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Association between cruciferous vegetable intake and risk of colorectal cancer among men in Shanghai, China

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ASSOCIATION BETWEEN CRUCIFEROUS VEGETABLE INTAKE AND RISK OF COLORECTAL CANCER AMONG MEN IN SHANGHAI, CHINA

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EPIDEMIOLOGY

ABSTRACT

Colorectal cancer (CRC) is a major global health concern and the intake of certain foods, such as cruciferous vegetables, has been studied for their potential protective effects against cancer development. The observed association between cruciferous vegetable consumption and CRC has been inconsistent, possibly related to glutathione *S*-transferase (*GST*) gene polymorphisms. Therefore, we aimed to (1) evaluate the association between fruits and vegetables, including cruciferous vegetables, on the risk of CRC; (2) determine factors associated with urinary isothiocyanate (ITC), a biomarker of cruciferous vegetable intake; and (3) evaluate the association between cruciferous vegetable consumption and CRC and to estimate the potential interaction between cruciferous vegetable intake and *GST* gene polymorphisms using data from the Shanghai Men's Health Study (SMHS).

In the SMHS cohort, 398 cases of CRC (236 colon and 162 rectum) were observed as of December 31, 2010. Fruit consumption was inversely associated with the risk of CRC while vegetable intake was not significantly associated with risk. Similarly, cruciferous vegetable intake was not significantly associated with colorectal, colon, or rectal cancer risk.

Using data from nested case-control studies within the Shanghai Men's and Women's Health Studies, usual cruciferous vegetable intake as assessed by a food frequency questionnaire was weakly correlated with urinary ITC level, while recent crucif-

erous vegetable intake was more strongly correlated with urinary ITC. Overall, the *GST* genotypes were not associated with urinary ITC level, but significant differences according to genotype were observed among current smokers and participants who provided an afternoon urine sample.

In the nested case-control study from the SMHS, CRC risk was not associated with cruciferous vegetable intake, whether measured by self-report or by urinary ITC, nor with *GST* gene variants. No statistical interactions were detected between cruciferous vegetable intake and *GST* gene variants on the odds of CRC. Stratifying by timing of urine sample collection (morning versus afternoon) and excluding colorectal cancer cases diagnosed in the first two years of follow-up did not materially alter the results.

In conclusion, this study does not support an association between cruciferous vegetable intake and CRC in a population of middle-aged and older men in Shanghai, China.

Keywords: China, colorectal cancer, cruciferous vegetables, *GST* gene, men

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INTRODUCTION

Colorectal cancer

Colorectal cancer, which includes cancer located in the colon or rectum, is a significant global concern with an estimated 1,234,108 cases and 609,051 deaths worldwide in 2008. Of these cases and deaths, approximately 18% occurred in China (1). Worldwide, incidence and mortality rates from colorectal cancer have been increasing, especially in areas that previously experienced a lower colorectal cancer burden (2). Currently in China, colorectal cancer ranks fifth for cancer incidence and mortality for both sexes (1) and the incidence of colorectal cancer has consistently been increasing over time (3-5). The increase in colorectal cancer cases in China has been hypothesized to be related to the rapid economic development experienced since the late 1970s which included increased exposure to the Western diet and lifestyle. Western diets have been characterized as diets with increased intakes of calories, fat and proteins and decreased intakes of complex carbohydrates and fiber (6). Lifestyle changes, such as decreases in physical activity and changes in occupational settings, are also associated with Westernization.

Although a number of secondary and tertiary prevention strategies are available to decrease the incidence and mortality from colorectal cancer, primary prevention is important to alleviate some of the burden on the medical system to diagnose and treat the increasing numbers of colorectal cancer patients. Research on the association between potentially modifiable factors, like diet and physical activity, with colorectal cancer is essential to determine an appropriate strategy for primary prevention.

