British Journal of Nutrition (2020), 124, 548–557
doi:10.1017/S0007114520001348

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

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(Submitted 30 September 2019 – Final revision received 20 March 2020 – Accepted 14 April 2020 – First published online 20 April 2020)

Abstract

Cruciferous vegetables contain high levels of glucosinolates (GSL) and isothiocyanates (ITC). ITC are known to induce glutathione *S*-transferases (GST) and thus exert their anticarcinogenic effects. This study explored the combined effects of cruciferous vegetable, GSL and 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. OR and 95 % CI were assessed by multivariable logistic regression. Higher cruciferous vegetable, GSL and ITC intakes were inversely associated with breast cancer risk, with adjusted OR of 0·48 (95 % CI 0·35, 0·65), 0·54 (95 % CI 0·40, 0·74) and 0·62 (95 % CI 0·45, 0·84), respectively. Compared with women carrying the *GSTP1* rs1695 wild AA genotype and high cruciferous vegetable, GSL or ITC intake, carriers of the AA genotype with low cruciferous vegetable, GSL and ITC intake had greater risk of breast cancer, with adjusted OR of 1·43 (95 % CI 1·01, 1·87), 1·34 (95 % CI 1·02, 1·75) and 1·37 (95 % CI 1·05, 1·80), respectively. Persons with the *GSTM1*-null genotype and lower intake of cruciferous vegetables, GSL and ITC had higher risk of breast cancer than those with the *GSTM1*-present genotype and higher intake, with OR of 1·42 (95 % CI 1·04, 1·95), 1·43 (95 % CI 1·05, 1·96) and 1·45 (95 % CI 1·06, 1·98), respectively. Among women possessing the *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 vegetable, GSL or ITC intake and *GST* polymorphisms on breast cancer risk.

Key words: **Cruciferous vegetables: Glucosinolates: Isothiocyanates: Glutathione *S*-transferase polymorphisms: Breast cancer**

Breast cancer is the most common cancer in women in the vast majority of 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 into 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

Abbreviations: GSL, glucosinolates; GST, glutathione *S*-transferase; ITC, isothiocyanates.

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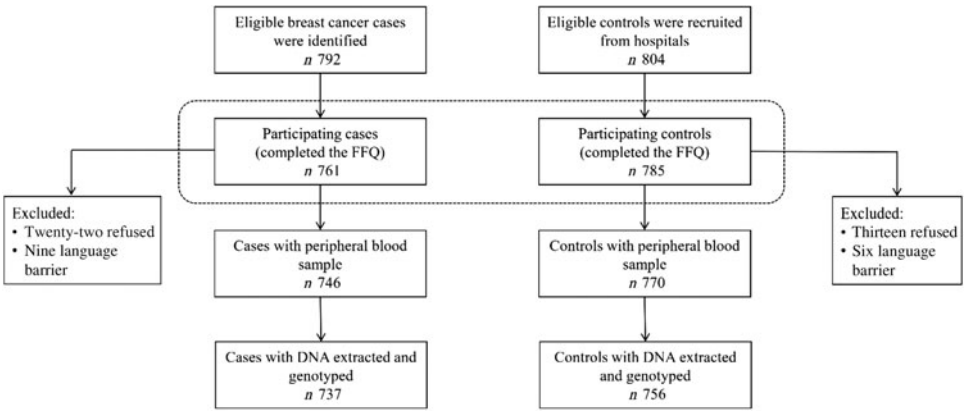

Fig. 1. Flow chart of the recruitment of breast cancer cases and controls.

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 (GST), which are members of the phase II enzyme systems. GST 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 GST. GST can catalyse 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.

GST 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 GST can take part in the pathogenesis of breast cancer[15]. 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 leads to a replacement 105 Ile to Val and causes 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 adjustment 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 vegetable 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* and *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 vegetable, GSL and ITC intake, *GST* polymorphisms and breast cancer risk. Here, we performed this case–control study to explore whether the inverse association between cruciferous vegetable, GSL and 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 Fig. 1.

