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Glutathione *S*-transferase M1 and P1 polymorphisms and risk of breast cancer and fibrocystic breast conditions in Chinese women

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Abstract

Enzymes encoded by the glutathione *S*-tranferase mu 1 (*GSTM1*) and pi 1 (*GSTP1*) genes, which are expressed in breast tissue, catalyze the detoxification of endogenous and exogenous electrophiles. Reduced enzyme activity, due to carriage of the *GSTM1* deletion or the *GSTP1* Ile105Val Val allele, may therefore affect susceptibility to breast cancer and related conditions. In a case-control study of Chinese women, we examined whether these polymorphisms were associated with risk of breast cancer and fibrocystic breast conditions. Women diagnosed with breast cancer (n=615) or fibrocystic breast conditions (n=467) were compared to women without clinical breast disease (n=878). We also examined whether these associations differed by menopausal status or by presence of proliferation in the extra-tumoral epithelium among women with breast cancer and in lesions among women with fibrocystic conditions. No overall association of either *GST* polymorphism with risk of breast cancer or fibrocystic breast conditions was observed. There was some evidence of slightly elevated cancer risk associated with carriage of the *GSTM1* null genotype and at least one *GSTP1* 105-Val allele (OR=1.33, 95% CI, 0.99–1.80), compared to carriage of the *GSTM1* non-null and *GSTP1* Ile/Ile genotypes. This relationship was stronger in women who have breast cancer with extra-tumoral tissue proliferation (OR=1.77, 95% CI, 1.03–3.04). Our results suggest that *GSTM1* and *GSTP1* genotypes do not individually influence susceptibility to breast cancer or fibrocystic breast conditions. The observed increased risk of breast cancer associated with joint carriage of the *GSTM1* null genotype and *GSTP1* 105-Val allele needs confirmation in other studies.

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Keywords

breast cancer; Chinese; fibrocystic breast conditions; glutathione *S*-transferase; polymorphism

INTRODUCTION

Glutathione *S*-transferases (GSTs) are enzymes that primarily catalyze the detoxification of exogenous and endogenous electrophilic compounds by conjugation with glutathione. Their substrates include known and suspected carcinogens, such as benzo(*a*)pyrene and other polycyclic aromatic hydrocarbons, reactive intermediates derived from estrogen metabolism, and byproducts of oxidative stress. These enzymes also function as peroxidases, isomerases, and thiol transferases and act in cellular processes unrelated to detoxification (reviewed by (Hayes et al. [1])).

Specific to breast cancer, the roles of the cytosolic GST class μ and π enzymes have been of particular interest, since both enzymes are frequently expressed in normal and tumor breast tissue (Howie et al. [2];Forrester et al. [3];Haas et al. [4]) and are encoded by genes that contain common, functional polymorphisms. The *GSTM1* gene (chromosome 1p13.3), encoding the GST class μ enzyme, contains a deletion polymorphism for which the homozygous deletion or “null” genotype corresponds to a total loss of enzyme function (Board [5]). In the *GSTP1* gene (chromosome 11q13), which encodes the GST class π enzyme, a base change (A>G) polymorphism (rs1695) causes an amino acid substitution of isoleucine (Ile) with valine (Val) at codon 105 that results in decreased levels of enzymatic activity (Zimniak et al. [6];Ali-Osman et al. [7]). Therefore, it has been hypothesized that differences in the metabolic capacity of either GST-specific enzyme might affect susceptibility to breast cancer, such that reduced GST activity, due to inheritance of the *GSTM1* null genotype or *GSTP1* Val allele, would predispose to increased cancer risk.

Evidence to support an association between *GSTM1* and *GSTP1* polymorphisms and breast cancer risk, however, has been weak. Numerous studies have detected no overall association between the *GSTM1* null genotype and risk of breast cancer (Zhong et al. [8];Ambrosone et al. [9];Kelsey et al. [10];Bailey et al. [11];Charrier et al. [12];Ambrosone et al. [13];Garcia-Closas et al. [14];Lizard-Nacol et al. [15];Curran et al. [16];Millikan et al. [17];Park et al. [18];Krajinovic et al. [19];Mitrunen et al. [20];Dialyna et al. [21];Gudmundsdottir et al. [22];da Fonte de Amorim et al. [23];Siegelmann-Danieliand Buetow [24];Zheng et al. [25];Zheng et al. [26];Khedhaier et al. [27];Park et al. [28];Vogl et al. [29];Egan et al. [30];Linhares et al. [31];van der Hel et al. [32];Cheng et al. [33];Wu et al. [34]), although a few have suggested the association might differ by menopausal status (Helzlsouer et al. [35];Charrier et al. [12];Park et al. [18];Mitrunen et al. [20];Park et al. [28]), smoking (Terry and Goodman [36]), alcohol use (Park et al. [18];Zheng et al. [37]), consumption of well-done meat (Zheng et al. [25]), and a combination of parity and age at first full-term pregnancy (Park et al. [38]). The majority of studies examining the *GSTP1* Ile105Val polymorphism and breast cancer risk have also yielded null results (Harries et al. [39];Helzlsouer et al. [35];Curran et al. [16];Millikan et al. [17];Dialyna et al. [21];Krajinovic et al. [19];Mitrunen et al. [20];Gudmundsdottir et al. [22];Zhao et al. [40];Kim et al. [41];Vogl et al. [29];Spurdle et al. [42]). The only study that has reported an increased risk of breast cancer associated with the *GSTP1* Val/Val genotype is a large case-control study conducted in Shanghai, China (Egan et al. [30]).

