Published in final edited form as:

Mol Carcinog. 2012 May ; 51(5): 379–388. doi:10.1002/mc.20799.

Urinary polyphenols, glutathione s-transferases copy number variation, and breast cancer risk: results from the Shanghai Women's Health Study

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Abstract

Background—*In vitro* studies have found that flavanol epigallocatechin (EGC) and flavonols, but not flavanol epicatechin (EC), activate glutathione s-transferases (GST), a family of phase II enzymes that detoxify reactive oxygen species, such as catechol estrogen metabolites. This study was designed to investigate prospectively whether urinary excretion of tea polyphenols interacts with *GST* polymorphisms to influence breast cancer risk.

Subjects and Methods—We conducted a study of 352 incident breast cancer cases and 701 individually-matched controls nested within the Shanghai Women's Health Study cohort of women aged 40–70 years at baseline. Liquid chromatography tandem mass spectrometry was used to measure urinary excretion of flavanols and flavonols. Real-time multiplex PCR was used to quantify the copy number variation in the *GSTM1* and *GSTT1* genes.

Results—Urinary excretion of flavonols and flavanols, particularly EGC ($p=0.02$), was significantly higher among women null for *GSTM1* than those positive for *GSTM1*. Flavonols and flavanols (EGC in particular) were associated with a reduced risk of breast cancer among those null for *GSTM1* and *GSTT1*, with a p -value of 0.04 for the interaction between EGC and *GSTM1* polymorphism. In contrast, among women possessing both *GSTM1* and *GSTT1*, breast cancer risk increased with levels of flavonols, particularly kaempferol.

Conclusions—The differential associations between polyphenols and breast cancer risk by *GST* polymorphisms, if confirmed, may provide a new avenue for the personalized prevention of breast cancer.

Keywords

tea polyphenols; flavonol; flavanol; genomic polymorphism; interaction

INTRODUCTION

The incidence rate of breast cancer among women in East Asia, such as China, is substantially lower than their counterparts in Western societies [1]. Migration studies have indicated the importance of lifestyle factors in the etiology of breast cancer [2]. In addition to well-investigated soy foods[3,4], East Asian women also consume high levels of green tea, allium vegetables, other vegetables, and fruits, all of which contain high concentrations of polyphenol flavanols and flavonols. Growing evidence from *in vitro* and *in vivo* studies implicates a critical role of catechol estrogens in mammary carcinogenesis [5]. With a catechol ring, flavanols and flavonols may be the only two common groups of polyphenols that are structurally similar to reactive estrogen catechol metabolites (See Figure 1). Flavonols and flavanols have been shown to significantly affect catechol estrogen metabolism [6] apart from their anti-aromatase, antioxidant and anti-telomerase properties[7–9]. Previous cohort studies, however, have not provided support for a strong association between consumption of green tea, one major source for flavonols and flavanols, and breast cancer risk, with an overall RR (95% CI) of 0.85(0.66–1.09) in the meta-analysis of cohort studies [10], whereas an overall OR (95% CI) of 0.81 (0.75–0.88) was found in the meta-analysis of case-control studies [11]. We also found in the Shanghai Women's Health Study (SWHS) that green tea drinking was overall not related to risk of breast cancer. However, showed it is possible that green tea drinking may delay onset of breast cancer[12].

Another possible explanation is that, in these studies, intakes of flavonols and flavanols from other fruits and vegetables were not considered. Culinary preparation, such as peeling and heating, may lead to substantial loss of polyphenols, which primarily exist in the outer sections of fruits and vegetables. Furthermore, absorption rates of individual polyphenols differ greatly [13]. Many polyphenols can be absorbed only after metabolism by gut microflora, and a dramatic inter-person variation in the bioavailability of polyphenols has been observed after identical intake of polyphenols[13,14].

Very recently, we found there were no overall dose-response associations between urinary excretion of major polyphenols, including flavonols and flavanols, and risk of breast cancer, although urinary excretion rates of polyphenol flavanols were significantly higher among controls than breast cancer cases, in a nested case-control study within the SWHS[15]. Subsequently, a nested case-control study conducted in Japan also did not find dose-response associations between tea polyphenols and risk of breast cancer [16].