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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 one cigarette per d for more than six 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 min per d in the previous 5 years. Regular drinkers were defined as alcohol drinking at least once per week in the past 5 years. BMI was computed by dividing weight (kg) by height squared (m^2). Women were classified as postmenopausal if they had cessation of menstrual periods 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 and heavy manual). The mean metabolic equivalent task value was calculated to self-reported activity in the Compendium of Physical Activities[27,28]. Metabolic equivalent task hours per week (numbers of days per week × numbers of hours per d × metabolic equivalent task for a certain activity) were calculated for the typical duration (h/d) and frequency (d/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 was classified into three subtypes: (1) luminal subtype (oestrogen receptor and/or progesterone receptor positive); (2) human epidermal growth factor receptor (HER2)-positive subtype (oestrogen receptor negative and progesterone receptor negative and HER2 positive) and (3) basal-like subtype (oestrogen receptor negative and progesterone receptor negative and HER2 negative).

Dietary assessment

Data on cruciferous vegetable consumption were collected from the study subjects using a validated eighty-one-item 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 sizes of foods to better estimate the consumed amounts of food. Nutrient intake values were calculated using the 2002 Chinese Food Composition Table[30]. Dietary GSL was computed according to a food composition database which summarised eighteen 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°C until experiments. TIANamp Genomic DNA Kit (Tiangen Biotech) 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 >5 % in Chinese population. Genotyping of *GSTP1* polymorphism in rs1695 was conducted using a custom-by-design 48-Plex SNP scan Kit (Genesky Biotechnologies Inc.) 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′-GTTGGG-CTCAAATATAGGGTGG-3′ and FwT1 5′-TTCCTTACTGGTC-CTCACATCTC-3′ and 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°C for 5 min and then thirty-five cycles of 95°C for 45 s, 58°C for 45 s, 72°C for 45 s and a final polymerisation step at 72°C for 7 min. 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 2× PCR Premix Taq (TaKaRa®), 1 µl of each primer (Sangon Biotech®) and water free of nucleases to complete the 50 µl reaction volume. The electrophoresis in ethidium bromide 1·5 % agarose gel (TaKaRa®) was used to analyse 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 OR between cruciferous vegetable 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]. The type I error rate was <0·05 ($\alpha = 0{\cdot}05$), the power of test was 90 % ($\beta = 0{\cdot}10$) 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 χ^2 tests were used for categorical variables (such as educational level, income and smoking habit) to test differences between cases and controls. Dietary cruciferous vegetable, GSL and ITC intake was adjusted for total energy intake by using the residual method[37]. Subjects were categorised 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. OR and 95 % CI 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 was used to evaluate whether *GSTP1* genotype fell within a standard distribution. Deviation from the Hardy–Weinberg equilibrium in genotype frequency was assessed with χ^2 test. Interactions between cruciferous vegetable, GSL and 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 $P < 0{\cdot}05$ 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 ninety-four were diagnosed with carcinoma *in situ*. When categorised according to hormone receptor status, 501 were luminal subtype, ninety-six were Her-2+ subtype and twenty-five were basal-like subtype. Compared with 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, breast-feeding history and history of using oral contraceptive.

Among control subjects, mean intake was 156·31 (SD 78·64) g/d for energy-adjusted total cruciferous vegetables, 115·75 (SD 65·33) mg/d for energy-adjusted GSL and 34·88 (SD 21·64) μmol/d 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 vegetable 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 ($P_{trend} < 0{\cdot}001$). GSL intake was also significantly inversely associated with the risk of breast cancer (highest *v.* lowest quartile OR 0·54; 95 % CI 0·40, 0·74, $P_{trend} < 0{\cdot}001$). Similarly, individuals with high consumption of ITC had significantly lower risk of breast cancer (highest *v.* lowest quartile adjusted OR 0·62; 95 % CI 0·45, 0·84, $P_{trend} = 0{\cdot}001$).

