doi:10.4149/neo_2015_059

Joint effects between urinary selenium and polymorphisms in methylation related genes on breast cancer risk

W. LIN[1], Y. L. CEN[1], Y. LIN[2], F. X. SU[3], B. H. WU[4], L. Y. TANG[5,*], Z. F. REN[1,*]

[1]The School of Public Health, Sun Yat-sen University, Guangzhou, 510080, China; [2]The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, 510080, China; [3]The Second Affiliated Hospital, Sun Yat-sen University, Guangzhou, 510120, China; [4]The Guangdong Prevention and Treatment Center for Occupational Diseases, Guangzhou 510300, China; [5]The Third Affiliated Hospital, Sun Yat-sen University, Guangzhou, 510630, China

**Correspondence: renzef@mail.sysu.edu.cn, tangly@mail.sysu.edu.cn*

Received September 29, 2014 / Accepted December 3, 2014

The aim of this study was to explore the associations of urinary selenium and polymorphisms in methylation related genes with breast cancer risk and the interactions on the risk.

The present study involved in 240 female patients with incident breast cancer and 246 age-matched controls in two affiliated hospitals of Sun Yat-sen University in Guangzhou, China, from October 2009 to July 2010. *DNMT1* rs2228611, *MTHFR* rs1801133, and *MTR* rs1805087 were genotyped using a matrix-assisted laser desorption/ionization time-of-flight mass spectrometry platform. Urinary concentration of selenium was measured by inductively coupled plasma mass spectrometry.

Women with urinary selenium in the second tertile had a significant reduced breast cancer risk compared to those with urinary selenium in the lowest tertile [OR (95%CI): 0.50 (0.30, 0.81)]. *DNMT1* rs2228611, *MTHFR* rs1801133, and *MTR* rs1805087 were not associated with breast cancer risk. Women with the third tertile of urinary selenium had a significant reduced breast cancer risk compared to those with the lowest tertile among women only with CC genotype [OR (95%CI): 0.55 (0.30, 1.00)] but not CT/TT genotypes [OR (95%CI): 1.58 (0.73, 3.42)] of *MTHFR* rs1801133 (*P* for interaction=0.044).

Our results suggested that selenium was associated with a decreased risk of breast cancer and this beneficial effect was limited to women with CC genotype of *MTHFR* rs1801133.

Key words: urinary selenium, single nucleotide polymorphism, DNA methyltransferase 1, methylene tetrahydrofolate reductase, methionine synthase, breast cancer

Selenium is an essential micronutrient required for human health and plays a unique role in several metabolic pathways of human biology, such as thyroid hormone metabolism, antioxidant defense systems, and immune function [1]. It also down-regulates the expression of estrogen receptor alpha (ERα) that enhance proliferation of breast cancer cells [2, 3]. It has been found that selenium suppresses breast tumor growth in a mice model [4]. Some epidemiological studies have reported that high levels of selenium in whole blood, serum, plasma, and diet are associated with a decreased breast cancer risk [5-12].

One of the suggested mechanisms of selenium as an anticarcinogen is the function in the regulation of DNA methylation [13-15]. Selenium supplementation has been reported to reduce the global DNA methylation [16, 17] and associate with a decrease of DNA methyltransferase 1 (DNMT1) expression [18-20]. DNMT1 catalyzes the process of DNA methylation, which uses S-adenosylmethionine as a methyl donor. S-adenosylmethionine is synthesized from methionine by methionine adenosyltransferase [21]. Methionine can be regenerated from homocysteine by methionine synthase (MTR) [22]. During the process of methionine synthesis, homocysteine obtains a methyl group from 5-methyltetrahydrofolate which is catalyzed by MTR [22]. 5-methyltetrahydrofolate is the product converted from 5, 10-methylenetetrahydrofolate by methylenetetrahydrofolate reductase (MTHFR), a crucial enzyme for one-carbon metabolism [23]. Futhermore, the level of homocysteine was found to be associated with selenium level [24, 25]. Therefore, the association of selenium with breast cancer risk may

be modified by methylation related genes, such as *DNMT*1, *MTHFR*, and *MTR*.

In the present study, we attempted to explore the interactions of urinary selenium and the polymorphisms in methylation related genes (*DNMT1* rs2228611, *MTHFR* rs1801133 and *MTR* rs1805087) on the risk of breast cancer in a Chinese breast cancer case-control study.

Table 1. Characteristics of breast cancer cases and controls

Variable	Case n(%)	Control n(%)	*P*-value [b]
Age			
≤40	50 (20.8)	50 (20.3)	
~60	146 (60.8)	141 (57.3)	
60~	44 (18.3)	55 (22.4)	0.539
Mean±SD	50.1±11.7	51.6±12.0	0.150 [c]
Education			
Junior middle school or below	102 (42.5)	66 (26.8)	
Senior middle school	74 (30.8)	118 (48.0)	
College or above	58 (24.2)	62 (25.2)	**<0.01**
Unknown	6 (2.5)	0 (0.0)	
Marital status			
Never married	14 (5.8)	11 (4.5)	
Married/living as married	208 (86.7)	214 (87.0)	
Separated/widow	11 (4.6)	21 (8.5)	0.200
Unknown	7 (2.9)	0 (0.0)	
Body mass index (kg/m^2)			
<22	102 (42.5)	90 (36.9)	
22~	72 (30.0)	93 (38.1)	
25~	64 (26.7)	61 (25.0)	0.175
Unknown	2 (0.0)	0 (0.0)	
Age at menarche (years)			
≤12	41 (17.1)	53 (21.5)	
12.1~13.9	89 (37.1)	110 (44.7)	
≥14	100 (41.7)	80 (32.5)	0.060
Unknown	10 (4.2)	3 (1.2)	
Menopausal status			
Premenopausal	135 (56.3)	105 (42.7)	
Postmenopausal	103 (42.9)	141 (57.3)	**<0.01**
Unknown	2 (0.8)	0 (0.0)	
Age at menopause (years) [a]			
≤45.0	23 (22.3)	24 (17.0)	
~50.0	36 (35.0)	61 (43.3)	
>50	35 (34.0)	47 (33.3)	0.390
Unknown	9 (8.7)	9 (6.4)	
Parity			
0	30 (12.5)	19 (7.7)	
1	99 (41.3)	152 (61.8)	
≥2	111 (46.2)	75 (30.5)	**<0.01**
Family history of breast cancer			
Absent	233 (97.1)	239 (97.2)	
Present	5 (2.1)	7 (2.8)	0.598
Unknown	2 (0.8)	0 (0.0)	

[a] Postmenopausal women only.
[b] *P* for Chi-square test between case and control groups.
[c] *P* for student's t-test between case and control groups.