Because of the structural similarities, catechol polyphenols may compete with catechol estrogens for metabolism enzymes. Catechol estrogens and polyphenols, particularly non-gallated green tea polyphenols, are mainly glucuronidated, methylated, or sulfated while only a small portion of them are conjugated by the family of glutathione S-transferases (GSTs) [17,18]. However, previous studies indicate that reactive oxygen species generated during oxidative metabolism of catechol estrogens, such as semiquinones and quinones, may play a critical role in the breast carcinogenesis [19,20] while GSTs catalyze the conjugation of semiquinones and quinones to glutathione (GSH)[21–23]. GSTM1 and GSTT1 are two major GST family members and their genes exhibit deletion polymorphisms, which lead to a complete loss of enzyme activities [24,25]. The deletion mutations in *GSTM1* and *GSTT1* have been investigated for the associations with breast cancer in a large number of studies. However, these studies have generated inconsistent results [26–33]. In addition to GSTT and GSTM, there are other members in the GST family, such as GSTA and GSTP.

Several *in vitro* studies have reported that flavanol epigallocatechin (EGC) [34] and flavonols (e.g. quercetin and kaempferol) affect the activities of some GSTs [21], whereas

no effect has been found for flavonol epicatechin (EC) on any GSTs [34]. However, the studies on the interactions between catechol polyphenols and GSTs have been very limited. We hypothesized that EGC, quercetin and kaempferol, but not EC, interacts with *GST* polymorphisms to affect breast cancer risk, and tested this hypothesis in a large nested case-control study within the Shanghai Women's Health Study.

SUBJECTS AND METHODS

The Shanghai Women's Health Study

Details on the establishment of the SWHS have been reported elsewhere [23]. In brief, at baseline from March 1997 to May 2000, a cohort of 74,942 Chinese women between 40 and 70 years of age was formed, with a 92% participation rate in Shanghai. Participants were interviewed by trained interviewers to elicit information on demographic characteristics, medical history, anthropometrics, usual dietary habits, physical activities, and other lifestyle factors. The study was approved by all relevant institutional review boards in China and the United States. All participants provided informed written consent.

Cohort follow-up and outcome ascertainment—The SWHS participants were followed for the occurrence of cancer by a combination of active surveys conducted every two to three years and annual record linkage to the population-based Shanghai Cancer Registry and death certificates. Nearly all cohort members were successfully followed. The response rates were 99.8% (2000–2002) for the first in-person follow-up survey, 98.7% (2002–2004) for the second, and 96.7% (2004–2007) for the third follow up. All possible incident cancer cases were verified by home visits and medical record review.

Sample collection, storage and processing—For 87.7% of the cohort participants, a spot urine sample was collected into a sterilized 100-ml cup containing 125 mg of ascorbic acid. The collected samples were kept in a portable, insulated bag with ice packs (at about 0–4°C) [23]. The urine samples were processed within 6 hours of collection, and the urine specimens were immediately stored at –80°C. Of the study participants, we collected genomic DNA samples from 65,765 women (87.7% collection rate).

Nested case-control design

Details on the nested case-control design have been described in our previous report [15]. A nested case-control study was conducted among 65,754 women who donated a urine sample at baseline or during the first follow-up approximately two years later. Among them, 436 cases with urine samples collected before cancer diagnosis were identified from Nov 1997 to Sep 2004. Two controls were randomly selected and matched with each case based on age at baseline (± 2 years), date at study enrollment (≤ 30 days), time (morning or afternoon) of urine collection, interval since last meal (≤ 2 hours), menopausal status (pre- or post-), and antibiotic use (yes/no) in the past week. The first 353 identified cases (with an average 6.1 years of follow-up) and their individually-matched controls were included in the present study [15]. Two controls were successfully matched with each of 349 cases, while 3 cases were matched with only one control each. One case was not matched with a control, and was therefore excluded. A total of 352 cases and 701 controls were included in this study.

Urinary excretion of tea polyphenols

Urinary tea polyphenol analyses were performed as described previously [15]. Tea polyphenols were measured by liquid chromatography tandem mass spectrometry as established recently in Dr. Franke's laboratory [35]. Final results were presented after adjusting for creatinine concentrations (ng/mg creatinine).