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, $P_{for\ trend} = 0{\cdot}095$; 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).

OR and 95 % CI for breast cancer risk according to *GST* polymorphisms and cruciferous vegetable intake are shown in Table 4. Overall, we found a combined effect between cruciferous vegetable 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 *v.* high cruciferous vegetable 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 vegetable intake, women with the *GSTM1* null genotype and lower intake had a higher risk of breast cancer, with an OR of 1·42 (95 % CI 1·04, 1·95) ($P_{for\ interaction}$ 0·398). Persons with *GSTT1* present genotype and low cruciferous vegetable 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 (Tables 5 and 6).

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Table 1. Socio-demographic and clinical characteristics of breast cancer in the study population (Mean values and standard deviations)

	Cases (*n* 737)		Controls (*n* 756)		
	Mean	SD	Mean	SD	*P*
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 (metabolic equivalent task-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/d)†	1413	374	1420	369	0·690
Total cruciferous vegetables (g/d)‡	141·24	77·30	156·31	78·64	<0·001
Glucosinolates (mg/d)‡	103·96	61·84	115·75	65·33	<0·001
Isothiocyanates (μmol/d)‡	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					
Primary school or below	191	25·92	183	24·21	<0·001
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					
Administrator/other white-collar worker	259	35·14	308	40·74	0·007
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					
Ever smoker	10	1·36	8	1·06	0·597
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					
Non-working	193	26·19	157	20·77	0·012
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					
Premenopausal	480	65·13	483	63·89	0·617
Postmenopausal	257	34·87	273	36·11	
Parity					
0	33	4·48	41	5·42	0·024
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			
Human epidermal growth factor receptor 2 positive	96	13·03			
Basal-like	25	3·39			
Unknown	115	15·60			
Breast cancer pathological type					
Carcinoma *in situ*	94	12·75			
Invasive tumour	643	87·25			

* Among women who have had a live birth.
† To convert kcal to kJ, multiply by 4·184.
‡ Consumption was adjusted for total energy intake by residual method.

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

	No. of cases/controls	Crude OR	95 % CI	Adjusted OR*	95 % CI	P_{trend}
Cruciferous vegetables (g/d)						
<103·35	260/189	1·00		1·00		<0·001
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	
GSL (mg/d)						
<70·54	257/189	1		1		<0·001
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	
ITC (μmol/d)						
<19·27	206/189	1		1		0·001
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.

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Table 3. Associations between glutathione *S*-transferase polymorphisms and breast cancer risk according to menopausal status (Odds ratios and 95 % confidence intervals)

	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. Breast cancer risk according to glutathione *S*-transferase gene polymorphisms and cruciferous vegetable intake (Odds ratios and 95 % confidence intervals)

	Cruciferous vegetable intake above median (≥145·72 g/d)					Cruciferous vegetable intake below median (<145·72 g/d)					
	Cases/controls	Crude OR	95 % CI	Adjusted OR*	95 % CI	Cases/controls	Crude OR	95 % CI	Adjusted OR*	95 % CI	$P_{interaction}$
GSTP1 rs1695											
AA	202/243	1		1		294/246	1·44	1·12, 1·85	1·43	1·01, 1·87	0·251
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											
Present	128/150	1		1		150/147	1·20	0·86, 1·66	1·21	0·85, 1·71	0·398
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											
Present	263/324	1		1		355/309	1·42	1·13, 1·77	1·42	1·12, 1·81	0·677
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. Breast cancer risk according to glutathione *S*-transferase gene polymorphisms and glucosinolate intake (Odds ratios and 95 % confidence intervals)

