Intake of total cruciferous vegetable and its contents of glucosinolates and isothiocyanates, *GST* polymorphisms, and breast cancer risk: a case-control study in China

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Running title: CV intake, *GST* polymorphisms and BC risk

Abstract

Cruciferous vegetables contained high levels of glucosinolates (GSL) and isothiocyanates (ITC). ITC is known to induce glutathione S-transferases (GSTs) thus exert its anticarcinogenic effects. This study explored the combined effects of cruciferous vegetables, GSL, ITC intake and *GST* polymorphisms on breast cancer risk. A total of 737 breast cancer cases and 756 controls were recruited into this case-control study. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were assessed by multivariable logistic regression. Higher cruciferous vegetables, GSL and ITC intake was inversely associated with breast cancer risk, with adjusted ORs (95% CIs) of 0.48 (0.35, 0.65), 0.54 (0.40, 0.74) and 0.62 (0.45, 0.84), respectively. Compared with women carrying *GSTP1* rs1695 wild AA genotype and high cruciferous vegetables, GSL or ITC intake, carriers of AA genotype with low cruciferous vegetables, GSL and ITC intake had greater risk of breast cancer, with adjusted ORs (95% CIs) of 1.43 (1.01, 1.87), 1.34 (1.02, 1.75) and 1.37 (1.05, 1.80), respectively. Persons with *GSTM1* null genotype and lower intake of cruciferous vegetables, GSL and ITC had higher risk of breast cancer than those with *GSTM1* present genotype and higher intake, with ORs (95% CIs) of 1.42 (1.04,1.95), 1.43 (1.05,1.96) and 1.45 (1.06,1.98), respectively. Among women possessing *GSTT1* present genotype, low intake of cruciferous vegetables, GSL or ITC was associated with higher risk of breast cancer. But these interactions were non-significant. This study indicated that there were no significant interactions between cruciferous vegetables, GSL or ITC intake and *GST* polymorphisms on breast cancer risk.

Keywords: Cruciferous vegetables; Glucosinolates; Isothiocyanates; *GST* polymorphism; Breast cancer

Introduction

Breast cancer is the most common cancer in women in the vast majority of the countries. In total, there are 2.1 million newly diagnosed female breast cancer cases in 2018, and ranks as the leading cause of cancer-related death among women in over 100 countries [(1)]. Breast cancer is also the most frequently diagnosed cancer in women in China. Although the incidence and mortality rates are not excessively high in China compared with those in Western countries, the burden is enormous relative to the large Chinese population [(2)].

Cruciferous vegetables have currently attracted great interest for their protective role of combating breast cancer [(3)]. The anticarcinogenic effect may be due to the high containing levels of glucosinolates (GSL) [(4)], which can be converted to isothiocyanates (ITC) and indole-3-carbinol by the catalytic action of plant myrosinase and gastrointestinal microflora. These major hydrolysis products of GSL exert their anticarcinogenic effects through several mechanisms, including the inhibition of carcinogen-activating enzymes, the facilitating effect of apoptosis, and the suppression of cell cycle progression [(5)]. Several epidemiological studies have indicated that high consumption of cruciferous vegetables may reduce the risk of breast cancer [(6-10)]. A previous study by our group also suggested that intake of cruciferous vegetables, GSL and ITC was inversely associated with breast cancer risk among Southern Chinese women[(11)].

The beneficial effect of cruciferous vegetables against breast cancer may attribute to the inherent metabolic activity. The GSL breakdown products, particularly ITC, can induce glutathione S-transferases (GSTs), which are members of the Phase II enzymes systems. GSTs can effectively detoxify electrophilic cancerogen activated by Phase I enzymes, thus destroy their ability to damage DNA and against the development of cancer [(12)]. Besides, ITC is not only inducers but also substrates for GSTs. GSTs can catalyze the conjugation of glutathione with ITC to accelerate membrane transport and excretion of ITC [(13)], thus, first increasing the availability of ITC but ultimately reducing the systemic concentrations of ITC, which adds more complexity to the anticarcinogenic mechanisms.

GSTs are a family that takes a crucial part in the detoxification of a large range of electrophilic cancerogen via conjugating with glutathione [(14)] and evidences showed that

GSTs can take part in the pathogenesis of breast cancer[15]. Glutathione S-transferase P1 (GSTP1) is a paramount GST enzyme found in the breast. A mutant of *GSTP1* gene from an A to a G nucleotide transition in exon 5 lead to a replacement 105 Ile to Val and cause the decreased enzyme activity of GSTP1 protein[16]. *GSTT1* and *GSTM1* polymorphisms are caused by a deletion of the genes, which leads to a complete lack of the encoding protein[17]. Previous studies suggested that the mutant of *GSTP1* and the deficiency in *GSTT1* and *GSTM1* may be related with the susceptibility to breast cancer, but the results remained inconsistent due to the sparse data of some studies or without considering adjusting for various potential confounders in multivariable models [18-20].