Also of related interest is the hypothesis that, since GST enzymes are induced by and metabolize isothiocyanates (ITCs) and other anti-carcinogenic compounds found naturally in plant foods, individuals with *GST* variants corresponding to low GST activity and a high intake

of fruits and vegetables are less susceptible to cancer than individuals with these variants and a low intake of fruits and vegetables, due to increased availability of these phytochemicals (Lampeand Peterson [43];Seow et al. [44]). Few studies to date have examined this potential interaction between *GST* variation, diet, and breast cancer risk (Fowke et al. [45];Ambrosone et al. [46];Ambrosone et al. [13]). In a study of Chinese women, an inverse relationship between urinary ITC levels and risk of breast cancer was found to be slightly more evident among *GST* null than non-null carriers (Fowke et al. [45]).

To our knowledge, neither *GST* polymorphism has been examined in relation to the risk of benign fibrocystic breast conditions. Although the etiology of these conditions is not well understood, women with proliferative lesions of the breast with or without atypia have a greater risk of developing breast cancer than women with non-proliferative lesions (Dupontand Page [47];Carter et al. [48];London et al. [49];Hartmann et al. [50]). Therefore, by also investigating whether either *GST* polymorphism is associated with fibrocystic breast conditions, particularly those characterized as proliferative, insight may be gained into whether GST-specific activity is influential early in mammary carcinogenesis.

In a case-control study of Chinese women enrolled in a randomized trial of breast self-examination, we examined whether the *GSTM1* deletion and *GSTP1* Ile105Val polymorphisms are associated with the risk of breast cancer and fibrocystic breast conditions, and whether these associations differ by either menopausal status or presence of proliferation in the extra-tumoral epithelium or benign lesions. We also explored whether the associations between *GST* genotype and risk of breast cancer and fibrocystic breast conditions varied by level of fruit or vegetable intake.

MATERIAL AND METHODS

Study Population and Setting

Participants of this case-control study were selected from the 266,064 current or retired female employees of the Shanghai Textile Industry Bureau, born between 1925 and 1958, who had been enrolled in a randomized trial of breast self-examination (BSE) in Shanghai, China, from October 1989 to October 1991. Details of this trial have been described elsewhere (Thomas et al. [51];Thomas et al. [52]). Briefly, at enrollment, a trained factory medical worker administered a baseline questionnaire to each participant. Participants were subsequently followed for vital status, continued employment in the textile industry, residence in Shanghai, and development of benign or malignant breast conditions until July 31, 2000. Within the follow-up period, those who reported a suspicious breast lump and other symptoms indicative of breast cancer to a factory medical worker were referred for further evaluation and treatment to one of the three major hospitals operated by the Shanghai Textile Industry Bureau or to other hospitals that had contractual agreements with individual factories. Active case finding procedures were supplemented by manual review of Shanghai Textile Industry Bureau cancer and death registry records and Shanghai Cancer Registry records, as well as by electronic linkage of the cohort to the Shanghai Cancer Registry. For all women with a histologically confirmed breast lesion, standardized review of pathology reports and other medical records was performed to ascertain information on lesion size and histologic classification. Histologic slides were also obtained for standardized review.

The present study was limited to those participants involved in case-control studies of benign and malignant breast conditions that were conducted during the course of the trial. Participants of these ancillary studies were re-interviewed in person, using a general risk factor questionnaire to elicit information on demographic characteristics, reproductive and gynecologic history, smoking and alcohol use, medical history, family history of breast cancer, and occupational and recreational physical activity, as well as a validated food frequency

questionnaire to elicit information on diet. At the time of interview, they were also asked to donate a blood sample.

Eligible cases, defined as women who had a breast biopsy and were diagnosed with either a fibrocystic breast condition or breast cancer, were selected from studies of mammary epithelial cell proliferation and breast cancer conducted between September 1995 and July 2000 (Wu et al. [53];Li et al. [54];Shannon et al. [55]). In these studies, 551 (89%) of the 622 eligible women with fibrocystic conditions and 376 (88%) of the 426 eligible women with breast cancer were interviewed. Of those interviewed, 470 (85%) women with fibrocystic conditions and 323 (86%) women with breast cancer provided a blood sample. Additionally, women with breast cancer diagnosed from November 1989 to September 1995 were selected from a study of *BRCA1* and *BRCA2* mutations, which was designed to retrospectively recruit diagnosed cases of breast cancer under age 45, along with an equal number of older cases (Suter et al. [56]). Retrospective recruitment was possible because in the BSE cohort, the average probability of survival from breast cancer for six years after diagnosis was greater than 80% (Thomas et al. [52]). Of the 830 women in the trial who developed breast cancer prior to September 1995, 153 (57%) of the 270 cases under age 45 and 147 (26%) of the 560 cases age 45 and older were interviewed and provided a blood sample. These retrospectively recruited cases were similar to all cases in the trial cohort on their baseline distributions of known risk factors for breast cancer, including parity, age at first birth, age at menarche, breastfeeding history, history of a breast lump, and family history of breast cancer. The food frequency questionnaire was not administered to this set of cases.

Eligible controls were defined as women in the trial who did not have a breast biopsy. For each benign or malignant case diagnosed between September 1995 and August 1997, a list of 20 randomly selected controls of the same age was generated, and these potential controls were contacted in the order listed until two of the same age and menstrual status as the case were successfully recruited. Using this approach, 367 controls (64% of those contacted) were interviewed. For each benign or malignant case diagnosed between September 1997 and July 2000, controls were randomly selected by frequency matching to cases on age (in 5-year groups) and hospital affiliation of their factories at baseline. Using this approach, 704 controls (82% of those selected) were interviewed. Of the 1,071 interviewed controls in total, 911 (85%) provided a blood sample. Controls were interviewed and had their blood drawn by the same study personnel as cases.