Patients and methods

Study population. Female patients, histologically diagnosed with breast cancer from October 2009 to July 2010 in the First and the Second Affiliated Hospital of Sun Yat-sen University, Guangzhou, China, were consecutively recruited in this study. Women with metastasized breast cancer or previous history of any cancers were excluded. A total of 270 eligible breast cancer patients completed in-person interviews with response rates of 75% to 85% depending on the hospitals during the study period. Cancer-free and age (within 5 years) frequency-matched female controls were recruited from the populations who attended health screening assessments at the same hospitals as the breast cancer cases in the same period. Of the 330 eligible controls, 81.8% completed in-person interviews. All the subjects must have resided in Guangzhou area for at least 5 years.

Data collection. The cases and the controls were interviewed face to face by trained interviewers using the same questionnaire. The following information was obtained: menstrual and reproductive history, life style, family history of cancer, height, weight, and demographic factors. Blood and midstream urine samples were collected from 240 cases (88.9% of those eligible) immediately after admission to the hospitals and from 246 controls (91.1% of those eligible) after the interview. The clinical characteristics of the breast cancer patients were collected from medical records and pathological reports. The statuses of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) from the breast cancer tissues were determined by pathologists using immunohistochemistry tests. Immunostaining was performed using anti-ER antibody SP1, anti-PR antibody 1E2 (Roche, Mannheim, Germany) and rabbit anti-human cerbB-2 oncoprotein (Dako Corp, Carpinteria, USA) as primary antibody. The definition of statuses of ER, PR, and HER2 were previously described in detail [26]. Informed consent was obtained for the interview and collection of the blood and urine specimens.

Laboratory protocol. All the blood and urine samples were placed in high-density polyethylene containers and stored at −80 °C until they were analyzed. Genomic DNA was extracted from the buffy coats of the participants using the TIANamp Genomic DNA Kit (TianGen Biotech Co., Ltd., Beijing, China) and genotyped using a matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry platform (Sequenom, San Diego, California, USA), according to the manufacturer's instructions. The details of the primers are described in Supplementary Table 1. Duplicate samples (5% of the total) were included for the evaluation of genotyping quality and the concordance rate was 100%. Genotyping was successfully performed among 233 (97.1%) cases and 236 (95.9%) controls for *DNMT1* rs2228611, 233 (97.1%)

cases and 237 (96.3%) controls for *MTHFR* rs1801133, 232 (96.7%) cases and 236 (95.9%) controls for *MTR* rs1805087. The observed genotype frequencies for *DNMT1* rs2228611 and *MTR* rs1805087 were in agreement with Hardy-Weinberg equilibrium in the controls (P=0.566 and 0.451, respectively). Although the P-value of *MTHFR* rs1801133 from Hardy-Weinberg equilibrium was 0.044, the minor allele frequency in the controls (27%) was close to those in other Chinese populations (26.2% or 28%) [27, 28].

Urine samples were processed at the Laboratory of Guangdong Testing Center of Occupational Hygiene, which is one of the members executing a national project to establish national standards of trace elements in biological specimens in China. Selenium in urine was quantified using inductively coupled plasma mass spectrometry (ICP-MS) (Agilent7500ce ICP-MS, Agilent Technologies, McMillan, TX) with the isotope of 82Se. Batches of assays contained samples from both cases and controls in a random fashion and the operator was unaware of the participant group. Immediately before analysis, urine samples were diluted 1:9 with a dilute nitric acid solution (0.5%) in trace metal clean polypropylene autosampler tubes. Metal quantification was done using external standards (Spex Industries, Metuchen NJ) with internal normalization. An internal standard was online added to every sample. Values were acquired in peak jumping mode with a minimum of three replicate analyses done on each sample after a 30-s uptake and a 25-s stabilization period. A 15-s rinse [0.5% (v/v) nitric acid+0.01% (v/v) Triton] between samples virtually eliminated carryover and improved quantification limits. Analytic batches consisted of 50 urine samples along with 5 quality-control samples. Quality control samples included both bench and blind samples. Sample concentrations were blank-baseline corrected using the mean of three batch specific matrix blanks. The limits of detection were 0.5ug/L for selenium, and one sample (0.2%) was below the limit for the metal. Urine standard reference from Bio-Rad (Bio-Rad, Hercules, CA) was used for external calibration. The coefficients of variation (CV) between duplicates for urinary selenium were below 10%, averaging 6.4%. In addition, a second aliquot of each urine sample was shipped to the clinical examination center in Guangdong Prevention and Treatment Center for Occupational Diseases, for the measurement of creatinine concentration by an enzymatic method. Urinary concentrations (ug/L) of selenium were divided by individual creatinine concentration (g/L) to correct for variability in urine dilution and kidney function according to a previously detailed methodology [29].

