Null Results in Brief

MTR and *MTRR* Polymorphisms, Dietary Intake, and Breast Cancer Risk

Martha J. Shrubsole,[1] Yu-Tang Gao,[2] Qiuyin Cai,[1] Xiao Ou Shu,[1] Qi Dai,[1] Fan Jin,[2] and Wei Zheng[1]

[1]Division of General Internal Medicine and Public Health, Department of Medicine and Vanderbilt-Ingram Cancer Center, Vanderbilt University, Nashville, Tennessee and [2]Department of Epidemiology, Shanghai Cancer Institute, Shanghai, China

Introduction

Methionine, the precursor for the universal methyl donor, *S*-adenosylmethionine, is produced through the irreversible transfer of a methyl group from 5-methyltetrahydrofolate. This reaction is regulated by two enzymes, methionine synthase (*MTR*) and methionine synthase reductase (*MTRR*). *MTR* is polymorphic at nucleotide 2,756 (A-to-G) and has been associated with decreased plasma homocysteine levels (1-3). *MTRR* is polymorphic at nucleotide 66 (A-to-G) and the variant has a lower affinity for *MTR* (4) and is inconsistently associated with homocysteine level (5-7), although it is a risk factor for neural tube defects (8) and Down syndrome (9) in conditions of higher homocysteine. There is no report on either *MTR* or *MTRR* in relation to breast cancer risk. In an extension of our previous reports that folate intake was inversely associated with breast cancer risk (10) and that this association was particularly strong among women with the *methylenetetrahydrofolate reductase* (*MTHFR*) *677TT* genotype (11), we investigated whether these associations may be modified by *MTR* and *MTRR* genotypes.

Materials and Methods

The Shanghai Breast Cancer Study is a large population-based case-control study conducted in urban Shanghai, China. Detailed study methods have been published previously (12). In brief, cases ages between 25 and 64 years were identified through a rapid case-ascertainment system supplemented by the population-based Shanghai Cancer Registry. Controls were identified from the Shanghai Resident Registry and frequency matched to the expected age distribution of cases by 5-year age groups. All subjects completed an in-person interview. Dietary intakes were assessed using a 76-item food frequency questionnaire that captured >85% of food intake in Shanghai (13). Blood samples were collected from 1,193 (82%) cases and 1,310 (84%) controls and used in this study for genotyping assays. *MTRR* Ile^{22}Met (A66G, rs1801394) genotyping was done using the Taqman 5′-Nuclease Assay (C_3068176_10; Applied Biosystems, Foster City, CA). *MTR* Asp^{919}Gly (A2756G, rs1805087) genotyping was done by BioServe Biotechnologies Ltd. (Laurel, MD) using Masscode assay (14). The consistency rate of quality control samples was 100% for *MTR A2756G* and 96% for *MTRR A66G*.

All dietary intake analyses only included cases (92.1%) and controls (91.3%) who did not use alcohol regularly or take vitamin supplements. Unconditional logistic regression models were used to calculate odds ratios (OR) and their 95% confidence intervals (95% CI) after adjusting for potential confounding variables. Diet was categorized into tertiles based on the control distribution. Energy was adjusted using the standard multivariate method (15). Stratified analyses were used to evaluate the potential modifying effect of *MTHFR* genotypes and folate and folate cofactor intakes.

Results

The frequencies of the *MTR A2756G* and *MTRR A66G* alleles were 0.10 and 0.24, respectively, among the controls who were not statistically different from the cases (data not shown). The genotype distributions among both cases and controls did not differ from the predicted distribution under Hardy-Weinberg equilibrium. Risk of breast cancer did not differ statistically by the *MTR* or *MTRR* genotypes either overall or by menopausal status nor did the genotypes modify the null association with *MTHFR C677T* genotype (Table 1). Likewise, there were no clear differences in risk for breast cancer and the joint *MTHFR-MTR-MTRR* genotypes.

The joint associations of *MTR* and *MTRR* genotypes and dietary folate and folate cofactor intake with breast cancer risk are presented in Table 2. Low intake of folate was associated with an increased risk among all genotypes, and the strength of the association did not differ by genotype. Risk associated with the genotypes was not statistically significantly different than one within all strata of methionine, vitamin B_{12}, and vitamin B_6 intakes.