Fruit and vegetable consumption

It has been estimated that more than 50% of colorectal cancer deaths could be avoidable by changes in diet (7). A number of studies have focused on the association between fruits and vegetables with the risk of colorectal cancer. One meta-analysis which included both case-control and cohort studies found a reduction in the risk of colorectal cancer of 6% for high fruit intake and 9% for high vegetable intake, however evidence for the association was stronger in case-control studies than in cohort studies and for the association with colon cancer rather than rectal cancer (8). A more recent meta-analysis which included data from only cohort studies found an inverse association between fruits (pooled RR: 0.90; 95% CI: 0.83, 0.98) and vegetables (pooled RR: 0.91; 95% CI: 0.86, 0.96) with the risk of colorectal cancer, again principally restricted to colon cancer, but this inverse association was observed only in studies in Europe and North America with no association observed in Asian populations (9). A non-linear association with colorectal cancer risk was observed in these cohort studies which suggests a threshold effect for fruit and vegetable intake (9). Additional research exploring this association between fruits and vegetables with the incidence of colorectal cancer within Asian populations that incorporates the possible non-linear association is therefore needed.

Cruciferous vegetable intake and isothiocyanate

One specific group of vegetables, cruciferous vegetables, has also been studied for prevention of colorectal cancer. Cruciferous vegetables include arugula, bok choy, broccoli, Brussels sprouts, cabbage, cauliflower, Chinese cabbage, collard greens, horseradish, kale, kohlrabi, mustard, radish, rutabaga, turnips, wasabi and watercress (10).

Cruciferous vegetables contain nutrients similar to other vegetables, like chlorophyll and fiber, but additionally contain glucosinolates (β-thioglucoside *N*-hydroxysulfates). For fresh cruciferous vegetables, the glucosinolates are converted to isothiocyanate (ITC) by a myrosinase catalyst that is activated after cellular damage from cutting or chewing of the vegetable. During cooking, the internal myrosinase enzyme is degraded; however myrosinase enzyme activity is also present in the large intestine. There, the glucosinolates from cooked cruciferous vegetables are converted to ITC (11).

In general, the most widely proposed mechanisms by which cruciferous vegetables and ITCs may inhibit carcinogenesis and/or suppress survival and proliferation of cancerous cells are the inhibition of phase-I enzymes (carcinogen activating) and the activation of phase-II enzymes (carcinogen detoxifying) during carcinogen metabolism (12). Specifically, cytochrome *P*450 (CYP) has been established as an important enzyme in phase I-dependent metabolism of xenobiotics which can create carcinogenic intermediates (13) and certain ITCs have been found to inhibit certain carcinogen activating CYP enzymes (14). Conversely, ITCs have been observed to activate a number of phase-II enzymes including quinone reductase-1, UDP-glucuronosyltransferases, and thioredoxin reductase, among others (15). These phase-II enzymes are able to conjugate carcinogenic intermediates and create a water soluble byproduct which can then be excreted from the body (16). ITCs have been also observed to reduce oxidative stress, inhibit cell proliferation, induce differentiation and exhibit anti-inflammatory and anti-infection effects (12).

In epidemiological studies, the association between cruciferous vegetable intake, assessed either by a dietary recall method or by urinary ITC, and colorectal cancer has not yielded consistent results. In one early case-control study conducted in the United

States, an inverse association with colorectal cancer was observed for a high intake of cruciferous vegetables compared to low intake, but only among men (4^{th} versus 1^{st} quartile; men: odds ratio [OR]: 0.3, 90% CI: 0.1, 0.8) (17). In contrast, in a prospective cohort study in the Netherlands, an inverse association with colon cancer, but not with rectal cancer, was observed only among women with a high intake of cruciferous vegetables (5^{th} versus 1^{st} quintile; women: incidence rate ratio [IRR]: 0.51, 95% CI: 0.33, 0.80) (18). A case-control study of men in Shanghai, China, found a statistically significant inverse association with colorectal cancer for the highest three quartiles of ITC compared to the lowest quartile of ITC only for colorectal cancer cases whose urine samples were collected at least 5 years prior to diagnosis (2^{nd} through 4^{th} versus 1^{st} quartile; OR: 0.70; 95% CI: 0.49, 0.99) (19). Other studies have found weak inverse associations or independence between cruciferous vegetable consumption and colorectal cancer (20-24). Some of the variability in the findings may be due to different intakes of cruciferous vegetables between populations or variations in genetic polymorphisms between individuals, especially glutathione *S*-transferase (*GST*) gene polymorphisms.