Statistical analysis. The differences in demographic characteristics and common risk factors for breast cancer between the cases and controls were tested using the Chi-square test (for categorical variables) or Student's t-test (for continuous variables). The urinary selenium showed a non-normal distribution and Mann-Whitney U test was used as a continuous variable. It was then divided into tertiles based on the level among controls and Chi-square test was used. Hardy-Weinberg equilibrium for the three SNPs was evaluated by a goodness-of-fit Chi-square test to compare the observed genotype frequency with the expected one among the controls. Multivariate logistic regression models were used to assess the associations of urinary levels of selenium and the SNPs with breast cancer risk, controlling for age and for suspected or established risk factors (age, body mass index, age at menarche, marital status, education, parity, menopausal status, and family history of breast cancer), which were defined categorically except age. Models were fit using levels of selenium as categorical (tertiles) and continuous variables, and frequencies of genotypes as categorical variables. For evaluating the dose-response association between selenium levels or genotypes of SNPs and breast cancer risk, tests for trend were performed by entering the categorical variable as continuous variable in the model. The odds ratios (ORs), 95% confidence intervals (CIs), and P-values were calculated. Stratified analyses for the associations between urinary selenium and the risk of breast cancer were performed by genotypes of the three SNPs. The mutant homozygotes and heterozygotes were combined assuming a dominant model. Multiplicative models were applied to evaluate the interactions between urinary selenium and genotypes on breast cancer risk. We tested for multiplicative interactions by including the product terms in multivariate logistic regressions. All statistical tests were 2-tailed with P<0.05 considered to be significant. Statistical analyses were performed using SPSS 13.0 (Chicago, IL).

Results

A total of 240 breast cancer cases and 246 control subjects were included in the analyses. Demographic characteristics and breast cancer related factors for the study population were reported elsewhere [30]. Breast cancer patients, as compared with similarly aged controls, were more likely to be premenopausal, nulliparous, and low educated. They were comparable in marital status, body mass index, age at menarche, age at menopause, and family history of breast cancer (Table 1).

Creatinine-adjusted levels (median, ug/g) of selenium were not normally distributed and stated as median (25th, 75th) μg/g creatinine, which were 34.08 (26.80, 46.38) in cases and 37.13 (29.25, 46.98) in controls. In multivariate logistic models, urinary selenium was not significantly associated with breast cancer risk as a continuous variable [OR (95% CI): 0.97 (0.89, 1.04), per 10 ug/g increase]; whereas women with the second tertile of selenium concentration, but not the highest tertile, had a significantly decreased risk of breast cancer as compared with those in the lowest tertile [OR (95% CI): 0.50 (0.30, 0.81)] (Table 2). This protective association occurred only among premenopausal women [OR (95%CI): 0.43 (0.21, 0.89)] but not postmenopausal women [OR (95%CI): 0.56 (0.25, 1.22)], although the interaction was not significant (P for interaction = 0.808). The distribution of the genotypes of *DNMT1* rs2228611, *MTHFR* rs1801133, and *MTR* rs1805087

were not significantly different between cases and controls either as a whole (Table 2) or stratified by menopausal status (all *P*-values > 0.05).

Then we evaluated the joint effects of selenium and the genotypes (assuming dominant models) on breast cancer risk. The associations between selenium and breast cancer risk were similar to the combined across the strata of the genotypes of *DNMT1* rs2228611 and *MTR* rs1805087 (Table 3). For the comparison of the second tertile to the lowest tertile of selenium, the risk of breast cancer was decreased among women with both CC [OR (95%CI): 0.44 (0.22, 0.86)] and CT/TT [OR (95%CI): 0.58 (0.26, 1.30)] genotypes of *MTHFR* rs1801133 (*P* for interaction=0.684). For the comparison of the highest tertile to the lowest tertile of selenium, the risk of breast cancer was significantly decreased among the women with the CC genotype of *MTHFR* rs1801133 [OR (95%CI): 0.55 (0.30, 1.00)] but increased among the women with the CT/TT genotypes [OR (95%CI): 1.58 (0.73, 3.42)], and the interaction was significant (*P* for interaction=0.044) (Table 3).

Discussion

In the present study, we found that the middle level of urinary selenium was significantly associated with a decreased breast cancer risk, particularly among premenopausal women. A significant interaction between urinary selenium and the genotypes of MTHFR rs1801133 on the risk of breast cancer was observed.

The main source of selenium intake is via the diet, which is typically provided by cereals, meat, fish, dairy products, and so on [31]. For the differences of dietary habits and environmental exposure in regions, selenium intake varies greatly in different areas. In the present study, the urinary selenium concentration adjusted for urinary creatinine in the controls (median, 37.13 ug/g) is much higher than those in New Zealand (mean, approximate 21.65 ug/g) [32] and Norway (median, 26.1 ug/g) [33]. It was reported that the beneficial range of selenium level may be narrow and the health effects exhibit a U-shape relation with cancer risk [34], supporting our results that only the second tertile but not the highest

Table 2. Multivariate odds ratio of breast cancer risk associated with urinary selenium and three methylation related SNPs

Variables	Case n (%)	Control n (%)	P-value [b]	OR (95%CI) [d]	OR (95%CI) [e]
Urinary selenium [a]					
Median (ug/g)	34.08	37.13	0.081 [c]		
T1	107 (44.6)	82 (33.3)		1.00 (reference)	1.00 (reference)
T2	53 (22.1)	82 (33.3)		**0.51 (0.32,0.80)**	**0.50 (0.30,0.81)**
T3	80 (33.3)	82 (33.3)	**0.009**	0.77 (0.50,1.17)	0.79 (0.50,1.25)
Continuous (per 10 ug/g increase)				0.96 (0.89,1.03)	0.97 (0.89,1.04)
P for trend				0.189	0.264
DNMT1(rs2228611)					
GG	107 (44.6)	120 (48.8)		1.00 (reference)	1.00 (reference)
GA	109 (45.4)	94 (38.2)		1.31 (0.89,1.91)	1.19 (0.78,1.80)
AA	17 (7.1)	22 (8.9)	0.29	0.86 (0.43,1.71)	0.84 (0.39,1.80)
Unknown	7 (2.9)	10 (4.1)			
P for trend				0.623	0.872
MTHFR(rs1801133)					
CC	143 (59.6)	131 (53.3)		1.00 (reference)	1.00 (reference)
CT	70 (29.2)	82 (33.3)		0.78 (0.52,1.16)	0.87 (0.58,1.35)
TT	20 (8.3)	24 (9.8)	0.406	0.74 (0.39,1.40)	0.66 (0.33,1.32)
Unknown	7 (2.9)	9 (3.7)			
P for trend				0.180	0.188
MTR(rs1805087)					
AA	183 (76.3)	188 (76.4)		1.00 (reference)	1.00 (reference)
AG	44 (18.3)	44 (17.9)		1.04 (0.65,1.66)	1.13 (0.68,1.85)
GG	5 (2.1)	4 (1.6)	0.930	1.41 (0.37,5.41)	1.72 (0.39,7.54)
Unknown	8 (3.3)	10 (4.1)			
P for trend				0.678	0.449