So far, two studies conducted in the USA [21, 22] have examined the role of *GST* gene polymorphisms in relation to cruciferous vegetables intake and the risk of breast cancer. To date, only one case-control study has been conducted in Shanghai, China to examine the association between cruciferous vegetable intake, *GSTP1* polymorphism and breast cancer risk [23]. This study also investigated the association between breast cancer and urinary ITC levels, biomarkers for recent cruciferous vegetable intake[24], and the interactions with *GSTM1*, *GSTT1*, *GSTP1* genotypes [10]. As a vast country, there are great differences in lifestyle and dietary habits between different geographic regions in China[25]. Therefore, more studies are needed to clarify the associations between cruciferous vegetables, GSL, ITC intake, *GST* polymorphisms and breast cancer risk. Here, we performed this case-control study to explore whether the inverse association between cruciferous vegetables, GSL, ITC intake and breast cancer risk observed in Southern Chinese women [11] was modified by *GSTP1*, *GSTM1* and *GSTT1* polymorphisms.

Materials and methods

Study population

This ongoing case-control study was performed to recruit breast cancer cases and controls from September 2011. The details of the study methods and design have been described previously [26]. Briefly, eligible women aged 25–70 years, native of Guangdong Province or

having lived in Guangdong for at least 5 years with histologically confirmed, incident, primary breast cancer diagnosed no more than 3 months before the interview, were recruited from three major hospitals in Guangzhou. Potential participants were excluded if they could not understand or speak Mandarin or Cantonese or had a history of cancer. In total, we recruited 792 eligible cases, of which 737 were both successfully interviewed and provided blood specimen, resulting in a response rate of 93%.

Simultaneously, controls were recruited from the same hospitals as cases, age-frequency matched (5-year interval) with cases. The eligibility criteria for control subjects were similar with cases except that they had no past-history of cancer. They were selected from the Departments of Vascular Surgery, Ear-Nose-Throat, Plastic and Reconstructive Surgery, and Orthopedics and Microsurgery. Totally, 756 controls out of 804 eligible controls were recruited, yielding a response rate of 94%. Details of the recruitment of breast cancer cases and controls are shown in Figure 1.

The present study was performed according to the Declaration of Helsinki. The procedures and protocols of this study were approved by The Ethical Committee of School of Public Health, Sun Yat-sen University. Written informed consent forms were obtained from all participants.

Data collection

Trained interviewers performed face-to-face interviews by using a structured questionnaire, which comprised socio-demographic factors, anthropometric factors, lifestyle factors (e.g., alcohol drinking, smoking and physical activity), reproductive information and family history of cancer among first-degree relatives. In the present study, subjects were classified as non-smokers and ever smokers (including regular smokers and former smokers). Someone who had smoked at least 1 cigarette per day for more than 6 consecutive months was defined as a regular smoker. Former smokers were those who reported being regular smokers in the past, but not having smoked in the past 6 months. Passive smokers were non-smokers exposed to the exhalations of smokers for at least 15 minutes per day in the previous 5 years. Regular drinkers were defined as alcohol drinking at least once per week in the past 5 year.

Body mass index (BMI) was computed by dividing weight (kg) by height squared (m) ^{2}. Women were classified as postmenopausal if they had cessation of menstrual period for more than 12 months. Data on current occupational activity were evaluated based on self-reported employment status and the physical activity level at work (non-working, sedentary, standing, manual, heavy manual). The mean metabolic equivalent (MET) value was calculated to self-reported activity in the Compendium of Physical Activities [27, 28]. MET hours per week (numbers of days per week×numbers of hours per day×MET for a certain activity) were calculated for the typical duration (h/day) and frequency (day/week) of household activities (cooking, mopping and so on) and recreational activities (walking, jogging, running, climbing, playing basketball and so on) during the previous year. Relevant personal medical history, medical diagnosis, histological findings, and hormone receptor status were obtained from the hospital medical records. According to the hormone receptor status, the breast cancer were classified into three subtypes: 1) Luminal subtype (estrogen receptor (ER) and/or progesterone receptor (PR) positive); 2) HER2 positive subtype (ER negative and PR negative and HER2 positive); 3) Basal-like subtype (ER negative and PR negative and HER2 negative).

Dietary assessment

Data on cruciferous vegetables consumption were collected from the study subjects using a validated 81-item food frequency questionnaire (FFQ) which evaluated the dietary habits of all the individuals during the past year before the interview. Ten kinds of cruciferous vegetables frequently consumed in Guangdong Province were included in the FFQ. The validity and reliability of FFQ have been reported previously [29]. Each participant was asked to report the average frequency of each type of food they consumed over the past year. Participants were provided with food photographs about different portion size of foods to better estimate the consumed amounts of food. Nutrient values intake was calculated using the 2002 Chinese Food Composition Table [30]. Dietary GSL was computed according to a food composition database which summarized 18 published studies to form a database on GSL contents in cruciferous vegetables [31]. The intake of ITC was calculated by using previously published ITC concentrations in cooked cruciferous vegetables [32].

Genotype of polymorphisms

Fasting blood samples were collected on the second day after admission to the hospital for cases and controls, and were stored at -80℃ until experiments. TIANamp Genomic DNA Kit (Tiangen Biotech, Beijing, China) was used to extract genomic DNA from the peripheral blood according to the manufacturer's instructions.