Included in the present investigation were the 615 women with breast cancer, 467 women with fibrocystic breast conditions, and 878 women without clinical breast disease who were interviewed and for whom DNA was available for genotyping of the *GSTM1* and *GSTP1* polymorphisms. Reasons for excluding 43 women (8 with breast cancer, 3 with fibrocystic breast conditions, and 32 controls) who were interviewed and provided a blood sample in the ancillary studies were evidence of sample contamination and mismatch of genotyping results from paired buffy coat and whole blood aliquot DNA samples. An additional control lacked sufficient DNA for the genotyping of both polymorphisms. For three women, sufficient DNA was available for the genotyping of the *GSTM1* polymorphism only. Informed consent was obtained from each woman prior to interview. The study was approved by the Institutional Review Board of the Fred Hutchinson Cancer Research Center, the Station for the Prevention and Treatment of Cancer of the Shanghai Textile Industry Bureau in accordance with assurance filed with the Office of Human Research Protections of the U.S. Department of Health and Human Services, and the Human Genetics Resources Administration of China.

Histologic Classification of Breast Tissue

Benign and malignant breast conditions diagnosed from September 1995 to July 2000 were histologically confirmed by a single reference pathologist who had no knowledge of the

original diagnosis. For women who had sufficient benign mammary gland tissue, either in benign biopsy or in extra-tumoral areas, benign changes were classified according to the scheme developed by Stalsberg (Aaman et al. [57]), which is similar to Page's classification for benign breast disease (Dupontand Page [47]). The minimum requirement for classification was five scanning power fields of benign mammary gland tissue, excluding areas of fibroadenoma. Benign changes, except fibroadenomas, were classified into one of three different types: atypical ductal hyperplasia, atypical lobular hyperplasia, or moderate apocrine atypia were classified as atypia; either moderate or florid ductal hyperplasia or moderate or predominant sclerosing adenosis and no atypia were classified as proliferative changes; and mild or no ductal hyperplasia and mild or no sclerosing adenosis were classified as non-proliferative changes. In the present study, 337 (72%) of the 467 women with fibrocystic conditions and 241 (75%) of the 320 women with breast cancer, who were diagnosed since September 1995, had sufficient tissue available for classification.

Genotyping Assays

Genomic DNA was isolated from buffy coat using a salt-precipitation (Miller et al. [58]) or a phenol-chloroform method (Suter et al. [56];Bell et al. [59];Maniatis et al. [60]), or from whole blood (red cell solution) using the QIAamp® DNA Blood Midi Kit (Qiagen, Alameda, CA). DNA concentration was determined by UV spectrophotometry.

Genotypes for *GSTM1* were determined according to Chen *et al.*(Chen et al. [61]) To verify that the null genotypes were due to a deletion of *GSTM1* alleles, and not PCR failure, human *β-globin* was co-amplified in each PCR (Bell et al. [62]). We included PCR without DNA template as a negative control and in-house genomic DNA samples of known genotypes as positive controls. A 20 uL reaction was used, consisting of 25 ng genomic DNA, 1× *Taq* PCR Master Mix (containing *Taq* DNA polymerase and a final concentration of 1× Qiagen PCR buffer, 1.5 mM $MgCl_2$, 200 μM of each dNTP; Qiagen), a final concentration of 450 nM of each forward ('5-GAA CTC CCT GAA AAG CTA AAG C) and reverse (5'-GTT GGG CTC AAA TAT ACG GTG G) *GSTM1* primer(Bell et al. [62]) (Qiagen) and 3 nM of each forward (5'- CAA CTT CAT CCA CGT TCA CC) and reverse (5'-GAA GAG CCA AGG ACA GGT AC) *β-globin* primer(Bell et al. [62]) (Qiagen). PCRs were performed in an MJ Research Thermal Cycler PTC-100. Reaction conditions were initial denaturation at 94°C, 5 min, followed by 40 cycles of 94°C, 1 min; 60°C, 1 min; 72°C, 2 min, with a final extension of 72° C, 5 min. PCR product sizes were verified by electrophoresis using a 3% NuSieve high-resolution agarose gel (ISC Bioexpress, Kaysville, UT).

SNaPshot™ (Applied Biosystems (ABI), Foster City, CA) assays were used to determine *GSTP1* genotypes. Genomic DNA was amplified for the polymorphic site in a multi-plexed PCR with *COMT*, *CYP1A2*, and ER polymorphisms. For each assay, a reaction without DNA template was included as a negative control and in-house genomic DNA samples of known genotypes as positive controls. A 20 uL reaction contained 100 ng genomic DNA, a final concentration of 1× *Taq* PCR Master Mix (Qiagen), and 210 nM of each forward (5'-ACC CCA GGG CTC TAT GGG AA) and reverse (5'-TGA GGG CAC AAG AAG CCC CT) *GSTP1* PCR primer (Harries et al. [39]) (Qiagen). Reactions were performed in a GeneAmp® PCR System 9700 (ABI). Reaction conditions were initial denaturation at 94°C, 5 min, followed by 40 cycles of 94°C, 45 s; 60°C, 45 s; 72°C, 30 s, with a final extension of 72°C, 5 min. PCR performance was verified by agarose gel electrophoresis (1% agarose, ISC Bioexpress). Products were purified with a SAP/*Exo*I (SAP: Amersham/Pharmacia, Piscataway, NJ; *Exo*I: New England Biolabs, Beverly, MA) treatment to remove unincorporated dNTPs and primers, respectively, according to the ABI Prism® SnaPshot™ Kit instructions. Thermal cycling was also conducted according to the SNaPshot™ Kit using a GeneAmp® PCR System 9700 (ABI). A final concentration of 0.125 μM of the *GSTP1*

SnaPshot primer (5'-$(GATC)_4$GTC ACA TAG TTG GTG TAG ATG AGG GAG A) was used in each reaction. After thermal cycling, unincorporated [F]ddNTPs were removed using SAP as per the SNaPshot™ protocol. The products were then mixed with HiDi formamide (ABI) and GeneScan™-120 LIZ™ Size Standard (ABI) and electrophoresed on an ABI Prism® 3100 Genetic Analyzer according to the SNaPshot™ protocol. Resulting data were analyzed using ABI GeneScan™ 3.7 and GeneMapper™ 2.0 genotyping software.