Glutathione *S*-transferase (*GST*) gene polymorphisms

Glutathione *S*-transferases are phase II enzymes that metabolize a variety of compounds in preparation for elimination from the body including ITC. ITC therefore can initiate or increase GST activity and is then later metabolized by GST (25). A number of genetic polymorphisms, including *GSTM1* and *GSTT1*, have been identified to affect the activity of the GST enzymes. Individuals with a homozygous deletion of both copies of the *GSTM1* or *GSTT1* gene do not produce the GSTM1 or GSTT1 enzyme, respectively (25). Therefore, individuals with these deletions may have decreased overall GST activi-

ty which could lead to lengthened exposure to ITC prior to elimination. Individuals with a null genotype may experience increased anti-carcinogenic effects from ITC due to the longer exposure to ITC prior to elimination. In Asian populations, it has been estimated that approximately 53% and 47% have homozygous deletions of *GSTM1* and *GSTT1*, respectively, while about 25% have homozygous deletions of both genes (26).

Previous epidemiological studies have assessed the potential interaction between *GSTM1* and/or *GSTT1* gene polymorphisms with cruciferous vegetable intake and the effect of the interaction on colorectal cancer risk, but no consistent associations have been observed. Interaction in this case would indicate that the effect of ITC would depend on the *GSTM1* and/or the *GSTT1* genotype (27). In a case-control study in Singapore, participants with dual *GSTM1* and *GSTT1* null genotypes were observed to have reduced risk of colorectal cancer with increased cruciferous vegetable intake, but individually, the *GSTM1* and *GSTT1* genotypes did not appear to have a significant interaction with cruciferous vegetable intake (21). However, in the United Kingdom, a significant interaction between *GSTT1*, but not *GSTM1*, with cruciferous vegetable intake was observed on the effect of colorectal cancer and the combination of the two polymorphisms did not yield a statistically significant interaction (22). Other studies found some or no interactions between the *GSTM1* and *GSTT1* genotypes and cruciferous vegetable intake on the risk of colorectal cancer (23;24;28-30).

Shanghai Men's Health Study (SMHS)

This dissertation primarily utilizes data from the Shanghai Men's Health Study (SMHS), a prospective, population-based cohort study conducted in Shanghai, China whose design has been published in detail previously (31). The SMHS recruited men

aged 40 to 74 years old without a previous history of cancer between March 2002 and June 2006. Out of the 82,043 eligible men, 61,482 were included in the cohort for a participation rate of 74.1%. Data on demographic and lifestyle characteristics, dietary and physical activity habits, and medical history were collected from all participants and biological samples were obtained. The men were followed up with interviews and through record linkage with the population-based Shanghai Cancer Registry for incident cancer diagnoses and the Shanghai Municipal Vital Statistics Unit for mortality.

Implications for current research

The research presented in this dissertation represents important contributions to the scientific literature on the topic of the association between fruits and vegetables, with a focus on cruciferous vegetables, and the incidence of colorectal cancer considering potential interactions with genetic polymorphisms in the *GST* gene.

In the first manuscript, we consider the overarching association between the consumption of fruits and vegetables, and various sub-groups of fruits and vegetables, including cruciferous vegetables, with the risk of colorectal cancer. Due to the previous indications of a non-linear association, we categorize fruit and vegetable intake both in quantiles of intake, but also as a continuous, potentially non-linear variable. Furthermore, we assess potential interactions between fruit and vegetable intake with smoking status, body mass index, and physical activity.

In the second manuscript, using nested case-control data from both the Shanghai Women's and Men's Health Studies, we determine factors associated with urinary ITC levels. Specifically, we assess the correlations between self-reported cruciferous vegeta-

ble intake and urinary ITC and the urinary ITC levels stratified by *GST* genotype, in addition to correlations with demographic and health status variables.