SNP for *GSTP1* rs1695 was selected because it causes functional mutation located in exons and the minor allele frequency (MAF) >5% in Chinese population. Genotyping of *GSTP1* polymorphism in rs1695 was conducted using a custom-by-design 48-Plex SNPscan Kit (Genesky Biotechnologies Inc., Shanghai, China) as previously described [(33)].

Multiplex PCR protocol was used to examine the absence or presence of the *GSTM1* and *GSTT1* genes. The absence of the specific fragment indicated the corresponding null genotype. The primers used for amplification of 215 bp for *GSTM1* allele and 480 bp in case of *GSTT1* allele were: FwM1 5'-GAACTCCCTGAAAAGCTAAAGC-3', RevM1 5'-GTTGGGCTCAAATATAGGGTGG-3' and FwT1 5'-TTCCTTACTGGTCCTCACATCTC-3', RevT1 5'- TCACCGGATCATGGCCAGCA-3'. The primer pair for a co-amplification of 268 bp of β Globin gene was used as a positive control for target DNA. A gradient thermocycler (Bio-Rad®) was used for PCR reactions: 95℃ for 5 minutes and then 35 cycles of 95℃ for 45s, 58℃ for 45s, 72℃ for 45s and a final polymerization step at 72℃ for 7min. A total amount of 100 ng of genomic DNA was obtained, and it was amplified in a total volume of 50 µl reaction mixture containing 25 µl 2xPCR Premix Taq (TaKaRa®), 1 µl of each primer (Sangon Biotech®) and water free of nucleases to complete the 50µl reaction volume. The electrophoresis in ethidium bromide 1.5% agarose gel (TaKaRa®) was used to analyze the amplification products; the null genotypes were considered in the absence of respective amplification products (215 bp for *GSTM1* and 480 bp for *GSTT1*).

For quality control, the laboratory staff was blind to the identity of the study subjects. Totally, 737 cases and 756 controls were included in the study. The genotyping concordance rates for *GSTP1*, *GSTM1* and *GSTT1* were 100%, 99.3% and 99.3%, respectively.

Statistical analysis

We assumed that people with higher consumption of cruciferous vegetables represented 25% of the general population, the estimated odds ratios (ORs) between cruciferous vegetables intake and breast cancer risk was 0.49[34]; the minor allele frequency for *GSTP1* rs1695 is 40%, the rate for homozygous deletion of *GSTM1* and *GSTT1* is 45% and 64%[35], and the estimated relative risks were 1.40[6], 1.34[36] and 1.47[20], respectively. The type I error rate was less than 0.05 ($\alpha = 0.05$), the power of test was 90% ($\beta = 0.10$) and the response rate was 80%. Based on these assumptions, we required a sample size of 670 cases.

Student's t tests were used for continuous variables (such as BMI, age, age at menarche, household and recreational activities) and Chi-square tests were used for categorical variables (such as educational level, income, smoking habit) to test differences between cases and controls. Dietary cruciferous vegetables, GSL and ITC intake was adjusted for total energy intake by using the residual method [37]. Subjects were categorized into quartiles based on the distribution of cruciferous vegetables or nutrients among the controls. The lowest quartile served as the reference group in the analyses. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated to evaluate the association between cruciferous vegetables, nutrients or *GST* genotype and breast cancer risk. To control for potential confounders, the following variables were included in the unconditional logistic regression model: age, BMI, educational level, occupation, regular drinking, passive smoking, occupational activity, household and recreational activities, parity and first-degree relative with cancer. Confounding factors were selected based on comparing characteristics between the cases and controls. Tests for trend were assessed by entering the categorical variables as continuous parameters in the models.

Hardy-Weinberg equilibrium (HWE) was used to evaluate whether *GSTP1* genotype fell within a standard distribution. Deviation from the HWE in genotype frequency was assessed with Chi-square test. Interactions between cruciferous vegetables, GSL, ITC intake, and *GST* polymorphisms were assessed by adding the multiplicative interaction terms (dietary intake×genotype) to the multivariable models as indicator variables. In the present study,

significance was defined as $P<0.05$ and statistical tests were two-tailed. All statistical analyses mentioned before were carried out using IBM SPSS Statistics, version 21.0.

Results

Table 1 shows the characteristics of the study population. Among 737 breast cancer cases, 643 patients were diagnosed with invasive breast cancer and 94 were diagnosed with carcinoma in situ. When categorized according to hormone receptor status, 501 were Luminal subtype, 96 were Her-2+ subtype and 25 were Basal-like subtype. Compared to controls, a higher proportion of breast cancer patients tended to have higher BMI and more live births, less engaged in white-collar or white-collar occupation, less educated and more likely to have a first-degree relative with cancer. Breast cancer patients were more likely to drink regularly and be exposed to second-hand smoke, engaged in more occupational activities and fewer household and recreational activities. All of the above-referenced variables were considered as potential confounders and were adjusted in the subsequent multivariable analyses. Age was also adjusted in the model since matching was on 5-year intervals. No significant differences were observed between cases and controls on age, age at menarche, age at first live birth, marital status, income, menopausal status, smoking habit, breastfeeding history and history of using oral contraceptive.