Statistical Analysis

Individual matching on age was not retained in the analyses, and cases of breast cancer or fibrocystic conditions were each compared to the full set of controls in all analyses. Frequencies of selected characteristics were adjusted to the age distribution of controls using direct methods (Szkloand Nieto [63]). Age-adjusted odds ratios (OR) and 95% confidence intervals (CI) were estimated using unconditional logistic regression to assess the risk of fibrocystic breast conditions and breast cancer associated with *GSTM1* and *GSTP1* genotypes. *GSTM1* and *GSTP1* genotypes were examined separately and jointly in relation to each disease outcome. These analyses were repeated, stratifying on menopausal status and on the presence or absence of proliferation in the fibrocystic lesion(s) (for women with fibrocystic conditions) or in the extra-tumoral epithelium (for women with breast cancer). In these analyses, proliferation was defined as the presence of either atypia or proliferative changes.

For the subgroup of women with dietary data included in our study, greater consumption of fruits and vegetables has been associated with decreased risk of fibrocystic breast conditions (Wu et al. [53]) and breast cancer (Shannon et al. [55]). To explore whether diet influences the relationship between *GSTM1* or *GSTP1* genotype and risk of either fibrocystic breast conditions or breast cancer, analyses stratified on intake level of fruits, vegetables, and related botanical groups were conducted using conditional logistic regression. Intake levels were based on the quartile distribution of each food or botanical group among controls. The general fruit group included lychee, persimmon, pineapple, and grapes, along with fruits in the following botanical groups: *Cucurbitaceae* (watermelon); *Musaceae* (bananas), *Rosaceae* (apples, apricots, peaches, pears); and *Rutaceae* (oranges, tangerines). The general vegetable group included spinach, wild rice stem, lettuce, yellow sweet potatoes (or yams), mushrooms, bamboo shoots, lotus rhizomes, taro root, corn, and seaweed, along with vegetables in the following botanical groups: *Cruciferae* (bok choy, cabbage, Chinese cabbage, watercress, broccoli, Chinese broccoli, cauliflower, radish or turnip); *Cucurbitaceae* (winter squash, wax gourd, gherkin, pumpkin); *Liliaceae* (asparagus, garlic, garlic stalk, onions, chives, scallions, Chinese chives); *Solanaceae* (eggplant, other potatoes, tomato, hot pepper, red or green pepper); and *Umbelliferae* (celery, carrots). To control for possible population differences in diet over time, analyses were conditioned on year of interview. Analyses examining fruit and vegetable intake were further adjusted for age and total energy intake, while those examining botanical groups were further adjusted for age and total fruit and vegetable intake. Confounding by other variables was assessed by examining whether their inclusion in the models altered risk estimates of the main independent variable by 10% or more. Effect modification was assessed by adding a cross-product (interaction) term between genotype and intake level to the model including their main effects. All analyses were performed using SAS 9.1 (SAS Institute, Inc., Cary, NC).

RESULTS

The women with breast cancer were somewhat older than controls, whereas the women with fibrocystic conditions tended to be younger. Age-adjusted distributions of selected characteristics for cases of breast cancer, cases of fibrocystic conditions, and controls are presented in Table 1. Compared to controls, the women with breast cancer were more likely

to be nulliparous, post-menopausal, have a prior history of breast lumps, and have a family history of breast cancer; the women with fibrocystic conditions were more likely to be pre-menopausal, have a prior history of breast lumps, and have a family history of breast cancer.

Genotyping of the *GSTM1* and *GSTP1* polymorphisms was successfully conducted, with respective call rates of 100% and 99.8%. Genotype frequencies of both polymorphisms were consistent with Hardy-Weinberg equilibrium. Observed frequencies were also similar to those previously reported for Chinese populations (Shen et al. [64];Zhong et al. [65]). Among controls, the frequency of the *GSTM1* null genotype was 49% and the frequency of the *GSTP1* Val allele was 19%.

GSTM1 and *GSTP1* genotypes were not associated with risk of either breast cancer or fibrocystic breast conditions (Table 2). There was some suggestion of a modest increase in breast cancer risk, however, for women with the *GSTM1* null genotype and at least one *GSTP1* Val allele (OR 1.33, 95% CI, 0.99–1.80). No striking differences in risk were observed according to menopausal status.

As shown in Table 3, risk of breast cancer associated with the *GSTM1* null (vs. non-null) genotype appeared to be slightly higher among women with than without proliferative elements in the extra-tumoral epithelium. In addition, risk of breast cancer in women carrying both the *GSTM1* null genotype and at least one *GSTP1* Val allele, relative to women carrying the *GSTM1* non-null and *GSTP1* Ile/Ile genotypes, was somewhat greater in women with concurrent proliferation in the extra-tumoral epithelium (OR=1.77, 95% CI, 1.03–3.04) than in women without concurrent proliferation (OR=1.40, 95% CI, 0.83–2.36), although this difference was not statistically significant. *GSTM1* and *GSTP1* genotypes, either individually or in combination, did not appear to be associated with risk of fibrocystic conditions, regardless of proliferation status.

The relationships between *GST* genotype and risk of breast cancer or fibrocystic breast conditions did not vary across intake levels of fruits or vegetables (Table 4). Likewise, the risk estimates for breast cancer or fibrocystic breast conditions associated with *GST* genotype did not differ across intake levels of all botanical groups studied (data not shown), including *Cruciferae* (Table 4).

DISCUSSION

In this case-control study of Chinese women, we found no association of either the *GSTM* deletion or *GSTP1* Ile105Val polymorphism with the risk of breast cancer or fibrocystic breast conditions among pre- or post-menopausal women. However, when the *GSTM1* and *GSTP1* genotypes were examined in combination, there was some suggestion of a slight increase in breast cancer risk for women carrying the *GSTM1* null genotype and at least one *GSTP1* Val allele. This association was somewhat stronger in the subset of women who had breast cancer with concurrent extra-tumoral epithelial proliferation.