[a] Tertile of selenium concentration(in ug/g):T1,<32.69;T2,32.69~42.61;T3,>42.61.
[b] *P* for Chi-square test between case and control groups.
[c] *P* for Mann-Whitney U test of urinary selenium concentration between case and control groups.
[d] Adjusted for age of breast cancer.
[e] Adjusted for age, body mass index, age at menarche, marital status, menopausal status,education, parity and family history of breast cancer.

tertile of urinary selenium were significantly associated with breast cancer risk compared with the lowest tertile. Unfortunately, we can only assume that the highest tertile may be beyond the beneficial range of urinary selenium in the present study because there has not been a standard normal range of urinary selenium yet.

Although studies on the association between urinary selenium and breast cancer are absent, there are reports that showed the relation between breast cancer and selenium in other specimens [7, 8, 10, 11, 35-40], and the results of which are consistent with ours to some extent. For example, an increased selenium concentration in serum or plasma was found to be related to a significantly reduced risk of breast cancer among Malaysian and Sweden populations [8, 37] and a non-significant lower risk of breast cancer among Indian population [10]. Van den Brandt et al. found that toenail selenium in the third quintile was significantly associated with a decreased breast cancer risk compared to that in the lowest quintile [RR (95%CI): 0.62 (0.40, 0.96)] in a Netherlands cohort study [40]. In addition, the present study found that the inverse association between urinary selenium and breast cancer risk occurred particularly among premenopausal women, which was partially supported by the facts that selenium can depress the expression of ERα mRNA, reduce estrogen receptor-ligand binding, and disrupt estrogen receptor signaling [2, 3], because ERα is able to promote breast tumor growth [41-43] and premenopausal women due to more estrogens to bind with the receptors before a decline in menopause [44] .

The present results did not show a significant association between breast cancer risk and any of the three loci, *DNMT1* rs2228611, *MTHFR* rs1801133, and *MTR* rs1805087, which are consistent with that from some [45-48] but not all of the similar studies [49-53]. The reasons for these inconsistencies may be the different populations and various sample sizes, particularly the small sample size for the present study. However, different environmental factors may also contribute to the inconsistency. For example, in the present study,

Table 3. Joint effect between urinary selenium and three methylation-related SNPs on breast cancer risk

Genotypes	Urinary selenium	Cases n (%)	Controls n (%)	OR (95%CI) [c]	OR (95%CI) [d]
DNMT1(rs2228611)					
GG	T1	47 (43.9)	37 (30.8)	1.00 (reference)	1.00 (reference)
	T2	26 (24.3)	44 (36.7)	**0.47 (0.25,0.90)**	0.50 (0.25,1.01)
	T3	34 (31.8)	39 (32.5)	0.70 (0.37,1.31)	0.65 (0.33,1.29)
GA/AA	T1	59 (46.8)	43 (37.1)	1.00 (reference)	1.00 (reference)
	T2	25 (19.8)	35 (30.2)	0.55 (0.28,1.05)	**0.39 (0.18,0.84)**
	T3	42 (33.3)	38 (32.8)	0.84 (0.47,1.53)	0.94 (0.49,1.81)
P for interaction [a]				0.802	0.707
P for interaction [b]				0.702	0.548
MTHFR(rs1801133)					
CC	T1	69 (48.3)	41 (31.3)	1.00 (reference)	1.00 (reference)
	T2	31 (21.7)	39 (29.8)	**0.49 (0.26,0.90)**	**0.44 (0.22,0.86)**
	T3	43 (30.1)	51 (38.9)	**0.51 (0.29,0.90)**	**0.55 (0.30,1.00)**
CT/TT	T1	37 (41.1)	39 (36.8)	1.00 (reference)	1.00 (reference)
	T2	20 (22.2)	40 (37.7)	0.54 (0.27,1.09)	0.58 (0.26,1.30)
	T3	33 (36.7)	27 (25.5)	1.35 (0.68,2.68)	1.58 (0.73,3.42)
P for interaction [a]				0.837	0.684
P for interaction [b]				**0.035**	**0.044**
MTR(rs1805087)					
AA	T1	83 (45.4)	63 (33.5)	1.00 (reference)	1.00 (reference)
	T2	42 (23.0)	67 (35.6)	**0.49 (0.29,0.81)**	**0.47 (0.27,0.82)**
	T3	58 (31.7)	59 (30.9)	0.77 (0.47,1.27)	0.78 (0.46,1.32)
AG/GG	T1	22 (44.9)	17 (35.4)	1.00 (reference)	1.00 (reference)
	T2	9 (18.4)	12 (25.0)	0.62 (0.21,1.84)	0.49 (0.12,2.04)
	T3	18 (36.7)	19 (39.6)	0.78 (0.31,1.96)	0.98 (0.32,2.99)
P for interaction [a]				0.727	0.585
P for interaction [b]				0.984	0.945

[a] Between genotypes of DNA methylation related genes and urinary selenium (T2 vs T1).

[b] Between genotypes of DNA methylation related genes and urinary selenium (T3 vs T1).

[c] Adjusted for age of breast cancer.

[d] Adjusted for age, body mass index, age at menarche, marital status, education, parity, menopausal status, and family history of breast cancer.

when stratified by tertiles of urinary selenium, women with CT/TT genotypes of *MTHFR* rs1801133 had different ORs (95% CIs) of 0.56 (0.29, 1.11), 0.60 (0.26, 1.40), 1.89 (0.89, 4.02) among women with the first, second, and third tertiles of urinary selenium, respectively, compared with those with the CC genotype.