Among control subjects, mean (±SD) intake was 156.31±78.64 g/day for energy-adjusted total cruciferous vegetables, 115.75±65.33 mg/day for energy-adjusted GSL, and 34.88±21.64 μmol/day for energy-adjusted ITC. Compared with controls, cases tended to have lower dietary intake of total cruciferous vegetables, GSL and ITC (Table 1).

There was an inverse association between total cruciferous vegetables intake and breast cancer risk in the present study (Table 2). The OR for the highest quartile of intake in comparison with the lowest quartile was 0.48 (95% CI=0.35-0.65) after adjusting for potential confounding factors ($P_{trend}<0.001$). GSL intake was also significantly inversely associated with the risk of breast cancer (highest vs. lowest quartile: OR=0.54; 95% CI=0.40-0.74, $P_{trend}<0.001$). Similarly, individuals with high consumption of ITC had

significantly lower risk of breast cancer (highest vs. lowest quartile: adjusted OR=0.62; 95% CI=0.45-0.84, P_{trend}=0.001).

The *GSTP1* distribution was in accordance with Hardy-Weinberg equilibrium among controls. The *GSTP1* G allele was prevalent among 18.1% of cases and 19.8% of controls. The *GSTM1* null genotypes were observed in 65.3% of cases and 60.7% of controls, and the *GSTT1* null genotypes were observed in 16.1% of cases and 16.3% of controls. No significant associations were found between *GSTP1*, *GSTM*1 and *GSTT1* genotypes and breast cancer risk (for *GSTP1*, adjusted OR=0.75, 95% CI= 0.43-1.30 for the GG genotype compared with the referent AA genotype, $P_{\text{for trend}}$ =0.095; for *GSTM1*, adjusted OR=1.06, 95% CI= 0.85-1.33 for the null genotype compared with the present genotype; for *GSTT1*, adjusted OR=0.93, 95% CI= 0.70-1.24 for the null genotype compared with the present genotype) . Stratified analysis by menopausal status showed that there were no significant associations between *GSTP1*, *GSTM*1 and *GSTT1* genotypes and breast cancer risk neither in premenopausal nor postmenopausal women (Table 3).

ORs and 95%CIs for breast cancer risk according to *GST* polymorphisms and cruciferous vegetables intake are shown in Table 4. Overall, we found a combined effect between cruciferous vegetables intake and *GST* polymorphisms in relation to breast cancer risk. However, there were no statistically significant interactions. Among individuals with the *GSTP1* rs1695 wild AA genotype, the OR for low versus high cruciferous vegetables intake was 1.43 (95% CI=1.01- 1.87) (*P* for interaction 0.251). Compared with women carrying the *GSTM1* present genotype with higher cruciferous vegetables intake, women with the *GSTM1* null genotype and lower intake had a higher risk of breast cancer, with an OR (95% CI) of 1.42 (1.04-1.95) (*P* for interaction 0.398). Persons with *GSTT1* present genotype and low cruciferous vegetables intake had a 42% greater risk of breast cancer than did persons with present genotype and high intake (*P* for interaction 0.677). There were no statistically significant interactions between GSL and ITC intake and *GST* polymorphisms in relation to breast cancer risk (Table 5 and Table 6).

Discussion

The aim of this case-control study was to examine the combined associations between cruciferous vegetables, GSL, ITC intake and *GST* polymorphisms and breast cancer risk. The results confirmed that higher intake of cruciferous vegetable, GSL and ITC was inversely associated with the risk of breast cancer. There were no overall associations between *GSTP1*, *GSTM1*, or *GSTT1* polymorphisms and breast cancer risk. Combined effects were observed between cruciferous vegetables intake and *GST* polymorphisms in relation to breast cancer risk, but there were no statistically significant interactions.

The frequency of *GSTP1* G allele was 18.1% among controls in the present study. It was in accordance with the reported frequency of *GSTP1* G allele from three studies in China [23, 38, 39] . Previous studies suggested that the functional mutation of the *GSTP1* rs1695 polymorphisms may reduce the activity of GST-π enzyme deactivating and detoxifying carcinogens, thus may increase cancer vulnerability[40]. Nevertheless, our data showed no significant association between the *GSTP1* homozygous mutant GG genotype and breast cancer risk, which was consistent with a 2016 meta-analysis of 36 case-control studies including 20615 cases and 20481 controls[19], and studies from China [39] and Cyprus [41], but contrary to studies from Shanghai [23] and Zhejiang [38] of China which found that the *GSTP1* GG genotype was significantly associated with greater risk of breast cancer (ORs = 2.23 and 1.50, respectively, GG *vs.* AA). The *GSTM1* and *GSTT1* null genotypes were prevalent among 60.7% and 16.3% of controls in the present study, which was consistent with the rate of *GSTM1* null genotype (59.1%) , but much lower than that of *GSTT1* null genotype (51.9%) in Shanghai Women's Health Study [42]. Given the activity of GSTs enzyme toward carcinogen detoxification, the deficiency of GST-μ and GST-θ enzyme activity caused by deletions in *GSTM1* and *GSTT1* genes may compromise an individual's ability to deactivate carcinogens, leading to be involved in carcinogenesis[17]. A 2018 meta-analysis of 53 studies for *GSTM1* polymorphism and 44 studies for *GSTT1* polymorphism found that *GSTM1* and *GSTT1* null genotypes were risk factors for breast cancer (ORs were 1.22 and 1.07, respectively) [18]. However, our data demonstrated that *GSTM1* and *GSTT1* polymorphisms were not significantly associated with the risk of breast cancer. Consistent with our results,