Our results are fairly consistent with those of most prior studies examining the risk of breast cancer associated with both *GST* polymorphisms, the majority of which have been conducted in Caucasian populations. Of the few studies that have been conducted in Korea (Park et al. [18];Park et al. [28]), China (Egan et al. [30]), and Taiwan (Cheng et al. [33];Wu et al. [34]), none have found an overall association between the *GSTM1* polymorphism and breast cancer risk, although those in Korea have found an increase in risk associated with carriage of the null genotype among pre-menopausal women. Of the two Asian studies that have examined breast cancer risk associated with the *GSTP1* Ile105Val polymorphism, one Korean study detected no overall association (Kim et al. [41]). The other, conducted in Shanghai (like the present investigation) including 1,144 cases and 1,221 controls, found women with the *GSTP1* Val/

Val genotype to be at an almost two-fold increased risk (Egan et al. [30]). Lower erythrocyte GST π activity has been correlated with carriage of the *GSTP1* Val/Val genotype in healthy Chinese individuals (Zhong et al. [65]), supporting the plausibility of this relationship. Therefore, our observed lack of a positive association between the *GSTP1* Val/Val genotype and breast cancer risk could be attributed to limited statistical power, particularly since this genotype is rare in the Shanghai population (~3%).

Although these individual *GSTM1* and *GSTP1* polymorphisms were not associated with breast cancer risk, the putative at-risk genotypes of these polymorphisms in combination appeared to be associated with a slight elevation in risk. Since GST enzymes are able to target similar substrates, it may be important to study the combined effect of the *GST* genes (and possibly even other genes involved in carcinogen metabolism). While the few studies evaluating the joint effects of *GSTM1*, *GSTP1*, and often *GSTT1* genotypes on breast cancer risk have yielded inconsistent results (Curran et al. [16];Egan et al. [30];Gudmundsdottir et al. [22];Helzlsouer et al. [35];Millikan et al. [17];Mitrunen et al. [20];Vogl et al. [29];Spurdle et al. [42]), several (including the study conducted in Shanghai) have suggested a modest increase in risk of breast cancer associated with carriage of the *GSTM1* null and *GSTP1* Ile/Val or Val/Val genotypes, in combination with the *GSTT1* non-null genotype (Egan et al. [30];Gudmundsdottir et al. [22];Helzlsouer et al. [35]). Joint carriage of the *GSTM1* null genotype and *GSTP1* Val allele has also been associated with significantly lower GST activity in Chinese individuals (Zhong et al. [65]). Larger studies are required, however, to more thoroughly evaluate and establish whether combinations of *GST* genotypes can distinguish women at risk for breast cancer.

In this study, we did not find any clear differences in the association between *GST* genotype and risk of breast cancer or fibrocystic breast conditions by intake level for various foods and botanicals. Overall, the evidence from epidemiologic studies examining the hypothesis of a biological interaction between GST activity and diet in relation to risk of breast cancer has been limited. In a study conducted in Shanghai, inverse associations between urinary ITC levels and breast cancer risk were found, particularly in women carrying the *GSTM1* or *GSTT1* null (as opposed to the non-null) genotype, although the interactions were not statistically significant (Fowke et al. [45]). In studies of Caucasian women, no interactions between dietary intake of cruciferous vegetables or antioxidants, *GST* genotype, and breast cancer risk were observed (Ambrosone et al. [46];Ambrosone et al. [13]).

Any true modest risk of breast cancer or fibrocystic breast conditions associated with these *GST* polymorphisms could be obscured if occurring only in the presence of a specific exposure. The present investigation was somewhat limited in its capacity to assess possible gene-environment interactions.

For example, although findings of a recent meta-analysis have suggested that only *GSTM1* null carriers are at slightly increased risk of breast cancer due to smoking (Terryand Goodman [36]), we were unable to examine if risk related to smoking is modified by *GSTM1* genotype, given the extremely low prevalence of smoking in our study population (<3%). Similarly, a large proportion of women included in this study were pre-menopausal, thereby reducing our ability to detect associations confined to postmenopausal women. However, only a few studies have reported a relationship between the *GSTM1* null genotype and post-menopausal breast cancer risk (Helzlsouer et al. [35];Charrier et al. [12];Mitrunen et al. [20]), and there is little evidence that the risk of breast cancer associated with the *GSTP1* Ile105Val variant differs by menopausal status (Egan et al. [30]).

Additional limitations of the study should be considered in interpreting our results. While the diagnosis of all cases was confirmed by biopsy, only for cases diagnosed after September 1995 did we attempt to further classify lesions by proliferation status. Sufficient tissue for further

classification was available for 72% of women diagnosed with fibrocystic breast conditions and 75% of women diagnosed with breast cancer, but this represented only 39% of the total number of breast cancer cases included in our investigation. As a result, there was limited statistical power to examine differences in risk according to the presence or absence of concurrent proliferation. Also with the small amount of tissue obtained by biopsy, even with the requirement of having five scanning fields, the histology of benign changes was likely misclassified in some patients because of the focal distribution of such changes in the breast, thus resulting in risk estimates biased closer to the null.

Nonetheless, we had the unique opportunity to assess whether *GSTM1* and *GSTP1* genotypes are associated with the risk of benign and malignant breast conditions and to evaluate whether these associations vary by proliferation status in a racially homogeneous population. The lack of association between *GST* genotypes and risk of fibrocystic breast conditions suggest that *GST* μ and π activity is not influential in the development of these benign conditions, although no prior studies have been conducted to corroborate our findings. The modest association observed between the combined *GST* genotypes and breast cancer risk, particularly in the subset of women with concurrent proliferation in the adjacent non-cancerous epithelium, suggests that reduced GST μ and π activity plays some role in the development of mammary tumors, presumably through less effective carcinogen inactivation.