We found further that the interaction between urinary selenium and *MTHFR* rs1801133 on breast cancer risk was significant; selenium exerted its protective effect only in CC genotypes of *MTHFR* rs1801133 but tends to increase the risk of breast cancer among individuals in the highest tertile with CT/TT genotypes (Table 3). It has been reported that selenium can inhibit DNMTs [19, 20] and develop an anticancer effect by causing promoter demethylation in some specific tumor suppressor genes, such as in von Hippel-Lindau (VHL) [54] and cellular stress response 1 (CSR1) [19]. In addition, inhibition of DNMTs by selenium has been found to be concomitant with genomic DNA hypomethylation [19, 55], which promotes tumor formation [56]. Meanwhile, the mutant CT/TT genotypes of *MTHFR* rs1801133 have a conspicuous reduction of its activity [57, 58] and possess a lower degree of genomic DNA methylation [59]. Furthermore, the mutant allele (T) of *MTHFR* rs1801133 was found to significantly elevate plasma homocysteine level [57, 58], which promotes breast cancer progression [60-62]. Therefore, a high level of selenium may cooperate with the mutant allele (T) of *MTHFR* rs1801133 and synergically increase breast cancer risk. However, the exact mechanisms of the interaction between selenium and *MTHFR* rs1801133 remain to be deciphered.

There are several potential limitations in the presented study. Firstly, this case-control study was hospital-based and the patients and controls might not be representative samples of the total patients and the whole population in Guangzhou area, respectively. However, the patients and controls were comparable to some extent because they were from the same hospitals in the same period and more likely to resemble each other with the selective factors that led to the use of the facilities and the effects of selection bias were minimized [63]. Secondly, we evaluated selenium level only in urine but not in other specimens and it might provide only limited information for selenium level in vivo. However, urine is the major route of selenium excretion and urinary selenium was found to be closely associated with selenium intake [31]. Moreover, because adult females usually keep stable eating habits and have slight changes of selenium intake [64, 65], so the urine concentration of selenium is a useful indicator for stable selenium level [32, 66, 67]. Thirdly, we did not consider external exposures, such as dietary and occupational factors, which are related to urinary levels of selenium. However, urine concentration is an integral internal measurement and can reflect multiple external exposures [68]. Finally, this is a retrospective study and we cannot determine whether the altered distribution of urinary selenium is a cause or a consequence of breast cancer. However, no obvious evidence suggests that breast cancer status would change the absorption, metabolism, and excretion of selenium. Moreover, the patients' urine samples were obtained immediately after their admission to hospitals before treatment began, so the urinary selenium levels may not be influenced by treatment or changed life styles due to disease status. Nevertheless, prospective studies with larger sample sizes are required to confirm our findings.

Conclusion

In summary, the presented work showed that urinary selenium in the second tertile was significantly associated with a reduced breast cancer risk, especially among premenopausal women. The association between urinary selenium and breast cancer risk was limited to women with CC genotype of *MTHFR* rs1801133. Our findings noted a possible link that selenium's beneficial effect may be more apparent in a subgroup of the females defined by genetic susceptibility for breast cancer.

Supplementary information is available in the online version of the paper.

Acknowledgements: This work was supported by the National Natural Science Foundation of China (grant number: 81273147, 81072383) and Guangdong Provincial Natural Science Foundation (grant number: S2012010009958). The authors would like to thank all the study participants and the nursing, secretary, and administrative staffs in the Breast Departments of The First Affiliated Hospital and The Second Affiliated Hospital, Sun Yat-sen University.

References:

[1] MEHDI Y, HORNICK JL, ISTASSE L, DUFRASNE I. Selenium in the environment, metabolism and involvement in body functions. Molecules 2013; 18: 3292–3311. http://dx.doi.org/10.3390/molecules18033292

[2] LEE SO, NADIMINTY N, WU XX, LOU W, DONG Y, et al. Selenium disrupts estrogen signaling by altering estrogen receptor expression and ligand binding in human breast cancer cells. Cancer Res 2005; 65: 3487–3492.

[3] SHAH YM, KAUL A, DONG Y, IP C, ROWAN BG. Attenuation of estrogen receptor alpha (ERalpha) signaling by selenium in breast cancer cells via downregulation of ERalpha gene expression. Breast Cancer Res Treat 2005; 92: 239–250. http://dx.doi.org/10.1007/s10549-005-3203-5

[4] CHEN YC, PRABHU KS, DAS A, MASTRO AM. Dietary selenium supplementation modifies breast tumor growth and metastasis. Int J Cancer 2013; 133: 2054–2064. http://dx.doi.org/10.1002/ijc.28224

[5] CHARALABOPOULOS K, KOTSALOS A, BATISTATOU A, CHARALABOPOULOS A, VEZYRAKI P, et al. Selenium in serum and neoplastic tissue in breast cancer: correlation with CEA. Br J Cancer 2006; 95: 674–676. http://dx.doi.org/10.1038/sj.bjc.6603292

[6] KUO HW, CHEN SF, WU CC, CHEN DR, LEE JH. Serum and tissue trace elements in patients with breast cancer in Taiwan.

Biol Trace Elem Res 2002; 89: 1–11. http://dx.doi.org/10.1385/BTER:89:1:1

[7] LOPEZ-SAEZ JB, SENRA-VARELA A, POUSA-ESTEVEZ L. Selenium in breast cancer. Oncology 2003; 64: 227–231. http://dx.doi.org/10.1159/000069312

[8] REJALI L, JAAFAR MH, ISMAIL NH. Serum selenium level and other risk factors for breast cancer among patients in a Malaysian hospital. Environ Health Prev Med 2007; 12: 105–110. http://dx.doi.org/10.1007/BF02898024

[9] SCHRAUZER GN, MOLENAAR T, MEAD S, KUEHN K, YAMAMOTO H, et al. Selenium in the blood of Japanese and American women with and without breast cancer and fibrocystic disease. Jpn J Cancer Res 1985; 76: 374–377.

[10] SINGH P, KAPIL U, SHUKLA NK, DEO S, DWIVEDI SN. Association between breast cancer and vitamin C, vitamin E and selenium levels: results of a case-control study in India. Asian Pac J Cancer Prev 2005; 6: 177–180.