reports from Philippines [43] and Mexico [44] also suggested that the deletion of *GSTM1* and *GSTT1* may not be risk factors for breast cancer susceptibility. Possible explanation for the different results of different studies might be that the genetic variability and lifestyle habits varied from different races [45]. Besides, the genetic predisposition to breast cancer associated with *GST* genotypes may be modified by some environmental factors [40], such as the consumption of cruciferous vegetables.

GSTs play a paramount role in the detoxification of a large range of electrophilic cancerogen. Not only can ITC induce GSTs enzyme activity Keap1-Nrf2 signaling pathway [46] but also GSTs conjugate ITC. Therefore, low GSTs enzyme activity may result in a greater extent of ITC exertion and reduce the protective effects of ITC [47]. To date, only three epidemiological studies have reported the associations between cruciferous vegetables intake, *GST* genotypes and breast cancer risk. One case-control study in the Long Island, USA found no interactions between cruciferous vegetable intake and *GSTP1*, *GSTM1*,*or GSTT1* polymorphisms on breast cancer risk [22]. Another case-control study including 208 breast cancer cases and 212 controls conducted in Caucasian-American women also suggested that the beneficial effect of broccoli was not modified by *GSTM1* and *GSTT1* genotypes [21]. One case-control study conducted in Shanghai, China found that women with the *GSTP1* GG genotype and lower cruciferous vegetables intake had a greater risk of breast cancer than did individuals with GA or AA genotypes and higher cruciferous vegetables intake (OR = 1.74, 95% CI=1.13, 2.67), but the interaction effect was non-significant ($P_{\text{interaction}}$ =0.331) [23]. This study also did not find statistically significant interaction between urinary ITC and *GST* genotypes and breast cancer risk ($P_{\text{interaction}}$ >0.05) [10]. Consistent with these results, although the present study found combined effects between cruciferous vegetables, GSL and ITC intake and *GST* polymorphisms in relation to breast cancer risk, there were no statistically significant interactions. For *GSTP1* rs1695, highest risk was observed in those consuming lower cruciferous vegetable, GSL and ITC intakes with the wild AA genotype as compared with high consumers with the wild AA genotype. The increased risk observed for carriers with *GSTM1* null genotype and with lower intakes of cruciferous vegetable, GSL and ITC as compared with carriers with *GSTM1* present genotype and higher intakes. Women carrying

the *GSTT1* present genotype with low cruciferous vegetables, GSL and ITC intake had a greater risk of breast cancer than did women with present genotype and high intake. Studies investigating other cancers did not show significant interaction effect of cruciferous vegetables or ITC intake and *GSTP1, GSTM1* or *GSTT1* polymorphisms on gastric cancer [48], oral cancer [49], kidney cancer [50], colorectal cancer [51] and colorectal adenoma [52]. But interactions were significant between urinary isothiocyanate concentrations and *GSTM1,* or *GSTT1* polymorphisms and lung cancer [53] and colorectal cancer [54]. Due to the small sample in some subgroups of the present study, further studies with larger sample size are needed to clarify this issue.

The major strengths of this study are the satisfactory reproducibility, reasonable validity of the FFQ and extensive collections of multiple known or suspected confounders. While there are also limitations warranted consideration. First, the results could be inevitably affected by recall bias and selection bias because of the hospital-based case-control studies design. To reduce selection bias, control subjects were excluded if they had any history of diseases potentially related to either dietary habits or breast cancer. The time-concordant period of hospitalization, identical catchment areas of all study subjects and the relatively high response rate helped to minimize selection bias. In addition, the allele frequencies in the present study were corresponded to previous studies in Chinese population [23, 38, 39], which suggested that selection bias may not be a serious problem. Besides, to reduce recall bias, cases were interviewed as soon as they were diagnosed with breast cancer (77.6% of the cases were interviewed within 3 days after hospitalization) and as far as possible before their surgery. Moreover, participants were provided with food photographs to better estimate the consumed amounts of food. Second, there remained residual confounding although various dietary and non-dietary confounders were adjusted in the multivariable models. Third, due to relatively small samples in stratification analysis, we did not have enough power to detect some associations with smaller effects. Further studies with larger sample size are needed to verify the present findings. Fourth, the food composition database used to calculate the consumption of ITC from vegetables was based on boiled vegetables. However, lack of collecting information on methods of cooking vegetables might lead to some information bias.