Discussion

We found that there was no statistically significant association between the risk for breast cancer and *MTR A2756G* or *MTRR A66G* genotypes. We further found that this association was not modified by *MTHFR C677T* genotypes or intakes of folate, methionine, vitamin B_{12}, or vitamin B_6. This is the first report of *MTR* and *MTRR* genotypes in relation to breast cancer risk. *MTR* has been associated with a reduced risk for both colorectal cancer (16, 17) and acute lymphoblastic leukemia (18) and increased risk for malignant lymphoma (19) and not associated with cancer risk for non-Hodgkin's lymphoma (18, 20) and uterine cancer (21). In the only two studies that have evaluated the *MTRR A66G* genotype in relation to cancer

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Requests for reprints: M.J. Shrubsole, Center for Health Services Research, Vanderbilt University Medical Center, Nashville, TN 37232-2587. Phone: 615-936-0812; Fax: 615-322-1754. E-mail: martha.shrubsole@vanderbilt.edu

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Table 1. *MTR*, *MTRR*, and *MTHFR* genotype frequencies and adjusted OR (95% CI) for breast cancer among Chinese women, Shanghai Breast Cancer Study, 1996 to 1998

Genotype	Total sample		*MTHFR* genotype					
			677CC		*677CT*		*677TT*	
	Cases/ Controls	Adjusted OR (95% CI)*	Cases/ controls	Adjusted OR (95% CI)*	Cases/ controls	Adjusted OR (95% CI)*	Cases/ Controls	Adjusted OR (95% CI)*
MTR A2756G								
AA	877/932	1.0 (Reference)	290/306	1.0 (Reference)	428/427	1.1 (0.9-1.3)	140/146	1.0 (0.8-1.4)
AG	181/195	1.0 (0.8-1.2)						
GG	8/11	0.8 (0.3-2.0)						
AG/GG			65/57	1.2 (0.8-1.8)	88/101	0.9 (0.7-1.3)	32/33	1.1 (0.7-1.8)
MTRR A66G								
AA	621/687	1.0 (Reference)	199/210	1.0 (Reference)	316/326	1.0 (0.8-1.3)	96/119	0.8 (0.6-1.2)
AG	393/422	1.0 (0.9-1.2)	133/139	1.0 (0.7-1.3)	182/188	1.0 (0.8-1.4)	64/58	1.2 (0.8-1.8)
GG	70/76	1.0 (0.7-1.4)	24/23	1.1 (0.6-2.0)	29/36	0.8 (0.5-1.4)	14/11	1.5 (0.6-3.4)
MTR/MTRR								
AA/AA	482/526	1.0 (Reference)	164/169	1.0 (Reference)	236/249	1.0 (0.7-1.3)	75/92	0.8 (0.6-1.2)
AA/AG or *GG*	107/108	1.0 (0.7-1.3)	31/31	1.1 (0.6-1.8)	59/58	1.0 (0.7-1.6)	16/20	0.8 (0.4-1.6)
AG or *GG/AA*	359/373	1.0 (0.9-1.3)	117/127	0.9 (0.7-1.3)	174/165	1.0 (0.8-1.5)	58/50	1.2 (0.8-1.9)
AG or *GG/AG* or *GG*	75/85	1.0 (0.7-1.4)	30/24	1.2 (0.7-2.2)	27/43	0.6 (0.4-1.1)	15/13	1.4 (0.6-3.0)

*All ORs are adjusted for age, history of fibroadenoma, waist-to-hip ratio, age at first live birth, physical activity, and total meat.

risk, there was no observed association with acute lymphoblastic leukemia, non-Hodgkin's lymphoma, or gastric cardia cancer (20, 22), although there was an increased esophageal cancer risk among those who did not consume alcohol (22).

MTR and MTRR are critical enzymes responsible for the biosynthesis of methionine, the precursor for methylation reactions, and the regeneration of tetrahydrofolate for nucleotide biosynthesis. Under conditions of adequate methionine, ~40% of homocysteine is remethylated to methionine through the activity of these enzymes (23). Alterations, therefore, in the function of these enzymes could have important effects on DNA methylation, synthesis, and repair. The *MTRR A66G* variant has a 3- to 4-fold lower affinity for MTR (4) and has been associated with altered blood or plasma levels of homocysteine, folate, or vitamin B_{12} in some but not all studies (5-7, 24, 25). Likewise, reports of *MTR A2756G* and

Table 2. Joint association of *MTR* and *MTRR* genotypes and folate and folate cofactor intake with breast cancer risk among Chinese women, Shanghai Breast Cancer Study, 1996 to 1998