Finally, in the third manuscript, we determine the association between cruciferous vegetable intake, as determined by a food frequency questionnaire and urinary ITC, and the risk of colorectal cancer within the nested case-control study from the SMHS in addition to the interaction between cruciferous vegetable intake and *GST* gene polymorphisms.

FRUIT AND VEGETABLE INTAKE AND THE RISK OF COLORECTAL CANCER: RESULTS FROM THE SHANGHAI MEN'S HEALTH STUDY

by

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ABSTRACT

Background: The observed associations between fruit and vegetable consumption with the risk of colorectal cancer (CRC) have been inconsistent in observational studies.

Objective: To evaluate the association of fruit and vegetable consumption with the risk of CRC among men in China.

Design: 61,274 male participants aged 40 to 74 years old were included. A validated food frequency questionnaire was administered to collect information on usual dietary intake, including 8 fruits and 38 vegetables commonly consumed by residents of Shanghai. Follow-up for diagnoses of colon or rectal cancer were available through December 31, 2010. Dietary intakes were categorized into quintiles and analyzed both as categorical and continuous variables. Multivariable-adjusted hazard ratios (HRs) and 95% confidence intervals (95% CIs) were calculated for CRC, colon, and rectal cancer using Cox proportional hazards models.

Results: After 390,688 person-years of follow-up, 398 cases of colorectal cancer (236 colon and 162 rectum) were observed in the cohort. Fruit consumption was inversely associated with the risk of CRC (5^{th} vs. 1^{st} quintile HR: 0.67; 95% CI: 0.48, 0.95; p trend = 0.03) while vegetable intake was not significantly associated with risk. The associations for sub-groups of fruits and legumes, but not other vegetable categories, were generally inversely associated with the risk of colon and rectal cancer.

Conclusions: Fruit intake was inversely associated with the risk of CRC while vegetable consumption was largely unrelated to the risk in middle age and older Chinese men.

Key words: Colorectal cancer; fruits and vegetables; cohort study; Chinese men

INTRODUCTION

Colorectal cancer is a major public health concern with over 1.2 million cases and approximately 609,051 deaths globally in 2008 (1). Worldwide, incidence and mortality rates from colorectal cancer have been on the rise (2) and in China, the incidence has consistently been increasing over the past two or three decades (3-5). This rise in incidence has been suggested to be attributed to the rapid economic development China has experienced since the late 1970s and the resultant increased exposure to the Western diet and lifestyle (3-5). Research on the possible associations between potentially modifiable factors, such as diet, with colorectal cancer is essential if we are to determine an appropriate strategy for primary prevention of colorectal cancer.

The suspected links between fruits and vegetable consumption and colorectal cancer risk have long been investigated, but the evidence has been inconsistent (6). The results have been so inconsistent that the 2007 World Cancer Research Fund and the American Institute for Cancer Research determined that current evidence suggests that the association between almost all fruits and vegetables with colorectal cancer risk is only "limited suggestive" (7). The association may vary by sub-site within the colorectum due to etiological differences which might explain some of the differences in findings across studies (8-12). A recent meta-analysis found that fruits and vegetables had a significant inverse association with colon cancer, but not rectal cancer (13). Effect modification by other lifestyle and dietary factors, like smoking and red meat consumption, has also been noted, although controversial (9;14).

In this report, we evaluated the association between intakes of fruits and vegetables and the risk of colorectal cancer in the Shanghai Men's Health Study (SMHS), a

large population-based cohort study, analyzing the consumption information both continuously and categorically. Using this method, we could also evaluate the presence of a non-linear association. Furthermore, we sought to assess potential interactions of fruit and vegetable intake with smoking status, BMI and physical activity.

METHODS

Study population

We used data collected for the SMHS with methods that have been described in detail previously (15). Briefly, the SMHS is a prospective, population based cohort study in Shanghai, China. Men aged 40 to 74 years old without a previous history of cancer were recruited between March 2002 and June 2006. Out of the 82,043 eligible men, 61,482 were included in the cohort for a participation rate of 74.1%. All participants were interviewed by a trained health professional. The baseline interview obtained information on demographic and lifestyle characteristics, dietary and physical activity habits, and medical history. Anthropometric measurements were taken following a standard protocol. All participants in the SMHS gave informed consent and the study received approval from the Institutional Review Boards of Vanderbilt University and the Shanghai Cancer Institute.