Besides, previous studies have shown that urinary ITC concentrations could be useful biomarker for cruciferous vegetable intake over the prior 24-48h period [24, 55]. However, this study did not measure urinary ITC due to the lack of collecting urine specimens. Further studies are expected to examine the interaction of urinary ITC and *GST* polymorphisms on breast cancer risk.

In conclusion, this study indicated that there were no significant interactions between cruciferous vegetables, GSL or ITC intake and *GST* polymorphisms in relation to breast cancer risk. The observed combined effects between cruciferous vegetables, GSL or ITC intake and *GST* polymorphisms on breast cancer risk need to be confirmed in other studies.

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Conflict of interest statement

The authors declare that they have no conflict of interest.

Authorship

The authors' responsibilities were as follows: Zhang NQ conducted the data collection, analyzed the data and wrote this paper. Feng XL, Zhang X and Luo H participated in the data collection and data entry. Mo XF and Lin FY were responsible for connecting and coordinating the field work. Zhang NQ, Zhan XX and Luo H did the experiment. Zhang CX constructed the project design, supervised and contributed to the manuscript writing.

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Table 1. Socio-demographic and clinical characteristics of breast cancer in the study population.

	Cases (n=737)		Controls (n=756)		*P*
	Mean	SD	Mean	SD	
Age (years)	47.60	9.36	47.46	9.33	0.773
BMI (kg/m^2)	23.01	3.60	22.65	3.14	0.047
Household and recreational activities (MET-h/week)	37.51	23.57	40.41	24.91	0.024
Age at menarche (years)	14.37	1.96	14.49	1.73	0.202
Age at first live birth (years) *	25.40	3.69	25.51	3.40	0.576
Total energy (kcal/day)	1413	374	1420	369	0.690
Total cruciferous vegetables (g/day) †	141.24	77.30	156.31	78.64	<0.001
Glucosinolates (mg/day) †	103.96	61.84	115.75	65.33	<0.001
Isothiocyanates (μmol/day) †	32.18	20.28	34.88	21.64	0.013
	n	%	n	%	
Marital status					
Married	692	93.89	700	92.59	0.317
Unmarried/divorced/widowed	45	6.11	56	7.41	
Educational level					<0.001
Primary school or below	191	25.92	183	24.21	
Junior high school	231	31.34	181	23.94	
Senior high school/secondary technical school	168	22.80	176	23.28	
College or above	147	19.95	216	28.57	
Occupation					0.007
Administrator/other white-collar worker	259	35.14	308	40.74	
Blue-collar worker	211	28.63	231	30.56	
Farmer/other	267	36.23	217	28.70	
Income (yuan/month)					
<2000	65	8.82	44	5.82	0.053
2001-5000	198	26.87	185	24.47	
5001-8000	248	33.65	289	38.23	
>8001	226	30.66	238	31.48	
Smoking habit					0.597
Ever smoker	10	1.36	8	1.06	
Non-smoker	727	98.64	748	98.94	
Passive smoker	410	55.63	356	47.09	0.001
Regular drinker	65	8.82	36	4.76	0.002
Occupational activity					0.012
Non-working	193	26.19	157	20.77	
Sedentary	283	38.40	302	39.95	
Standing	144	19.54	183	24.21	
Manual	67	9.09	80	10.58	
Heavy manual	50	6.78	34	4.50	
Menopausal status					0.617

Premenopausal	480	65.13	483	63.89	
Postmenopausal	257	34.87	273	36.11	
Parity					0.024
0	33	4.48	41	5.42	
1-2	531	72.05	580	76.72	
≥3	173	23.47	135	17.86	
Breast-feeding *	617	85.10	640	85.91	0.619
First-degree relative with cancer	91	12.35	67	8.86	0.029
Ever used an oral contraceptive	48	6.51	45	5.95	0.557
Breast cancer subtype					
Luminal	501	67.98			
Her-2+	96	13.03			
Basal-like	25	3.39			
Unknown	115	15.60			
Breast cancer pathological type					
Carcinoma in situ	94	12.75			
Invasive tumor	643	87.25			

MET, metabolic equivalent task.

*Among women who have had a live birth

†The consumption was adjusted for total energy intake by residual method.

Table 2. Risks for breast cancer according to quartiles of cruciferous vegetables, GSL and ITC intake (Odds ratios and 95% confidence intervals)

	No. Cases/Controls	Crude OR (95%CI)	Adjusted OR (95%CI) *	P_{trend}
Cruciferous vegetables (g/day)				<0.001
<103.35	260/189	1.00	1.00	
103.35-145.72	172/189	0.66 (0.50,0.87)	0.63 (0.47,0.85)	
145.72-195.35	165/189	0.64 (0.48,0.84)	0.69 (0.49,0.91)	
⩾195.35	136/189	0.52 (0.38,0.70)	0.48 (0.35,0.65)	
Glucosinolate (mg/day)				<0.001
<70.54	257/189	1	1	
70.54-105.04	166/189	0.65 (0.49,0.86)	0.69 (0.51,0.93)	
105.04-146.90	164/189	0.64 (0.48,0.85)	0.66 (0.49,0.89)	
⩾146.90	146/189	0.57 (0.43,0.76)	0.54 (0.40,0.74)	
Isothiocyanate (μmol/day)				0.001
<19.27	206/189	1	1	
19.27-30.71	209/189	1.01 (0.77,1.34)	0.92 (0.68,1.24)	
30.71-48.81	167/189	0.81 (0.61,1.08)	0.73 (0.53,0.99)	
⩾45.81	151/189	0.75 (0.55,0.98)	0.62 (0.45,0.84)	

* OR adjusted for age, educational level, occupation, BMI, passive smoking, regular drinking, household and recreational activities, occupational activity, parity and first-degree relative with cancer.