Genotyping Method for GSTs

Genomic DNA was extracted from buffy coat fractions of blood or exfoliated buccal cell sample using the Qiagen (Valencia, CA) DNA purification kit following the manufacturer's protocol. A duplex real-time PCR strategy was used to genotype copy number of the *GSTM1* and *GSTT1* genes using the method described in the SNP500 (<http://snp500cancer.nci.nih.gov/assays>, 26) with modifications. All sequences used in assay designs were obtained from Genbank: *GSTM1*, NM_000561 and *GSTT1*, NM_000853. The detailed genotyping method is described in detail elsewhere [36]. Quality control samples (water, Coriell DNA, as well as blinded and unblinded DNA samples) were included in genotyping assays. Coriell DNA samples containing the 0, 1, or 2 copies of *GSTM1* and *GSTT1* genes were included in each genotyping plate to serve as internal quality controls. The concordance rates for QC samples were 100%. Among 352 cases and 701 controls, only one control did not have genotyping data for *GSTM1*.

Statistical Analysis

Baseline covariates were compared between cases and controls to evaluate potential confounding factors. Urinary levels of polyphenols were categorized based on the tertile distribution in controls. Conditional logistic regression was used to analyze the association between urinary excretion levels of polyphenols and the risk of breast cancer. The models were adjusted for multiple covariates that might confound the relation between polyphenols and breast cancer. Potential confounding factors included in final regression models were age, education, age at menarche, age at first live birth, months of breastfeeding, body mass index, menopausal status, history of breast fibroadenoma, first-degree family cancer history, smoking status, total intake of red meat and isoflavones, and use of hormone replacement therapy. Furthermore, we mutually controlled for other urinary polyphenols. For example, urinary levels of flavanols and flavonols other than quercetin were additionally adjusted for in the analysis for urinary quercetin. Stratified analyses by *GSTM1* and *GSTT1* polymorphism were performed to evaluate whether the associations were modified by these genetic factors. Because this is a matched case-control design, stratum-specific ORs were derived from conditional regression with the inclusion of terms for main effects along with two interaction terms. By adding these interaction terms, case-control pairs were not broken, and all of the subjects were included in the model building [37]. Likelihood ratio tests were conducted to test for potential interactions. Tests for trend were performed by entering the categorical variables as ordinal variables in the model. Statistical analyses were performed with SAS 9.1 software (SAS Institute, Cary, NC). All of the reported P values were two-tailed, and statistical significance was set at 0.05.

RESULTS

Shown in Table 1 are the comparisons of potential confounding factors between cases and controls at baseline. Approximately half of the cases and controls were postmenopausal. The median age of cases at diagnosis was 52.8 years. Compared to controls, cases were more likely to have higher educational attainment, earlier age at menarche, later age at first live birth, a family history of breast cancer, and a history of breast fibroadenoma. Controls were more likely to smoke cigarettes, breastfeed longer, and have a higher intake of red meat and soy isoflavones compared with cases. The distribution of genotypes for *GSTT1* and *GSTM1* was consistent with Hardy–Weinberg equilibrium ($P=0.970$ for *GSTT1* and $p=0.786$ for *GSTM1* among controls). The frequencies of *GSTT1* null and *GSTM1* null genotypes in the control group were 51.9% and 59.1%, respectively. The distributions of *GSTM1* and *GSTT1* polymorphisms did not significantly differ by case-control status. Due to low frequencies for individuals who possessed two copies of *GSTM1* or *GSTT1*, we classified those with either

one copy or two copies into one group (i.e., *GSTT1* or *GSTM1* present) in the stratified analyses.

We compared the urinary excretion rates of flavonols and flavanols by *GSTM1* and *GSTT1* genotypes among controls (Table 2). Since EGC and EC may have different activities on GSTs, we have not conducted analysis on total flavanols (EGC + EC). Excretion rates of all polyphenols were elevated among women with the *GSTM1* null genotype compared to those with at least one copy of *GSTM1*, although the results were only significant for EGC ($p=0.02$) and of borderline significance for flavonols (K + Q) ($p=0.08$), even after adjusting for age and amount of green tea consumption. On the other hand, we did not find a significant difference by *GSTT1* genotype, although the urinary level of EGC was higher among women with the *GSTT1* null genotype than those with at least one copy of *GSTT1*.