We excluded participants who reported consuming an extreme daily total energy intake (< 500 or > 4,200 kcal; N = 63) and participants with unconfirmed cancer (N = 145), which left 61,274 participants for analysis.

Colorectal cancer ascertainment

Participants in the SMHS were followed up approximately every two to three years for cancer incidence, occurrence of other chronic diseases, and vital status by in-

home visits. Annual record linkage with the population based Shanghai Cancer Registry and the Shanghai Municipal Vital Statistics Unit was also conducted to identify incident cancer cases and decedents, respectively. Incident cancer cases were verified through home visits and medical charts were obtained to document detailed diagnostic information. Colorectal cancer was defined as a primary tumor with an ICD-9 code of 153 (malignant neoplasm of colon) or 154 (malignant neoplasm of rectum, rectosigmoid junction, and anus). Follow-up data up to December 31, 2010 was included in this analysis.

Fruit and vegetable consumption

Usual dietary intakes of 38 vegetable and 8 fruit items were assessed using a validated food frequency questionnaire (FFQ) at baseline. The SMHS FFQ captured about 89% of all average food intake in this population (16). The FFQ assessed how often (daily, weekly, monthly, yearly or never) the participant consumed a specific food or food group. If the participant had consumed that specific food or food group, he was then asked the amount of consumption for that time period. Then the average amounts of each food group were calculated by summing the intake for each food item. Nutrient intake was calculated using the Chinese Food Composition Tables (17).

The FFQ was tested for validity and reliability in this population and has been described in detail elsewhere (16). The correlation coefficients between the estimated intakes of fruits and vegetables from the FFQ compared to that from an average of 12 monthly 24 hour dietary recalls were 0.72 and 0.42, respectively. The FFQ data were used to categorize participants into quantiles of intake based on the distribution of consumption at baseline of participants who did not develop colorectal cancer, and were treated as a continuous variable to assess potential linear and non-linear associations. We

analyzed the data by total fruit, total vegetable, and total fruit and vegetable intake combined, as well as five vegetable subgroups (cruciferous, allium, green leafy, legumes, and other), one fruit subgroup (citrus), and one individual fruit category (watermelon) due to the high intake in this population. For the initial analyses, all groups were categorized into quintiles except for allium vegetables, citrus fruits and watermelon, which were categorized into tertiles due to the low variability of intake. For the analyses of interaction, all groups were categorized into tertiles to keep sufficient sample size for each analysis.

Other covariates of interest

Additional variables available for analysis included a number of demographic, dietary, behavioral and medical factors that were assessed from the baseline questionnaire, the follow-up questionnaire, and/or direct assessment. We selected covariates for adjustment based on the previous literature for their associations with colorectal cancer (9;13). Demographic variables of interest were age, education level, occupation, and annual per capita family income. Participants with data missing on education (N = 856; 1.4%), income (N = 127; 0.2%), or occupation (N = 69; 0.1%) were assigned to the most common categories as follows: high school education, income of 6,000-11,999 yuan per year, and occupation in manual work. Each participant's body mass index (BMI) was calculated from the interviewer measured height and weight of each participant at the baseline visit. Participants with missing data on BMI (N = 35; 0.1%) were set to the median value of BMI (23.67 kg/m^2). For interaction analyses, BMI was categorized as overweight/obese ($\geq$ 25.0 kg/m^2) versus underweight/normal weight ($<$ 25.0 kg/m^2). Behavioral characteristics under consideration were cigarette smoking, alcohol consumption, and amount of leisure time physical activity per week (metabolic equivalent (MET)

hours/day) and obtained from the baseline questionnaire. The sole participant missing data on cigarette smoking and alcohol consumption was categorized in the most common groups as a current smoker and a never drinker. For the interaction analyses, physical activity was categorized as no leisure time physical activity (0 MET hours/day) and some leisure time physical activity (> 0 MET hours/day). We determined history of diabetes mellitus and family history of colorectal cancer from the baseline questionnaire. Participants with missing data on family history of colorectal cancer (N = 36; 0.1%) were assumed to have no such family history. Dietary characteristics of interest were red meat, total meat and total energy intake which were all derived from the FFQ questionnaire.