Table 3. Associations between *GST* polymorphism and breast cancer risk according to menopausal status

	All women			Premenopause women			Postmenopause women		
	Cases/ Controls	Crude OR (95%CI)	Adjusted OR (95%CI) *	Cases/ Controls	Crude OR (95%CI)	Adjusted OR (95%CI) *	Cases/ Controls	Crude OR (95%CI)	Adjusted OR (95%CI) *
GSTP1									
rs1695									
AA	496/489	1	1	324/306	1	1	172/183	1	1
GA	215/234	0.91 (0.73,1.13)	0.84 (0.67,1.06)	136/151	0.85 (0.64,1.13)	0.74 (0.55,1.00)	79/83	1.01 (0.70,1.47)	1.07 (0.72,1.59)
GG	26/33	0.78 (0.46,1.32)	0.75 (0.43,1.30)	20/26	0.73 (0.40,1.33)	0.71 (0.37,1.37)	6/7	0.91 (0.30,2.77)	0.81 (0.26,2.55)
GSTM1									
Present	278/297	1	1	186/186	1	1	92/111	1	1
Null	459/459	1.07 (0.87,1.32)	1.06 (0.85,1.33)	294/297	0.99 (0.76,1.28)	0.95 (0.72,1.25)	165/162	1.23 (0.87,1.75)	1.31 (0.91,1.92)
GSTT1									
Present	618/633	1	1	402/407	1	1	216/226	1	1
Null	119/123	0.99 (0.75,1.31)	0.93 (0.70,1.24)	78/76	1.04 (0.74,1.47)	0.97 (0.67,1.41)	41/47	0.91 (0.58,1.44)	0.87 (0.53,1.41)

*OR adjusted for age, educational level, occupation, BMI, passive smoking, regular drinking, household and recreational activities, occupational activity, parity and first-degree relative with cancer.

Table 4. OR and 95%CIs for breast cancer risk according to *GST* gene polymorphisms and cruciferous vegetable intake

	Cruciferous vegetables intake above median (≥145.72 g/day)			Cruciferous vegetables intake below median (<145.72 g/day)			$P_{interaction}$
	Cases/ Controls	Crude OR (95%CI)	Adjusted OR (95%CI) *	Cases/ Controls	Crude OR (95%CI)	Adjusted OR (95%CI) *	
GSTP1							0.251
rs1695							
AA	202/243	1	1	294/246	1.44 (1.12,1.85)	1.43 (1.01,1.87)	
GA	97/115	1.02 (0.73,1.41)	0.94 (0.66,1.33)	118/119	1.19 (0.87,1.64)	1.10 (0.78,1.54)	
GG	9/20	0.54 (0.24,1.22)	0.49 (0.21,1.14)	17/13	1.57 (0.75,3.32)	1.61 (0.72,3.59)	
GSTM1							0.398
Present	128/150	1	1	150/147	1.20 (0.86,1.66)	1.21 (0.85,1.71)	
Null	180/228	0.93 (0.68,1.26)	0.93 (0.68,1.28)	279/231	1.42 (1.06,1.90)	1.42 (1.04,1.95)	
GSTT1							0.677
Present	263/324	1	1	355/309	1.42 (1.13,1.77)	1.42 (1.12,1.81)	
Null	45/54	1.03 (0.67,1.58)	0.97 (0.62,1.52)	74/69	1.32 (0.92,1.91)	1.24 (0.84,1.83)	

* OR adjusted for age, educational level, occupation, BMI, passive smoking, regular drinking, household and recreational activities, occupational activity, parity and first-degree relative with cancer.