We evaluated the association between urinary flavonols and flavanols and risk of breast cancer by the *GSTM1* genotype (Table 3). Consistent with results observed in table 2, urinary excretion of EGC, but not EC, significantly interacted with *GSTM1* genotype (P for interaction, 0.04). Among those with the *GSTM1* null genotype, increased urinary excretion of EGC was associated with a reduced risk of breast cancer, with ORs (95% CI) of 0.66 (0.41–1.05) and 0.64 (0.41–1.01) for the middle and highest tertile of excretion levels, respectively, compared to women in the lowest tertile of EGC (p for trend, 0.058). Conversely, among women with one or two copies of *GSTM1*, EGC was positively associated with the risk of breast cancer (p for trend, 0.10). Similarly, urinary excretion of flavonols, particularly kaempferol, also was associated with an increased risk of breast cancer among those with at least one copy of *GSTM1* (P for trend, 0.05).

We did not find any significant interactions between urinary flavonols or flavanols and *GSTT1* genotype in relation to breast cancer risk (Table 4). However, similar to patterns of associations observed for *GSTM1*, breast cancer risk also increased with levels of flavonols, particularly kaempferol, among women with at least one copy of *GSTT1* (p for trend=0.04 for flavonols and p for trend=0.05 for kaempferol). Further, we found that urinary excretion of EGC (p for interaction, $P<0.01$) and flavonols (p for interaction, 0.03) significantly interacted with joint genotypes of *GSTM1* and *GSTT1* in relation to breast cancer. Among women null for both *GSTM1* and *GSTT1*, breast cancer risk decreased with increasing levels of flavonols (p for trend, 0.03), with OR of 0.49 (0.25–0.93) among those in the highest tertile of flavonols. Conversely, among those with at least one copy for both *GSTM1* and *GSTT1*, breast cancer risk rose with increasing levels of urinary flavonols, primarily kaempferol (p for trend, 0.04). Breast cancer risk for women in the highest tertile of kaempferol have more than doubled (OR=2.41 (1.04–5.59) that for women in the lowest tertile (data not shown).

DISCUSSION

To our knowledge, this is the first study examining the association between polyphenols and *GST* polymorphisms in relation to cancer risk. In humans, eight different subfamilies of cytosolic GST isoenzymes have been identified [38]. A large proportion of all populations [39–42] possess a homozygous deletion of the *GSTM1* gene (encoding GST μ) or the *GSTT1* gene (encoding GST θ), leading to a complete absence of these isoenzymes [38]. However, *GSTM1* and *GSTT1* polymorphisms have not been consistently linked to breast cancer risk directly in previous epidemiologic studies [26–33]. Similarly, we recently reported that there was no overall significant association between urinary flavonols and flavanols and breast cancer risk [15].

In the current nested case-control study, we found significantly higher urinary excretion of flavonols and EGC among healthy women carrying the *GSTM1* null genotype than those positive for *GSTM1*. Furthermore, we found that breast cancer risk associated with urinary excretion of EGC and flavonols may be modified by *GSTM1* and *GSTT1* polymorphism. Among women carrying the null genotype for both *GSTM1* and *GSTT1*, breast cancer risk was reduced with increased levels of flavonols and EGC. Among women possessing at least one copy of both *GSTM1* and *GSTT1*, flavonols, particularly kaempferol, were significantly associated with an increased risk of breast cancer. Our finding that *GST* polymorphisms may modify the associations of flavonols and EGC with breast cancer may provide a possible interpretation [12] for the inconsistent results on green tea drinking, a major source of EGC and flavonols, and breast cancer risk in previous cohort studies [10,15,43].

We found that the inverse association between urinary flavonols and EGC and breast cancer risk only appeared among those null for *GSTM1*, particularly null for both *GSTM1* and *GSTT1*. This finding is very similar to that for another phytochemical, isothiocyanates. In three previous studies, the inverse association between urinary isothiocyanates and cancer of lung, breast and colorectum was only observed among those null for *GSTM1* and *GSTT1* [44–47]. One possible explanation is that for those null for both *GSTM1* and *GSTT1*, the EGC [34] and isothiocyanates can activate other forms of GSTs, such as GSTP and GSTA [48].