[11] SUZANA S, CHAM BG, AHMAD RG, MOHD RR, FAIRULNIZAL MN, ET AL. Relationship between selenium and breast cancer: a case-control study in the Klang Valley. Singapore Med J 2009; 50: 265–269.

[12] HARRIS HR, BERGKVIST L, WOLK A. Selenium intake and breast cancer mortality in a cohort of Swedish women. Breast Cancer Res Treat 2012; 134: 1269–1277. http://dx.doi.org/10.1007/s10549-012-2139-9

[13] DAVIS CD, UTHUS EO. Dietary folate and selenium affect dimethylhydrazine-induced aberrant crypt formation, global DNA methylation and one-carbon metabolism in rats. J Nutr 2003; 133: 2907–2914.

[14] DAVIS CD, UTHUS EO, FINLEY JW. Dietary selenium and arsenic affect DNA methylation in vitro in Caco-2 cells and in vivo in rat liver and colon. J Nutr 2000; 130: 2903–2909.

[15] FERGUSON LR, KARUNASINGHE N, ZHU S, WANG AH. Selenium and its‘ role in the maintenance of genomic stability. Mutat Res 2012; 733: 100–110. http://dx.doi.org/10.1016/j.mrfmmm.2011.12.011

[16] METES-KOSIK N, LUPTAK I, DIBELLO PM, HANDY DE, TANG SS, et al. Both selenium deficiency and modest selenium supplementation lead to myocardial fibrosis in mice via effects on redox-methylation balance. Mol Nutr Food Res 2012; 56: 1812–1824. http://dx.doi.org/10.1002/mnfr.201200386

[17] ZENG H, YAN L, CHENG WH, UTHUS EO. Dietary selenomethionine increases exon-specific DNA methylation of the p53 gene in rat liver and colon mucosa. J Nutr 2011; 141: 1464–1468. http://dx.doi.org/10.3945/jn.111.140715

[18] HU Y, MCINTOSH GH, LE LEU RK, NYSKOHUS LS, WOODMAN RJ, et al. Combination of selenium and green tea improves the efficacy of chemoprevention in a rat colorectal cancer model by modulating genetic and epigenetic biomarkers. PLoS One 2013; 8: e64362. http://dx.doi.org/10.1371/journal.pone.0064362

[19] XIANG N, ZHAO R, SONG G, ZHONG W. Selenite reactivates silenced genes by modifying DNA methylation and histones in prostate cancer cells. Carcinogenesis 2008; 29: 2175–2181. http://dx.doi.org/10.1093/carcin/bgn179

[20] DAVIS CD, UTHUS EO. Dietary selenite and azadeoxycytidine treatments affect dimethylhydrazine-induced aberrant crypt formation in rat colon and DNA methylation in HT-29 cells. J Nutr 2002; 132: 292–297.

[21] NORDGREN KK, PENG Y, PELLEYMOUNTER LL, MOON I, ABO R, et al. Methionine adenosyltransferase 2A/2B and methylation: gene sequence variation and functional genomics. Drug Metab Dispos 2011; 39: 2135–2147. http://dx.doi.org/10.1124/dmd.111.040857

[22] MILLER AL. The methionine-homocysteine cycle and its effects on cognitive diseases. Altern Med Rev 2003; 8: 7–19.

[23] SELHUB J. Homocysteine metabolism. Annu Rev Nutr 1999; 19: 217–246. http://dx.doi.org/10.1146/annurev.nutr.19.1.217

[24] ANGELOVA EA, ATANASSOVA PA, CHALAKOVA NT, DIMITROV BD. Associations between serum selenium and total plasma homocysteine during the acute phase of ischaemic stroke. Eur Neurol 2008; 60: 298–303. http://dx.doi.org/10.1159/000157884

[25] GONZALEZ S, HUERTA JM, ALVAREZ-URIA J, FERNANDEZ S, PATTERSON AM, et al. Serum selenium is associated with plasma homocysteine concentrations in elderly humans. J Nutr 2004; 134: 1736–1740.

[26] HE JR, TANG LY, YU DD, SU FX, SONG EW, et al. Epstein-Barr virus and breast cancer: serological study in a high-incidence area of nasopharyngeal carcinoma. Cancer Lett 2011; 309: 128–136. http://dx.doi.org/10.1016/j.canlet.2011.05.012

[27] YU CP, WU MH, CHOU YC, YANG T, YOU SL, et al. Breast cancer risk associated with multigenotypic polymorphisms in folate-metabolizing genes: a nested case-control study in Taiwan. Anticancer Res 2007; 27: 1727–1732.

[28] LIN WY, CHOU YC, WU MH, HUANG HB, JENG YL, et al. The MTHFR C677T polymorphism, estrogen exposure and breast cancer risk: a nested case-control study in Taiwan. Anticancer Res 2004; 24: 3863–3868.

[29] BARR DB, WILDER LC, CAUDILL SP, GONZALEZ AJ, NEEDHAM LL, et al. Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. Environ Health Perspect 2005; 113: 192–200. http://dx.doi.org/10.1289/ehp.7337

[30] SU Y, CHEN LJ, HE JR, YUAN XJ, CEN YL, et al. Urinary rubidium in breast cancers. Clin Chim Acta 2011; 412: 2305–2309. http://dx.doi.org/10.1016/j.cca.2011.08.035

[31] ROMAN M, JITARU P, BARBANTE C. Selenium biochemistry and its role for human health. Metallomics 2014; 6: 25–54. http://dx.doi.org/10.1039/C3MT00185G

[32] THOMSON CD, PACKER MA, BUTLER JA, DUFFIELD AJ, O‘DONAGHUE KL, et al. Urinary selenium and iodine during pregnancy and lactation. J Trace Elem Med Biol 2001; 14: 210–217. http://dx.doi.org/10.1016/S0946-672X(01)80004-3

[33] HOL PJ, VAMNES JS, GJERDET NR, EIDE R, ISRENN R. Dental amalgam affects urinary selenium excretion. Biol Trace Elem Res 2002; 85: 137–147. http://dx.doi.org/10.1385/BTER:85:2:137

[34] RAYMAN MP. Selenium and human health. Lancet 2012; 379: 1256–1268. http://dx.doi.org/10.1016/S0140-6736(11)61452-9

[35] MANNISTO S, ALFTHAN G, VIRTANEN M, KATAJA V, UUSITUPA M, et al. Toenail selenium and breast cancer-a

case-control study in Finland. Eur J Clin Nutr 2000; 54: 98–103. http://dx.doi.org/10.1038/sj.ejcn.1600902

[36] GHADIRIAN P, MAISONNEUVE P, PERRET C, KENNEDY G, BOYLE P, et al. A case-control study of toenail selenium and cancer of the breast, colon, and prostate. Cancer Detect Prev 2000; 24: 305–313.