Table 5. OR and 95%CIs for breast cancer risk according to *GST* gene polymorphisms and glucosinolates intake

	Glucosinolates intake above median (≥105.04 mg/day)			Glucosinolates intake below median (<105.04 mg/day)			$P_{interaction}$
	Cases/ Controls	Crude OR (95%CI)	Adjusted OR (95%CI) *	Cases/ Controls	Crude OR (95%CI)	Adjusted OR (95%CI) *	
GSTP1							0.415
rs1695							
AA	208/240	1	1	288/249	1.34 (1.04,1.72)	1.34 (1.02,1.75)	
GA	99/119	0.96 (0.69,1.33)	0.86 (0.61,1.21)	116/115	1.16 (0.85,1.60)	1.12 (0.80,1.58)	
GG	10/19	0.61 (0.28,1.34)	0.51 (0.23,1.16)	16/14	1.32 (0.63,2.77)	1.50 (0.66,3.37)	
GSTM1							0.667
Present	127/149	1	1	151/148	1.20 (0.86,1.66)	1.26 (0.89,1.78)	
Null	190/229	0.93 (0.72,1.72)	0.98 (0.72,1.35)	269/230	1.37 (1.02,1.84)	1.43 (1.05,1.96)	
GSTT1							0.263
Present	268/326	1	1	350/307	1.39 (1.11,1.73)	1.45 (1.14,1.85)	
Null	49/52	1.15 (0.75,1.75)	1.09 (0.70,1.70)	71/70	1.20 (0.83,1.73)	1.16 (0.78,1.71)	

* OR adjusted for age, educational level, occupation, BMI, passive smoking, regular drinking, household and recreational activities, occupational activity, parity and first-degree relative with cancer.

Table 6. OR and 95%CIs for breast cancer risk according to *GST* gene polymorphisms and isothiocyanates intake

	Isothiocyanates intake above median (≥30.71 μmol/day)			Isothiocyanates intake below median (<30.71 μmol/day)			$P_{interaction}$
	Cases/ Controls	Crude OR (95%CI)	Adjusted OR (95%CI) *	Cases/ Controls	Crude OR (95%CI)	Adjusted OR (95%CI) *	
GSTP1							0.513
rs1695							
AA	215/240	1	1	281/249	1.26 (0.98,1.62)	1.37 (1.05,1.80)	
GA	99/118	0.94 (0.68,1.30)	0.85 (0.61,1.20)	116/116	1.12 (0.81,1.53)	1.15 (0.82,1.62)	
GG	11/21	0.59 (0.28,1.24)	0.56 (0.25,1.22)	15/12	1.40 (0.64,3.05)	1.51 (0.65,3.52)	
GSTM1							0.336
Present	133/149	1	1	145/148	1.10 (0.79,1.52)	1.20 (0.85,170)	
Null	192/230	0.94 (0.69,1.27)	0.93 (0.68,1.27)	267/229	1.31 (0.97,1.75)	1.45 (1.06,1.98)	
GSTT1							0.363
Present	275/321	1	1	343/312	1.28 (1.03,1.60)	1.45 (1.15,1.85)	
Null	50/58	1.01 (0.67,1.52)	1.02 (0.66,1.56)	65/69	1.24 (0.85,1.80)	1.22 (0.82,1.82)	

* OR adjusted for age, educational level, occupation, BMI, passive smoking, regular drinking, household and recreational activities, occupational activity, parity and first-degree relative with cancer.

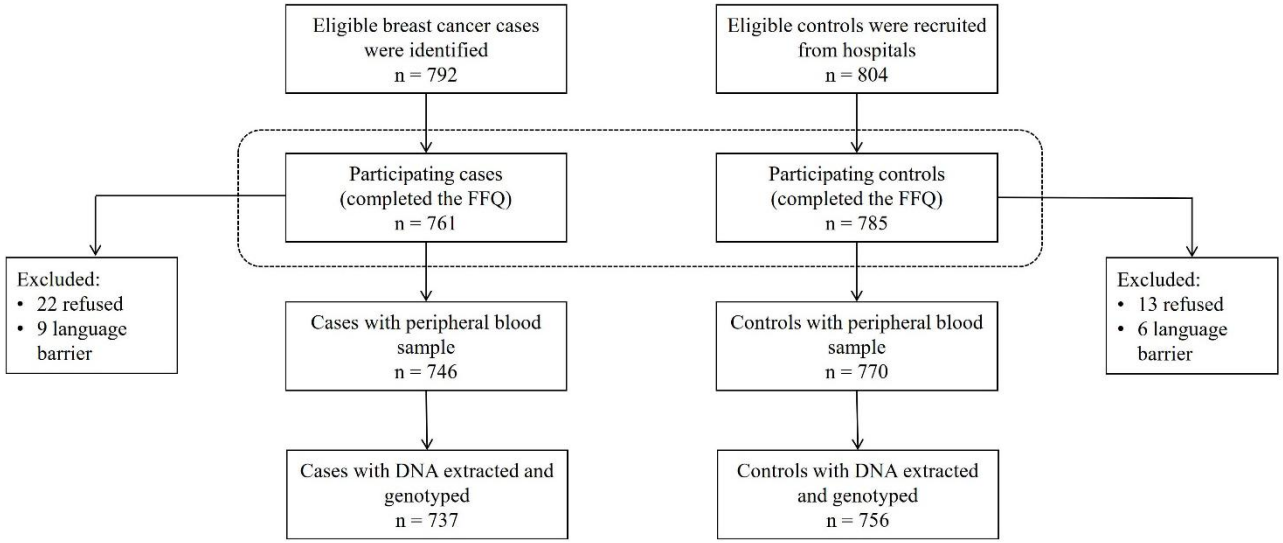

Figure 1. Flow chart of the recruitment of breast cancer cases and controls.