Isothiocyanates, as well as catechol estrogens, are substrates of GST enzymes [49]. EGC and flavanols are a special family of polyphenols, structurally similar to catechol estrogens [6]. Thus, it is conceivable that catechol polyphenols are substrates of GSTs as well. As a result, among those present for *GSTM1* and *GSTT1*, catechol polyphenols may be more rapidly excreted in urine through conjugation with glutathione (GSH), leading to a lower bioavailable level of cancer-inhibitory tea polyphenols. This possibility is supported by our finding that healthy women with *GSTM1* null genotype had higher urine levels of both flavonols and flavanol EGC than those with at least one copy of *GSTM1*. Furthermore, previous *in vitro* studies showed that flavanol EC did not affect activity of GST enzymes [21,34], which provides biologic support for why we did not find a significant interaction between EC and *GST* polymorphisms.

Individuals carrying the *GSTM1* and *GSTT1* null genotype may possess enzyme activity for other GST isoforms [50]. For example, a previous feeding study found that, compared to subjects positive for *GSTM1*, those with *GSTM1*-null had significantly higher levels of serum GST-alpha after a high cruciferous vegetable diet [51]. This finding indicates that loss of *GSTM1* function may lead to increased levels of isothiocyanates which induces expression of GST-alpha, while GST-alpha in turn possesses much less activity to degrade isothiocyanates than *GSTM1* [52]. Likewise, in an intervention trial without a placebo group, treatment of green tea polyphenols led to a statistically significant elevation (80%, $P = 0.004$) in GST activity among those in the lowest tertile of baseline GST activity, while there was a statistically significant reduction (20%, $P = 0.02$) in GST activity among those with the highest baseline GST activity [53]. It is possible that women positive for *GSTM1* and/or *GSTT1* possess relatively higher levels of GST activity compared to those null for both *GSTM1* and *GSTT1*. Under this circumstance, tea polyphenols may have a stronger inhibitory effect on GST activity among those positive for *GSTM1* and/or *GSTT1*.

The present study has several notable strengths. Levels of bioavailable polyphenols were measured using a sensitive method (LC/PDA/ESI-MS). Although consumption of green tea or other polyphenol-rich diets are unlikely to change, metabolism of polyphenols could be affected among incipient breast cancer patients [15]. We conducted sensitivity analyses by excluding women who were diagnosed with breast cancer within one year of urine collection

and found similar results. The parent study, the population-based Shanghai Women's Health Study, had high rates for baseline participation and follow-up, which minimized selection bias. However, one spot urine was used to measure urinary levels of polyphenols, which may not reflect adequately long-term exposure levels. The resulting misclassification from inter-person variation tends to be non-differential, which usually biases the results to the null. We also cannot exclude the possibility that the significant interactions occurred by chance. However, this is unlikely because the findings are not only supported by *in vitro* studies, but are consistent with the fact that urinary levels of flavonols and EGC differ by *GST* genotypes among controls in the current study, and in studies of isothiocyanates which also interact with *GST* polymorphisms.

The findings of the present study indicate the importance of gene-diet interactions in the development of breast cancer. These results, if confirmed, may provide a new avenue for the personalized prevention of breast cancer.

Acknowledgments

Sources of support: This study was supported by USPHS grant R01CA106591 and R37CA70867. Biological sample collection in the parent study was supported in part by NIH intramural program (N02 CP1101066) for the parent study. The authors thank Laurie Custer for the skillful performance of LC/MS assays. We also acknowledge the support by NIH grant S10 RR020890-01.

Definitions for abbreviations

EGC	epigallocatechin
EC	epicatechin
PCR	polymerase chain reaction
GST	glutathione s-transferases
COMT	catechol-O-methyltransferase
SWHS	Shanghai Women's Health Study
QC	quality control
OR	odds ratio
CI	confidence intervals

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Catechol Estrogens **2-OHE₁****Flavanols** **EGC****Flavonols** **Quercetin** **4-OHE₁** **EC** **Kaempferol****Figure 1.**

Chemical structure of catechol estrogens and flavanols and flavonols. **a)** EGC, EGC and Quercetin possess a catechol moiety in B ring; and **b)** although kaempferol does not have a catechol moiety, it has a very similar structure to quercetin. Previous studies found kaempferol was transformed to quercetin by cytochromes p450 in a time-dependent manner[54]

Comparison of breast cancer cases and controls by selected baseline demographic and risk factors, a nested case-control study within the Shanghai Women's Health Study (SWHS), 1997–2006