[37] HARDELL L, DANELL M, ANGQVIST CA, MARKLUND SL, FREDRIKSSON M, et al. Levels of selenium in plasma and glutathione peroxidase in erythrocytes and the risk of breast cancer. A case-control study. Biol Trace Elem Res 1993; 36: 99–108. http://dx.doi.org/10.1007/BF02783168

[38] HUNTER DJ, MORRIS JS, STAMPFER MJ, COLDITZ GA, SPEIZER FE, et al. A prospective study of selenium status and breast cancer risk. JAMA 1990; 264: 1128–1131. http://dx.doi.org/10.1001/jama.1990.03450090064026

[39] VAN T VP, VAN DER WIELEN RP, KOK FJ, HERMUS RJ, STURMANS F. Selenium in diet, blood, and toenails in relation to breast cancer: a case-control study. Am J Epidemiol 1990; 131: 987–994.

[40] VAN DEN BRANDT PA, GOLDBOHM RA, VAN T VP, BODE P, DORANT E, et al. Toenail selenium levels and the risk of breast cancer. Am J Epidemiol 1994; 140: 20–26.

[41] PLATET N, CATHIARD AM, GLEIZES M, GARCIA M. Estrogens and their receptors in breast cancer progression: a dual role in cancer proliferation and invasion. Crit Rev Oncol Hematol 2004; 51: 55–67. http://dx.doi.org/10.1016/j.critrevonc.2004.02.001

[42] SANCHEZ AM, FLAMINI MI, BALDACCI C, GOGLIA L, GENAZZANI AR, et al. Estrogen receptor-alpha promotes breast cancer cell motility and invasion via focal adhesion kinase and N-WASP. Mol Endocrinol 2010; 24: 2114–2125. http://dx.doi.org/10.1210/me.2010-0252

[43] LIAO XH, LU DL, WANG N, LIU LY, WANG Y, et al. Estrogen receptor alpha mediates proliferation of breast cancer MCF-7 cells via a p21/PCNA/E2F1-dependent pathway. FEBS J 2014; 281: 927–942. http://dx.doi.org/10.1111/febs.12658

[44] SOULES MR, SHERMAN S, PARROTT E, REBAR R, SANTORO N, et al. Stages of Reproductive Aging Workshop (STRAW). J Womens Health Gend Based Med 2001; 10: 843–848. http://dx.doi.org/10.1089/152460901753285732

[45] SHRUBSOLE MJ, GAO YT, CAI Q, SHU XO, DAI Q, et al. MTR and MTRR polymorphisms, dietary intake, and breast cancer risk. Cancer Epidemiol Biomarkers Prev 2006; 15: 586–588. http://dx.doi.org/10.1158/1055-9965.EPI-05-0576

[46] SUN MY, YANG XX, XU WW, YAO GY, PAN HZ, et al. Association of DNMT1 and DNMT3B polymorphisms with breast cancer risk in Han Chinese women from South China. Genet Mol Res 2012; 11: 4330–4341. http://dx.doi.org/10.4238/2012.September.26.1

[47] ZHONG S, XU J, LI W, CHEN Z, MA T, et al. Methionine synthase A2756G polymorphism and breast cancer risk: an up-to-date meta-analysis. Gene 2013; 527: 510–515. http://dx.doi.org/10.1016/j.gene.2013.06.054

[48] ZIVA CJ, STEGEL V, GERSAK K, NOVAKOVIC S. Lack of association between methylenetetrahydrofolate reductase genetic polymorphisms and postmenopausal breast cancer risk. Mol Med Rep 2011; 4: 175–179.

[49] HOSSEINI M. Role of polymorphism of methyltetrahydrofolate-homocysteine methyltransferase (MTR) A2756G and breast cancer risk. Pol J Pathol 2013; 64: 191–195. http://dx.doi.org/10.5114/pjp.2013.38138

[50] LI K, LI W, DONG X. Association of 677 C>T (rs1801133) and 1298 A>C (rs1801131) Polymorphisms in the MTHFR Gene and Breast Cancer Susceptibility: A Meta-Analysis Based on 57 Individual Studies. PLoS One 2014; 9: e71290. http://dx.doi.org/10.1371/journal.pone.0071290

[51] XIANG G, ZHENKUN F, SHUANG C, JIE Z, HUA Z, et al. Association of DNMT1 gene polymorphisms in exons with sporadic infiltrating ductal breast carcinoma among Chinese Han women in the Heilongjiang Province. Clin Breast Cancer 2010; 10: 373–377. http://dx.doi.org/10.3816/CBC.2010.n.049

[52] ZHANG J, QIU LX, WANG ZH, WU XH, LIU XJ, et al. MTHFR C677T polymorphism associated with breast cancer susceptibility: a meta-analysis involving 15,260 cases and 20,411 controls. Breast Cancer Res Treat 2010; 123: 549–555. http://dx.doi.org/10.1007/s10549-010-0783-5

[53] LU M, WANG F, QIU J. Methionine synthase A2756G polymorphism and breast cancer risk: a meta-analysis involving 18,953 subjects. Breast Cancer Res Treat 2010; 123: 213–217. http://dx.doi.org/10.1007/s10549-010-0755-9

[54] UTHUS E, BEGAYE A, ROSS S, ZENG H. The von Hippel-Lindau (VHL) tumor-suppressor gene is down-regulated by selenium deficiency in Caco-2 cells and rat colon mucosa. Biol Trace Elem Res 2011; 142: 223–231. http://dx.doi.org/10.1007/s12011-010-8764-4