Although information on hormone receptor status was not available in our study, assessing breast cancer risk associated with these *GST* genotypes by estrogen-receptor (ER) and progesterone-receptor (PR) status in future studies may be useful in clarifying whether GST activity influences breast cancer. Some, but not all studies examining the relationship between GST π activity and hormone receptor status in human breast carcinoma have found increased GST π expression in ER-negative and/or PR-negative tumors (Howie et al. [66];Forrester et al. [3];Gilbert et al. [67]). It is less well-established, however, whether GST μ expression is related to hormone receptor status.

In conclusion, *GSTM1* and *GSTP1* polymorphisms do not appear to individually influence susceptibility for breast cancer or fibrocystic breast conditions. The suggestive increase in risk of breast cancer associated with joint carriage of the *GSTM1* null genotype and *GSTP1* 105-Val allele, particularly among women with concurrent proliferation in the adjacent non-tumor tissue, needs to be confirmed in other studies.

Abbreviations

GST, glutathione *S*-transferase; BSE, breast self examination; PCR, polymerase chain reaction; OR, odds ratio; CI, confidence interval; ITC, isothiocynates.

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Table 1

Selected characteristics for controls and cases of breast cancer and fibrocystic breast conditions[a]

	Controls (n=879)[b]		Breast Cancer (n=615)		Fibrocystic Conditions (n=467)	
Characteristic/Factor	**n**	**(%)**	**n**	**(%)**	**n**	**(%)**
Age (years)						
< 40	12	(1.4)	78	(12.7)	62	(13.3)
40–44	376	(42.8)	169	(27.5)	200	(42.8)
45–49	171	(19.5)	97	(15.8)	123	(26.3)
50–54	64	(7.3)	40	(6.5)	22	(4.7)
55–59	38	(4.3)	47	(7.6)	12	(2.6)
60–64	106	(12.1)	99	(16.1)	15	(3.2)
≥ 65	112	(12.7)	85	(13.8)	33	(7.1)
Number of live births						
None	36	(4.1)	37	(6.2)	23	(4.9)
1	560	(64.0)	319	(57.3)	362	(63.9)
2	110	(12.6)	105	(16.2)	37	(11.3)
≥ 3	169	(19.3)	152	(20.3)	40	(19.9)
Age at menopause (years)						
Pre-menopausal	529	(60.2)	294	(53.1)	375	(64.3)
< 45	61	(6.9)	64	(10.9)	20	(5.6)
45–49	127	(14.5)	117	(17.7)	35	(12.7)
≥ 50	162	(18.4)	140	(18.3)	37	(17.4)
Ever smoked						
No	858	(97.6)	594	(97.5)	460	(98.7)
Yes	21	(2.4)	19	(2.5)	5	(1.3)
Years lived with smoking spouse						
None	324	(37.1)	217	(36.7)	157	(36.6)
1–15	249	(28.5)	184	(30.3)	167	(28.6)
16–20	156	(17.8)	75	(15.6)	89	(19.2)
≥ 21	145	(16.6)	128	(17.4)	45	(15.6)

	Controls		Breast Cancer		Fibrocystic Conditions	
	(n=879)[b]		(n=615)		(n=467)	
Characteristic/Factor	**n**	**(%)**	**n**	**(%)**	**n**	**(%)**
Prior breast lumps						
Never	828	(96.6)	549	(93.4)	395	(89.4)
Ever	29	(3.4)	38	(6.6)	50	(10.6)
First degree relative with breast cancer						
No	864	(98.3)	587	(95.8)	448	(96.5)
Yes	15	(1.7)	28	(4.2)	19	(3.5)

[a] For all characteristics except age, the direct age-adjusted percentages based on the age distribution of controls are presented for cases of breast cancer and fibrocystic conditions

[b] Includes one woman for whom sufficient DNA was unavailable for the genotyping of *GSTM1* and *GSTP1* polymorphisms

Table 2

Age-adjusted ORs and 95% CIs for *GSTM1* and *GSTP1* genotypes[a] (separate and combined) and the risk of breast cancer and fibrocystic conditions

		Controls		Breast Cancer				Fibrocystic Conditions			
Overall		**n**	**(%)**	**n**	**(%)**	**OR**	**(95% CI)**	**n**	**(%)**	**OR**	**(95% CI)**
GSTM1											
Not Null		450	(51)	294	(48)	1.00	(Referent)	223	(48)	1.00	(Referent)
Null		428	(49)	321	(52)	1.15	(0.94, 1.41)	244	(52)	1.11	(0.88, 1.39)
GSTP1											
Ile/Ile		569	(65)	378	(62)	1.00	(Referent)	313	(67)	1.00	(Referent)
≥1 Val		307	(35)	235	(38)	1.15	(0.93, 1.42)	152	(33)	0.93	(0.73, 1.19)
Ile/Val		277	(32)	215	(35)	1.17	(0.93, 1.45)	134	(29)	0.91	(0.71, 1.17)
Val/Val		30	(3)	20	(3)	1.00	(0.56, 1.79)	18	(4)	1.12	(0.61, 2.08)
GSTM1	*GSTP1*										
Not Null	Ile/Ile	293	(33)	183	(30)	1.00	(Referent)	144	(31)	1.00	(Referent)
Null	Ile/Ile	276	(32)	195	(32)	1.14	(0.88, 1.47)	169	(36)	1.20	(0.90, 1.59)
Not Null	≥1 Val	157	(18)	110	(18)	1.12	(0.83, 1.52)	78	(17)	1.04	(0.74, 1.47)
Null	≥1 Val	150	(17)	125	(20)	1.33	(0.99, 1.80)	74	(16)	1.00	(0.70, 1.41)
Premenopausal		n	(%)	n	(%)	OR	(95% CI)	n	(%)	OR	(95% CI)
GSTM1											
Not Null		265	(50)	141	(48)	1.00	(Referent)	174	(46)	1.00	(Referent)
Null		263	(50)	153	(52)	1.03	(0.77, 1.38)	201	(54)	1.14	(0.88, 1.49)
GSTP1											
Ile/Ile		353	(67)	181	(62)	1.00	(Referent)	251	(67)	1.00	(Referent)
≥1 Val		174	(33)	111	(38)	1.18	(0.87, 1.60)	122	(33)	0.97	(0.73, 1.29)
Ile/Val		156	(30)	100	(34)	1.20	(0.87, 1.64)	108	(29)	0.96	(0.71, 1.29)
Val/Val		18	(3)	11	(4)	1.04	(0.47, 2.29)	14	(4)	1.05	(0.51, 2.16)
GSTM1	*GSTP1*										
Not Null	Ile/Ile	180	(34)	89	(30)	1.00	(Referent)	114	(30)	1.00	(Referent)

