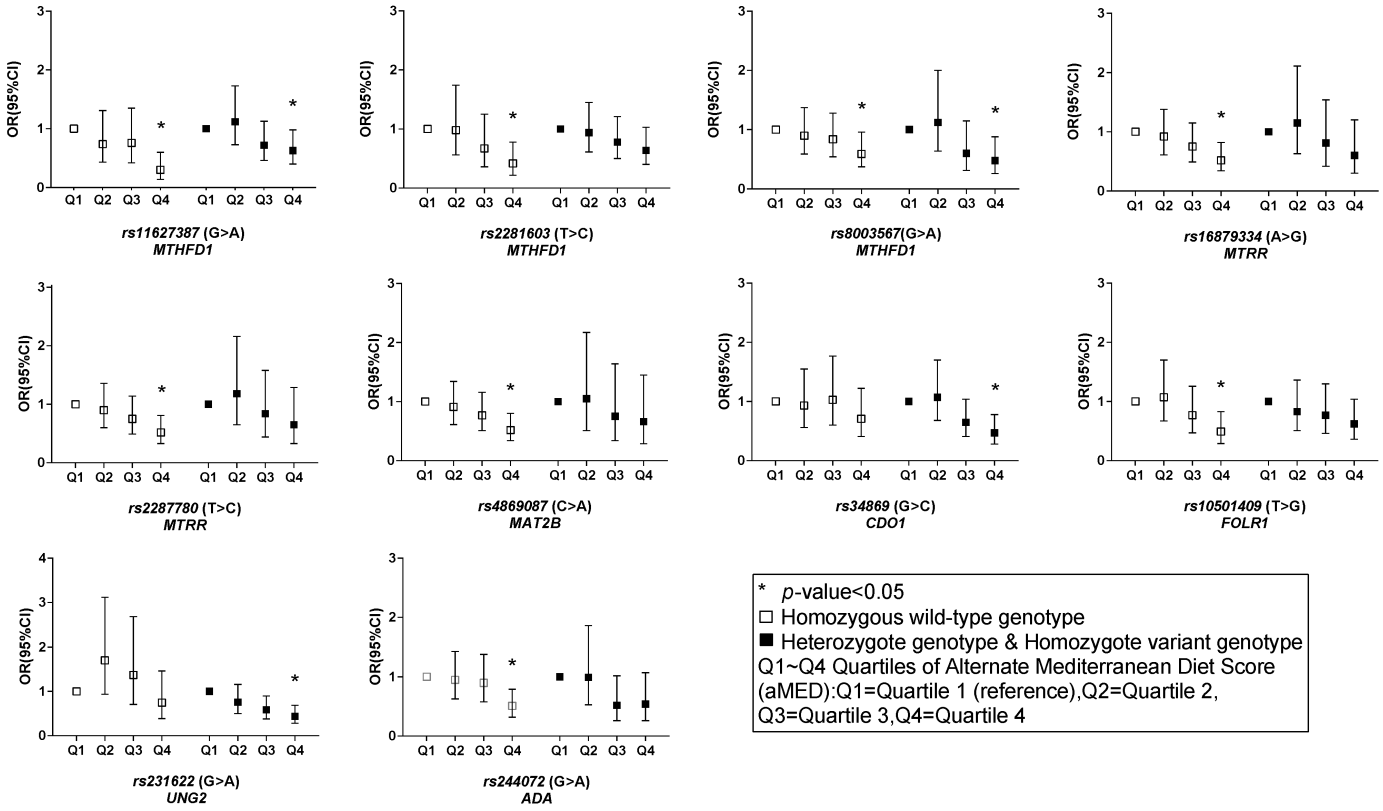

Figure 2

Associations between high adherence to Mediterranean dietary pattern (MDP) and breast cancer risk, stratified by SNPs genotypes.

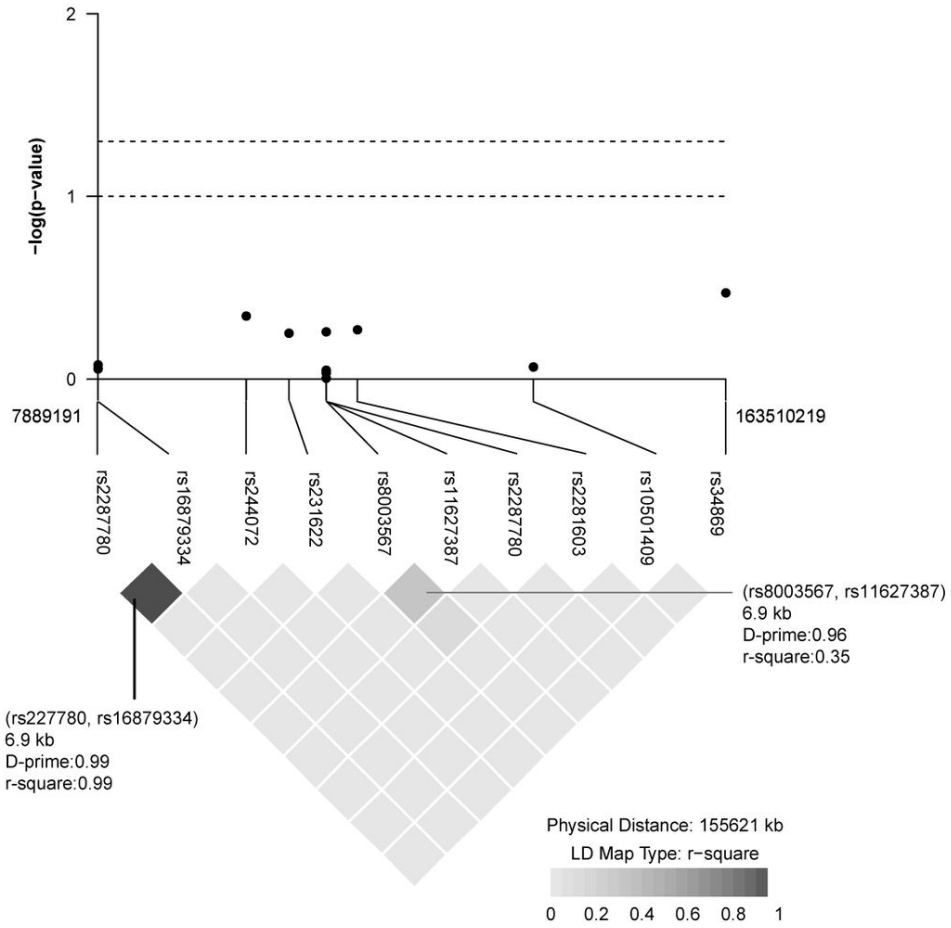

Figure 3

Logarithm-transformed P values for the association between breast cancer risk and single-nucleotide polymorphisms (SNPs) of one carbon-metabolism genes and pattern of linkage disequilibrium for tagging SNPs genotyped in carbon-metabolism genes.

Supplementary Files

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