[55] FIALA ES, STARETZ ME, PANDYA GA, EL-BAYOUMY K, HAMILTON SR. Inhibition of DNA cytosine methyltransferase by chemopreventive selenium compounds, determined by an improved assay for DNA cytosine methyltransferase and DNA cytosine methylation. Carcinogenesis 1998; 19: 597–604. http://dx.doi.org/10.1093/carcin/19.4.597

[56] GAUDET F, HODGSON JG, EDEN A, JACKSON-GRUSBY L, DAUSMAN J, et al. Induction of tumors in mice by genomic hypomethylation. Science 2003; 300: 489–492. http://dx.doi.org/10.1126/science.1083558

[57] FROSST P, BLOM HJ, MILOS R, GOYETTE P, SHEPPARD CA, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet 1995; 10: 111–113. http://dx.doi.org/10.1038/ng0595-111

[58] MISRA UK, KALITA J, SRIVASTAVA AK, AGARWAL S. MTHFR gene polymorphism and its relationship with plasma homocysteine and folate in a North Indian population. Biochem Genet 2010; 48: 229–235. http://dx.doi.org/10.1007/s10528-009-9312-9

[59] FRISO S, CHOI SW, GIRELLI D, MASON JB, DOLNIKOWSKI GG, et al. A common mutation in the 5,10-methylenetetrahydrofolate reductase gene affects genomic DNA methylation through an interaction with folate status. Proc Natl Acad Sci U S A 2002; 99: 5606–5611. http://dx.doi.org/10.1073/pnas.062066299

[60] LIN J, LEE IM, SONG Y, COOK NR, SELHUB J, et al. Plasma homocysteine and cysteine and risk of breast cancer in women. Cancer Res 2010; 70: 2397–2405. http://dx.doi.org/10.1158/0008-5472.CAN-09-3648

[61] WU X, ZOU T, CAO N, NI J, XU W, et al. Plasma homocysteine levels and genetic polymorphisms in folate metablism are associated with breast cancer risk in chinese women. Hered Cancer Clin Pract 2014; 12: 2. http://dx.doi.org/10.1186/1897-4287-12-2

[62] NAUSHAD SM, REDDY CA, KUMARASWAMI K, DIVYYA S, KOTAMRAJU S, et al. Impact of hyperhomocysteinemia on breast cancer initiation and progression: epigenetic perspective. Cell Biochem Biophys 2014; 68: 397–406. http://dx.doi.org/10.1007/s12013-013-9720-7

[63] WACHOLDER S, SILVERMAN DT, MCLAUGHLIN JK, MANDEL JS. Selection of controls in case-control studies. II. Types of controls. Am J Epidemiol 1992; 135: 1029–1041.

[64] MACDONALD HM, NEW SA, REID DM. Longitudinal changes in dietary intake in Scottish women around the menopause: changes in dietary pattern result in minor changes in nutrient intake. Public Health Nutr 2005; 8: 409–416. http://dx.doi.org/10.1079/PHN2005705

[65] SHI Z, YUAN B, HU G, DAI Y, ZUO H, et al. Dietary pattern and weight change in a 5-year follow-up among Chinese adults: results from the Jiangsu Nutrition Study. Br J Nutr 2011; 105: 1047–1054. http://dx.doi.org/10.1017/S0007114510004630

[66] HOJO Y. Evaluation of the expression of urinary selenium level as ng Se/mg creatinine and the use of single-void urine as a sample for urinary selenium determination. Bull Environ Contam Toxicol 1981; 27: 213–220. http://dx.doi.org/10.1007/BF01611010

[67] LONGNECKER MP, STRAM DO, TAYLOR PR, LEVANDER OA, HOWE M, et al. Use of selenium concentration in whole blood, serum, toenails, or urine as a surrogate measure of selenium intake. Epidemiology 1996; 7: 384–390. http://dx.doi.org/10.1097/00001648-199607000-00008

[68] NEEDHAM LL, CALAFAT AM, BARR DB. Uses and issues of biomonitoring. Int J Hyg Environ Health 2007; 210: 229–238. http://dx.doi.org/10.1016/j.ijheh.2006.11.002

doi:10.4149/neo_2015_059

Supplementary Information

Joint effects between urinary selenium and polymorphisms in methylation related genes on breast cancer risk

W. LIN[1], Y. L. CEN[1], Y. LIN[2], F. X. SU[3], B. H. WU[4], L. Y. TANG[5,*], Z. F. REN[1,*]

[1]The School of Public Health, Sun Yat-sen University, Guangzhou, 510080, China; [2]The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, 510080, China; [3]The Second Affiliated Hospital, Sun Yat-sen University, Guangzhou, 510120, China; [4]The Guangdong Prevention and Treatment Center for Occupational Diseases, Guangzhou 510300, China; [5]The Third Affiliated Hospital, Sun Yat-sen University, Guangzhou, 510630, China

**Correspondence: renzef@mail.sysu.edu.cn, tangly@mail.sysu.edu.cn*

Supplementary Table

Table 1. The primer sequences for three SNP in methylation related genes

SNPs		Primer sequences
DNMT1 rs2228611	F;	5' – ACGTTGGATGGTGTGCCCCAAACATAATCC – 3'
	R:	5' – ACGTTGGATGCTGGTTCAGCAAAACCAATC – 3'
	SBE*:	5' – TATTCCTTACCTTCAAGAGA – 3'
MTHFR rs1801133	F;	5' – ACGTTGGATGGAAGCACTTGAAGGAGAAGG – 3'
	R:	5' – ACGTTGGATGAGCCTCAAAGAAAAGCTGCG – 3'
	SBE*:	5' – AGGTGTCTGCGGGAG – 3'
MTR rs1805087	F;	5' – ACGTTGGATGTCTACCACTTACCTTGAGAG – 3'
	R:	5' – ACGTTGGATGCTTTGAGGAAATCATGGAAG – 3'
	SBE*:	5' – ACCTTGAGAGACTCATAATGG – 3'

*Primers sequences for single base extension.