		Controls		Breast Cancer				Fibrocystic Conditions			
Overall		**n**	**(%)**	**n**	**(%)**	**OR**	**(95% CI)**	**n**	**(%)**	**OR**	**(95% CI)**
Null	Ile/Ile	173	(33)	92	(32)	1.02	(0.71, 1.47)	137	(37)	1.21	(0.88, 1.68)
Not Null	≥1 Val	85	(16)	51	(17)	1.16	(0.75, 1.80)	59	(16)	1.06	(0.70, 1.59)
Null	≥1 Val	89	(17)	60	(21)	1.22	(0.80, 1.86)	63	(17)	1.09	(0.73, 1.62)
Postmenopausal		n	(%)	n	(%)	OR	(95% CI)	n	(%)	OR	(95% CI)
GSTM1											
Not Null		185	(53)	153	(48)	1.00	(Referent)	49	(53)	1.00	(Referent)
Null		165	(47)	168	(52)	1.23	(0.91, 1.67)	43	(47)	0.98	(0.62 1.55)
GSTP1											
Ile/Ile		216	(62)	197	(61)	1.00	(Referent)	62	(67)	1.00	(Referent)
≥1 Val		133	(38)	124	(39)	1.04	(0.76, 1.42)	30	(33)	0.80	(0.49, 1.30)
Ile/Val		121	(35)	115	(36)	1.05	(0.76, 1.45)	26	(28)	0.76	(0.46, 1.27)
Val/Val		12	(3)	9	(3)	0.87	(0.36, 2.12)	4	(4)	1.22	(0.38, 3.93)
GSTM1	*GSTP1*										
Not Null	Ile/Ile	113	(32)	94	(29)	1.00	(Referent)	30	(33)	1.00	(Referent)
Null	Ile/Ile	103	(30)	103	(32)	1.18	(0.80, 1.74)	32	(35)	1.15	(0.65, 2.03)
Not Null	≥1 Val	72	(21)	59	(18)	0.97	(0.63, 1.51)	19	(21)	1.00	(0.52, 1.90)
Null	≥1 Val	61	(17)	65	(20)	1.30	(0.84, 2.04)	11	(12)	0.69	(0.32, 1.48)

[a]Excludes individuals with missing genotypes

Table 3

Age-adjusted ORs and 95% CIs for *GSTM1* and *GSTP1* genotypes[a] (separate and combined) and risk of breast cancer and fibrocystic conditions, by proliferation status

	Controls		Breast Cancer								Fibrocystic Conditions							
			w/o concurrent proliferation				w/ concurrent proliferation				non-proliferative				proliferative			
	(n=878)		(n=125)				(n=116)				(n=154)				(n=183)			
	n	(%)	n	(%)	OR	(95% CI)	n	(%)	OR	(95% CI)	n	(%)	OR	(95% CI)	n	(%)	OR	(95% CI)
GSTM1																		
Not Null	450	(51)	56	(45)	1.00	(Referent)	49	(42)	1.00	(Referent)	70	(45)	1.00	(Referent)	87	(47)	1.00	(Referent)
Null	428	(49)	69	(55)	1.31	(0.90, 1.91)	67	(58)	1.45	(0.98, 2.15)	84	(55)	1.20	(0.85, 1.70)	96	(53)	1.12	(0.81, 1.54)
GSTP1																		
Ile/Ile	569	(65)	80	(64)	1.00	(Referent)	70	(60)	1.00	(Referent)	111	(72)	1.00	(Referent)	120	(66)	1.00	(Referent)
≥1 Val	307	(35)	45	(36)	1.03	(0.69, 1.52)	46	(40)	1.21	(0.81, 1.80)	43	(28)	0.75	(0.51, 1.10)	62	(34)	0.99	(0.70, 1.39)
Ile/Val	277	(32)	41 4	(33)	1.04	(0.69, 1.55)	41	(35)	1.19	(0.79, 1.80)	39	(25)	0.76	(0.51, 1.13)	52	(28)	0.92	(0.64, 1.32)
Val/Val	30	(3)	4	(3)	0.92	(0.31, 2.68)	5	(3)	1.33	(0.50, 3.55)	4	(3)	0.70	(0.24, 2.06)	10	(5)	1.60	(0.75, 3.41)
	(n=876)		(n=125)				(n=116)				(n=154)				(n=182)			
	n	(%)	n	(%)	OR	(95% CI)	n	(%)	OR	(95% CI)	n	(%)	OR	(95% CI)	n	(%)	OR	(95% CI)
GSTM1 *GSTP1*																		
Not Null Ile/Ile	293	(33)	39	(31)	1.00	(Referent)	32	(28)	1.00	(Referent)	48	(31)	1.00	(Referent)	60	(33)	1.00	(Referent)
Null Ile/Ile	276	(32)	41	(33)	1.13	(0.71, 1.81)	38	(33)	1.28	(0.77, 2.10)	63	(41)	1.32	(0.87, 2.00)	60	(33)	1.01	(0.68, 1.51)
Not Null ≥1Val	157	(18)	17	(14)	0.80	(0.44, 1.46)	17	(15)	0.98	(0.53, 1.83)	22	(14)	0.89	(0.51, 1.53)	26	(14)	0.83	(0.50, 1.37)
Null ≥1Val	150	(17)	28	(22)	1.40	(0.83, 2.36)	29	(25)	1.77	(1.03, 3.04)	21	(14)	0.85	(0.49, 1.49)	36	(20)	1.17	(0.73, 1.85)