	Glucosinolate intake above median (≥105·04 mg/d)					Glucosinolate intake below median (<105·04 mg/d)					
	Cases/controls	Crude OR	95 % CI	Adjusted OR*	95 % CI	Cases/controls	Crude OR	95 % CI	Adjusted OR*	95 % CI	$P_{interaction}$
GSTP1 rs16950											
AA	208/240	1		1		288/249	1·34	1·04, 1·72	1·34	1·02, 1·75	0·415
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											
Present	127/149	1		1		151/148	1·20	0·86, 1·66	1·26	0·89, 1·78	0·667
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											
Present	268/326	1		1		350/307	1·39	1·11, 1·73	1·45	1·14, 1·85	0·263
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. Breast cancer risk according to glutathione *S*-transferase gene polymorphisms and isothiocyanate intake (Odds ratios and 95 % confidence intervals)

	Isothiocyanate intake above median (≥30·71 μmol/d)					Isothiocyanate intake below median (<30·71 μmol/d)					
	Cases/controls	Crude OR	95 % CI	Adjusted OR*	95 % CI	Cases/controls	Crude OR	95 % CI	Adjusted OR*	95 % CI	$P_{interaction}$
GSTP1 rs1695											
AA	215/240	1		1		281/249	1·26	0·98, 1·62	1·37	1·05, 1·80	0·513
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											
Present	133/149	1		1		145/148	1·10	0·79, 1·52	1·20	0·85, 1·70	0·336
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											
Present	275/321	1		1		343/312	1·28	1·03, 1·60	1·45	1·15, 1·85	0·363
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.

Discussion

The aim of this case–control study was to examine the combined associations between cruciferous vegetable, GSL and ITC intake and *GST* polymorphisms and breast cancer risk. The results confirmed that higher intake of cruciferous vegetables, 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 vegetable 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 and 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 thirty-six case–control studies including 20 615 cases and 20 481 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 (OR 2·23 and 1·50, respectively, GG *v.* 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 GST enzyme towards 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 fifty-three studies for *GSTM1* polymorphism and forty-four studies for *GSTT1* polymorphism found that *GSTM1* and *GSTT1* null genotypes were risk factors for breast cancer (OR 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.

GST play a paramount role in the detoxification of a large range of electrophilic cancerogens. Not only can ITC induce GST enzyme activity Kelch-like ECH-associated protein 1 and nuclear factor erythroid 2-related factor 2 (Keap1-Nrf2) signalling pathway[46] but also GST conjugate ITC. Therefore, low GST 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 vegetable 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 vegetable intake had a greater risk of breast cancer than did individuals with GA or AA genotypes and higher cruciferous vegetable intake (OR 1·74, 95 % CI 1·13, 2·67), but the interaction effect was non-significant ($P_{interaction} = 0·331$)[23]. This study also did not find statistically significant interaction between urinary ITC and *GST* genotypes and breast cancer risk ($P_{interaction} > 0·05$)[10]. Consistent with these results, although the present study found combined effects between cruciferous vegetable, 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 vegetable, 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 vegetable 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 ITC 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 hospitalisation, identical catchment areas of all study subjects and the relatively high response rate helped to minimise 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 d after hospitalisation) 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 24–48 h 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 vegetable, GSL or ITC intake and *GST* polymorphisms in relation to breast cancer risk. The observed combined effects between cruciferous vegetable, GSL or ITC intake and *GST* polymorphisms on breast cancer risk need to be confirmed in other studies.

Acknowledgements

We gratefully acknowledge the contribution of the study participants; without them the study would not have been possible.

This work was supported by Danone Nutrition Research and Education Foundation (no. DIC2017-05). The funders had no role in the design, analysis or writing of this article.

The authors' responsibilities were as follows: N.-Q. Z. conducted the data collection, analysed the data and wrote this paper. X.-L. F., X. Z. and H. L. participated in the data collection and data entry. X.-F. M. and F.-Y. L. were responsible for connecting and coordinating the fieldwork. N.-Q. Z., X.-X. Z. and H. L. did the experiment. C.-X. Z. constructed the project design, supervised and contributed to the manuscript writing.

The authors declare that there are no conflicts of interest.

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