Table 1

Subject Characteristic	Cases (n=352)	Controls (n=701)	P value *
Age (years), mean $\pm$ SD	52.8 $\pm$ 8.8	52.9 $\pm$ 8.9	0.84
Income, %			0.66
Low	26.6	29.0	
Middle	38.0	37.9	
High	35.4	33.1	
Education, %			<0.01
Elementary school or below	13.6	21.5	
Middle or high school	68.8	67.2	
College or above	17.6	11.3	
Breast cancer in first-degree relative, %	4.6	1.4	<0.01
Ever had breast fibroadenoma, %	7.2	4.2	0.04
Age at menarche (years)	14.8 $\pm$ 1.9	15.0 $\pm$ 1.8	0.06
Age at first live birth (years), mean $\pm$ SD †	26.3 $\pm$ 4.1	25.6 $\pm$ 4.1	<0.01
Months of breastfeeding (month), mean $\pm$ SD †	13.7 $\pm$ 15.8	15.5 $\pm$ 16.2	0.08
Post-menopausal, %	48.7	49.6	0.79
Age at menopause (years), mean $\pm$ SD	49.7 $\pm$ 4.8	48.9 $\pm$ 4.0	0.06
Use of hormone replacement therapy, %	5.1	3.4	0.19
Physically active past 5 years, %	33.2	34.2	0.76
BMI (kg/m ²), mean $\pm$ SD	24.3 $\pm$ 3.5	24.4 $\pm$ 3.4	0.60
Waist-to-hip ratio, mean $\pm$ SD	0.82 $\pm$ 0.06	0.82 $\pm$ 0.05	0.97
Ever smoke regularly, %	0.8	2.7	0.05
Ever exposure to passive smoking, %	80.2	83.5	0.19
Ever drink alcohol regularly, %	1.7	3.0	0.21
Ever drink tea regularly, %	31.7	29.5	0.46
Amount of tea drunk (monthly 50 grams)	7.8 $\pm$ 15.5	7.6 $\pm$ 16.2	0.84
Use of ginseng regularly, %	29.5	26.5	0.32
GSTT			

Subject Characteristic	Cases (n=352)	Controls (n=701)	P value*
Null	186 (52.7 %)	364 (51.9 %)	0.81
Present	167 (47.3 %)	337 (48.1 %)	
d/i	138 (39.1 %)	282 (40.2 %)	
i/i	29 (8.2 %)	55 (7.8 %)	
GSTM			0.88
Null	207 (58.6 %)	414 (59.1 %)	
Present	146 (41.4 %)	286 (40.9 %)	
d/i	122 (34.6 %)	247 (35.3 %)	
i/i	24 (6.8 %)	39 (5.6 %)	
Dietary factors			
Daily energy intake (kcal), mean ±SD	1658.7 ±355.3	1698.7 ±393.9	0.11
Daily intake of fish, mean ±SD	49.5 ±38.8	53.6 ±50.6	0.18
Daily intake of red meat, mean ±SD	46.90 ±31.1	52.3 ±35.7	0.02
Daily intake of vegetables, mean ±SD	297.6 ±158.9	303.8 ±170.8	0.57
Daily intake of fruits, mean ±SD	265.9 ±163.0	269.7 ±175.0	0.73
Daily intake of isoflavones (mg), mean ±SD	29.6 ±20.2	32.5 ±25.4	0.07

* For χ^2 test (categorical variables) or Cochran Mantel Haenszel χ^2 test (ordinal categorical variables) or t test (continuous variables)

† Among parous women only

Table 2

Comparison of urinary polyphenols between GST null and present genotype among controls, a nested case-control study within the Shanghai Women's Health Study (SWHS), 1997–2006*

Urinary excretion rate (ng/mg creatinine)	GSTMI		P value [†]	P value [‡]
	Null (108)	Present (82)		
Flavanols	0.59 (0.08–3.12)*	0.19 (0.02–1.97)	0.05	0.06
epigallocatechin	0.32 (0.03–2.00)	0.07(0.00–1.20)	0.02	0.02
epicatechin	0.06 (0.00–1.17)	0.02 (0.00–0.79)	0.18	0.27
Flavonols	0.45 (0.14–1.00)	0.29 (0.10–0.70)	0.09	0.08
Kaempferol	0.19 (0.03–0.55)	0.10 (0.03–0.44)	0.16	0.16
Quercetin	0.19 (0.04–0.45)	0.09 (0.02–0.35)	0.10	0.19
<i>GSTII</i>				
	Null (98)	Present (92)		
Flavanols	0.70 (0.04–2.90)	0.29 (0.04–2.03)	0.38	0.55
epigallocatechin	0.23 (0.02–1.98)	0.14 (0.01–1.12)	0.41	0.74
epicatechin	0.03 (0.00–1.06)	0.04 (0.00–0.90)	0.97	0.90
Flavonols	0.38 (0.12–0.99)	0.34 (0.10–0.80)	0.41	0.49
Kaempferol	0.13 (0.03–0.58)	0.11 (0.03–0.42)	0.71	0.86
Quercetin	0.17 (0.05–0.40)	0.10 (0.02–0.40)	0.32	0.31