[a] Excludes individuals with missing genotypes

Table 4

OR and 95% CIs[a] for *GSTM1* and *GSTP1* genotypes and risk of breast cancer or fibrocystic conditions, in relation to intake of fruits, vegetables, and cruciferous vegetables

		***GSTM1* genotype**												
		Controls		**Breast Cancer**						**Fibrocystic Conditions**				
Food		**Not Null**	**Null**	**Not Null**			**Null**			**Not Null**			**Null**	
Times/year	**n**	**n**	**n**	**OR**	**(95% CI)**	**n**	**OR**	**(95% CI)**	**N**	**OR**	**(95% CI)**	**N**	**OR**	**(95% CI)**
Fruits														
<202	118	109	44	1.00	(Referent)	54	0.93	(0.47–1.85)	60	1.00	(Referent)	67	0.90	(0.47–1.71)
202–306	101	99	40	1.00	(Referent)	50	1.63	(0.71–3.73)	60	1.00	(Referent)	70	1.43	(0.68–2.99)
>306–435	113	102	43	1.00	(Referent)	49	0.91	(0.46–1.81)	50	1.00	(Referent)	50	0.68	(0.33–1.42)
>435	117	118	57	1.00	(Referent)	50	0.67	(0.38–1.17)	52	1.00	(Referent)	57	0.95	(0.53–1.70)
					P interaction = 0.15						P interaction = 0.69			
Vegetables														
<538	103	118	45	1.00	(Referent)	73	0.99	(0.51–1.92)	75	1.00	(Referent)	89	0.86	(0.46–60)
538–735	104	101	41	1.00	(Referent)	38	0.87	(0.44–1.73)	43	1.00	(Referent)	46	0.93	(0.45–1.94)
>735–956	119	100	39	1.00	(Referent)	31	0.59	(0.27–1.29)	48	1.00	(Referent)	44	0.56	(0.26–1.21)
>956	123	109	59	1.00	(Referent)	61	1.04	(0.60–1.78)	56	1.00	(Referent)	65	1.46	(0.83–2.58)
					P interaction = 0.63						P interaction = 0.47			
Cruciferae														
<162	91	128	38	1.00	(Referent)	55	0.75	(0.35–1.60)	61	1.00	(Referent)	74	0.91	(0.45–1.86)
162–251	105	105	45	1.00	(Referent)	56	0.99	(0.50–1.94)	60	1.00	(Referent)	63	0.78	(0.39–1.54)
>251–377	125	94	41	1.00	(Referent)	55	1.31	(0.70–2.46)	58	1.00	(Referent)	63	1.00	(0.54–1.84)
>377	128	101	60	1.00	(Referent)	37	0.55	(0.29–1.02)	43	1.00	(Referent)	44	1.07	(0.57–2.02)
					P interaction = 0.26						P interaction = 0.98			

	GSTP1 genotype												
Food	**Ile/Ile**	**≥1 Val**	**Ile/Ile**			**≥1 Val**			**Ile/Ile**			**≥1 Val**		
Times/year	**n**	**n**	**n**	**OR**	**(95% CI)**	**n**	**OR**	**(95% CI)**	**N**	**OR**	**(95% CI)**	**N**	**OR**	**(95% CI)**
Fruits														
<202	145	82	61	1.00	(Referent)	36	0.89	(0.44–1.82)	84	1.00	(Referent)	43	1.09	(0.55–2.15)
202–306	135	65	53	1.00	(Referent)	36	0.72	(0.31–1.67)	90	1.00	(Referent)	39	0.38	(0.16–0.89)
>306–435	138	76	59	1.00	(Referent)	33	0.87	(0.43–1.75)	70	1.00	(Referent)	30	0.52	(0.24–1.13)
>435	150	84	64	1.00	(Referent)	43	1.03	(0.59–1.80)	69	1.00	(Referent)	39	0.74	(0.41–1.34)
					P interaction = 0.95						P interaction = 0.24			
Vegetables														
<538	136	84	70	1.00	(Referent)	48	1.16	(0.59–2.29)	111	1.00	(Referent)	52	0.72	(0.37–1.40)
538–735	134	71	52	1.00	(Referent)	26	0.70	(0.34–1.47)	59	1.00	(Referent)	30	0.69	(0.31–1.52)
>735–956	152	67	37	1.00	(Referent)	32	1.27	(0.58–2.78)	64	1.00	(Referent)	28	0.53	(0.23–1.21)
>956	146	85	78	1.00	(Referent)	42	0.78	(0.44–1.37)	79	1.00	(Referent)	41	0.77	(0.43–1.37)
					P interaction = 0.47						P interaction = 0.90			
Cruciferae														
<162	142	77	55	1.00	(Referent)	38	0.79	(0.36–1.73)	92	1.00	(Referent)	43	0.39	(0.18–0.86)
162–251	127	82	61	1.00	(Referent)	39	0.89	(0.45–1.78)	78	1.00	(Referent)	45	1.12	(0.55–2.29)
>251–377	145	74	57	1.00	(Referent)	39	1.14	(0.60–2.19)	81	1.00	(Referent)	39	0.81	(0.42–1.55)
>377	154	74	64	1.00	(Referent)	32	0.76	(0.40–1.43)	62	1.00	(Referent)	24	0.55	(0.28–1.11)
					P interaction = 0.65						P interaction = 0.34			

[a]All analyses conditioned on year of interview. Analyses of fruit and vegetable intake further adjusted for age and energy, while analyses of *Cruciferae* (and other botanical groups, not shown) further adjusted for age and total fruit and vegetable intake.