Genotype	Cases/ controls	Adjusted OR (95% CI)*	Cases/ controls	Adjusted OR (95% CI)*	Cases/ controls	Adjusted OR (95% CI)*
	T1 (high)		T2		T3	
Daily folate intake						
MTR A2756G						
AA	236/292	1.0 (Reference)	299/298	1.5 (1.1-2.0)	280/276	1.7 (1.2-2.4)
AG/GG	46/56	1.0 (0.7-1.6)	61/51	1.8 (1.2-2.8)	58/65	1.6 (1.0-2.5)
MTRR A66G						
AA	166/209	1.0 (Reference)	205/210	1.4 (1.0-1.9)	196/199	1.6 (1.1-2.3)
AG	106/134	0.9 (0.7-1.3)	137/131	1.6 (1.1-2.2)	127/130	1.6 (1.1-2.4)
GG	17/25	0.8 (0.4-1.5)	26/16	2.4 (1.2-4.7)	22/27	1.4 (0.8-2.7)
Daily methionine intake						
MTR A2756G						
AA	312/292	1.0 (Reference)	233/295	0.9 (0.7-1.2)	270/279	1.2 (0.8-1.7)
AG/GG	52/49	1.0 (0.7-1.6)	56/55	1.1 (0.7-1.7)	57/68	1.0 (0.6-1.7)
MTRR A66G						
AA	220/209	1.0 (Reference)	166/216	0.8 (0.6-1.2)	181/193	1.2 (0.8-1.7)
AG	133/134	0.9 (0.7-1.3)	112/120	1.0 (0.7-1.5)	125/141	1.1 (0.7-1.7)
GG	19/24	0.7 (0.4-1.3)	25/22	1.4 (0.7-2.6)	21/22	1.2 (0.6-2.3)
Daily vitamin B_{12} intake						
MTR A2756G						
AA	285/308	1.0 (Reference)	273/276	1.1 (0.8-1.4)	257/282	1.2 (0.8-1.5)
AG/GG	55/46	1.3 (0.8-2.0)	53/64	0.9 (0.6-1.4)	57/62	1.2 (0.8-1.8)
MTRR A66G						
AA	200/205	1.0 (Reference)	182/208	0.9 (0.7-1.2)	185/205	1.1 (0.8-1.5)
AG	122/134	1.0 (0.7-1.3)	134/130	1.1 (0.8-1.5)	114/131	1.1 (0.7-1.5)
GG	23/28	0.9 (0.5-1.6)	19/23	0.8 (0.4-1.6)	23/17	1.6 (0.8-3.1)
Daily vitamin B_6 intake						
MTR A2756G						
AA	303/299	1.0 (Reference)	267/286	0.9 (0.7-1.2)	246/281	0.9 (0.6-1.3)
AG/GG	56/46	1.1 (0.8-1.8)	55/62	0.9 (0.6-1.3)	54/64	0.9 (0.6-1.4)
MTRR A66G						
AA	220/205	1.0 (Reference)	186/214	0.8 (0.6-1.1)	161/199	0.7 (0.5-1.1)
AG	128/142	0.8 (0.6-1.1)	122/120	0.9 (0.7-1.3)	120/133	0.9 (0.6-1.3)
GG	23/22	0.9 (0.5-1.6)	23/23	0.9 (0.5-1.8)	19/23	0.8 (0.4-1.6)

*All ORs are adjusted for age, history of fibroadenoma, waist-to-hip ratio, age at first live birth, physical activity, total meat intake, total energy intake, and intake of folate and/or its cofactors.

homocysteine are conflicting (7, 26). We did not find any statistically significant associations between these genotypes and breast cancer risk even among conditions of replete and low intake. Nor did we observe any associations when the low-activity *MTHFR*, the rate-limiting enzyme for the methionine cycle, was either present or absent. If these particular variants of these critical enzymes do indeed have important functional consequences, our data suggested that these consequences do not seem likely to alter one-carbon metabolism sufficiently to affect risk for breast cancer.

This case-control study is one of the largest and most comprehensive evaluations of genetic variants in enzymes involved in the remethylation of homocysteine. Potential biases are limited in this study because both cases and controls had very high participation rates (>90%) and high blood donation rates (>80%). Fruit and vegetable intake, the major contributors to folate, methionine, and vitamin B_6 intakes, did not significantly differ between cases and controls, and recall of diet would unlikely be related to genotype. We also observed little confounding when we carefully adjusted for known risk factors and any possible residual confounders would need to be very strong to alter the null associations observed in this study. We cannot exclude the possibility that these genotypes may be related to risk of breast cancer in an older population; however, the null association was not modified by menopausal status. Particular strengths of our study include the population-based design, the estimation of folate and cofactor intake in a population of nonusers of alcohol and vitamin supplements, and the large sample size that facilitated examination of modifying effects.

In summary, we found that *MTR* and *MTRR* genotypes are not likely to play an important independent role in breast cancer etiology. This is the first evaluation of these genotypes with breast cancer risk and future studies are warranted in populations with different nutrient intake.

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