* Median (25th percentile, 75th percentile)

[†] P value by Wilcoxon rank sum test

[‡] P value estimated using log transformed polyphenols after adjusting for age and amount of tea consumption.

Odds ratios (ORs) and 95% confidence intervals (CI) for risk of breast cancer associated with urinary excretion rate of polyphenols stratified by GSTM1 polymorphism, a nested case-control study within the Shanghai Women's Health Study (SWHS), 1997–2006

Table 3

Urinary excretion rate (ng/mg creatinine)	GSTM1 genotype	Urinary excretion rate of polyphenols by tertile*			
		T1(Low Tertile) †	T2	T3	P for interaction
Flavanols	Present	1.00	0.87(0.51–1.49)	1.16(0.67–2.01)	0.63
	Null	1.00	0.97(0.61–1.54)	1.01(0.63–1.62)	0.97
epicatechin	Present	1.00	0.52(0.28–0.99)	1.08(0.65–1.79)	0.93
	Null	1.00	0.63(0.37–1.08)	1.10(0.72–1.68)	0.83
epigallocatechin	Present	1.00	1.08(0.63–1.85)	1.63(0.92–2.90)	0.10
	Null	1.00	0.66(0.41–1.05)	0.64(0.41–1.01)	0.06
Flavonols	Present	1.00	1.51(0.87–2.61)	1.66(0.96–2.86)	0.07
	Null	1.00	0.98(0.63–1.54)	0.85(0.52–1.37)	0.51
kaempferol	Present	1.00	1.36(0.76–2.42)	1.78(1.00–3.19)	0.05
	Null	1.00	1.11(0.72–1.72)	0.91(0.56–1.46)	0.74
quercetin	Present	1.00	0.87(0.50–1.52)	1.34(0.79–2.28)	0.28
	Null	1.00	0.85(0.54–1.35)	0.97(0.62–1.53)	0.94

* Conditional logistic regression model adjusting for age, education, age at menarche (continuous), age at 1st live birth (continuous), months of breastfeeding (continuous), body mass index (continuous), menopause(yes/no), history of breast fibroadenoma (yes/no), first-degree family cancer history (yes/no), ever smoker (never/ever), total intake of red meat and isoflavones, and use of hormone replacement therapy.

† 33.3th and 66.7th percentiles for each polyphenols.

Odds ratios (ORs) and 95% confidence intervals (CI) for risk of breast cancer associated with urinary excretion rate of polyphenols stratified by GSTT1 polymorphism, a nested case-control study within the Shanghai Women's Health Study (SWHS), 1997–2006

Table 4

Urinary excretion rate (ng/mg creatinine)		GSTT1 genotype	Urinary excretion rate of polyphenols by tertile*			
			T1(Low Tertile) [†]	T2	T3	P for interaction
Flavanols	Present	Present	1.00	1.14(0.68–1.91)	1.38(0.81–2.33)	0.25
	Null	Null	1.00	0.76(0.47–1.25)	0.84(0.51–1.40)	0.51
epicatechin	Present	Present	1.00	0.56(0.30–1.03)	1.32(0.83–2.10)	0.29
	Null	Null	1.00	0.61(0.35–1.06)	0.90(0.56–1.45)	0.47
epigallocatechin	Present	Present	1.00	0.88(0.55–1.42)	0.90(0.53–1.52)	0.67
	Null	Null	1.00	0.75(0.45–1.24)	0.92(0.56–1.50)	0.76
Flavonols	Present	Present	1.00	1.30(0.78–2.16)	1.73(1.01–2.95)	0.04
	Null	Null	1.00	1.13(0.70–1.81)	0.81(0.49–1.32)	0.40
kaempferol	Present	Present	1.00	1.17(0.70–1.97)	1.73(0.99–3.02)	0.05
	Null	Null	1.00	1.28(0.80–2.06)	0.91(0.56–1.47)	0.76
quercetin	Present	Present	1.00	0.77(0.46–1.30)	1.20(0.73–1.97)	0.46
	Null	Null	1.00	0.94(0.58–1.54)	1.01(0.62–1.66)	0.93

* Conditional logistic regression model adjusting for age, education, age at menarche (continuous), age at 1st live birth (continuous), months of breastfeeding (continuous), body mass index (continuous), menopause(yes/no), history of breast fibroadenoma (yes/no), first-degree family cancer history (yes/no), ever smoker (never/ever), total intake of red meat and isoflavones, and use of hormone replacement therapy.

[†] 33.3th and 66.7th percentiles for each polyphenols.

Odds ratios (ORs) and 95% confidence intervals (CI) for risk of breast cancer associated with urinary excretion rate of polyphenols stratified by GST polymorphism additionally adjusted by each other (flavanol and flavonol), a nested case-control study within the Shanghai Women's Health Study (SWHS), 1997–2006*

Table 5

Urinary excretion rate (ng/mg creatinine)		Urinary excretion rate of polyphenols by tertile			
		T1(Low Tertile) †	T2	T3	P for Trend P for interactions
Flavanols	Double Present	1.00	1.24(0.58–2.67)	0.94(0.43–2.04)	0.78
	GSTT1 Null	1.00	0.56(0.25–1.26)	1.53(0.69–3.38)	0.32
	GSTM1 Null	1.00	1.10(0.54–2.24)	2.00(0.99–4.05)	0.06
	double Null	1.00	0.87(0.47–1.64)	0.57(0.30–1.09)	0.09
epicatechin	Double Present	1.00	0.40(0.16–1.00)	0.96(0.48–1.92)	0.77
	GSTT1 Null	1.00	0.71(0.29–1.75)	1.19(0.58–2.45)	0.73
	GSTM1 Null	1.00	0.73(0.32–1.63)	1.72(0.94–3.17)	0.08
	double Null	1.00	0.57(0.28–1.16)	0.74(0.41–1.33)	0.21
epigallocatechin	Double Present	1.00	1.82(0.86–3.85)	1.29(0.56–2.96)	0.54
	GSTT1 Null	1.00	0.60(0.26–1.34)	2.19(0.99–4.88)	0.08
	GSTM1 Null	1.00	0.53(0.27–1.04)	0.77(0.39–1.53)	0.38
	double Null	1.00	0.87(0.45–1.67)	0.58(0.31–1.09)	0.09
Flavonols	Double Present	1.00	1.86(0.86–4.02)	1.80(0.83–3.91)	0.14
	GSTT1 Null	1.00	1.25(0.57–2.74)	1.70(0.77–3.72)	0.20
	GSTM1 Null	1.00	1.00(0.51–1.96)	1.65(0.81–3.34)	0.15
	double Null	1.00	1.07(0.58–1.97)	0.49(0.25–0.93)	0.03
kaempferol	Double Present	1.00	1.53(0.67–3.51)	2.41(1.04–5.59)	0.04
	GSTT1 Null	1.00	1.31(0.58–2.96)	1.44(0.65–3.16)	0.36
	GSTM1 Null	1.00	0.99(0.49–1.98)	1.37(0.66–2.85)	0.35
	double Null	1.00	1.34(0.75–2.42)	0.66(0.36–1.24)	0.26
quercetin	Double Present	1.00	0.80(0.37–1.69)	1.08(0.51–2.26)	0.82
	GSTT1 Null	1.00	0.98(0.43–2.23)	1.78(0.81–3.92)	0.15
	GSTM1 Null	1.00	0.74(0.37–1.48)	1.33(0.70–2.52)	0.37
	double Null	1.00	0.91(0.49–1.67)	0.73(0.39–1.36)	0.34

* Conditional logistic regression model adjusting for age, education, age at menarche (continuous), age at 1st live birth (continuous), months of breastfeeding (continuous), body mass index (continuous), menopause(yes/no), history of breast fibroadenoma (yes/no), first-degree family cancer history (yes/no), ever smoker (never/ever), total intake of red meat and isoflavones, and use of hormone replacement therapy.

[†] 33.3th and 66.7th percentiles for each polyphenols