Statistical analysis

We calculated age-adjusted descriptive statistics by colorectal cancer case status. We applied Cox proportional hazards regression analysis to derive the hazard ratios (HRs) and 95% confidence intervals (95% CIs) to estimate the relative risk of colorectal cancer by quantiles of intake of total fruit, total vegetable, total fruit and vegetable, cruciferous vegetables, allium vegetables, green leafy vegetables, legumes, other vegetables, citrus fruits and watermelon with adjustment for age and total energy intake and other potential confounders. In the Cox regression analysis, the entry time was defined as the age at which the participant started in the SMHS and the exit time was the age at which the participant developed incident colorectal cancer or was censored (e.g. at death, loss to follow up, or on December 31, 2010, whichever occurred first). To evaluate linear trends, we entered the median level of intake for each fruit and/or vegetable category by quantile into the model as a continuous variable. We evaluated the proportional hazards assumption by including an interaction term between the fruit and/or vegetable categories

with the logarithm of time. No significant interactions were observed indicating that the proportional hazards assumption was not violated.

To determine whether the association between the quantiles of intake and colorectal cancer risk was affected by undiagnosed or prevalent colorectal cancer, we repeated the initial analyses excluding the first year of follow-up. We also carried out analyses by excluding participants who reported having a large increase or a large reduction in the intake of fruits and vegetables over the past 5 years. To assess interactions between the fruit and vegetable groups and smoking status (ever vs. never), BMI (overweight/obese vs. underweight/normal weight), and physical activity (none vs. at least some), we included an interaction term in the Cox model for the occurrence of colon and rectal cancer. The interaction was tested using the likelihood ratio test. In order to assess the potential non-linear association between fruits and/or vegetables and the risk of colorectal cancer, we analyzed fruit and vegetable intake as continuous variables by 20 g/day increment in the Cox regression analysis. We also conducted penalized spline regression analysis to test non-linearity of the associations. The Akaike information criterion method was used to select the appropriate degrees of freedom for the test of non-linearity (18). SAS 9.3 was used for all analyses except for the penalized splines models which were created using R. Statistical significance was set as a two-sided p value less than 0.05.

RESULTS

After 390,688 person-years of follow-up and a median follow-up of 6.3 years, 398 cases of colorectal cancer were observed. Of these cases, 236 cases were cancer of the colon and 162 cases were cancer of the rectum. Descriptive statistics by colorectal cancer case status are presented in **Table 1**. Age was highly associated with colorectal can-

cer case status ($p < 0.01$), with cases appreciably older than non-cases. After adjustment for age, colorectal cancer cases were similar to non-cases for mean consumption of fruits and vegetables and individual fruit and vegetable categories, education, income, occupation, cigarette smoking, alcohol consumption, leisure time physical activity, total energy, red meat and total meat intake, history of diabetes and family history of colorectal cancer ($p > 0.05$). However, colorectal cancer cases had a higher average BMI (24.24 vs. 23.72; $p < 0.01$) than non-cases.

For the risk of colorectal, colon and rectal cancers by categories of fruits and vegetables, many estimates were less than one, but few reached statistical significance. Similarly, most of the tests for trend were not statistically significant. An inverse association was observed between total fruits and vegetables and colorectal cancer with a potential dose-response effect (5^{th} vs. 1^{st} quintile HR: 0.71; 95% CI: 0.50, 1.01; p trend = 0.09) while there appeared to be no association between total vegetable intake and colorectal cancer (5^{th} vs. 1^{st} quintile HR: 1.00; 95% CI: 0.72, 1.41; p trend = 0.83). The associations between quintiles of fruit (5^{th} vs. 1^{st} quintile HR: 0.67; 95% CI: 0.48, 0.95; p trend = 0.03) and watermelon intake (3^{rd} vs. 1^{st} tertile HR: 0.77; 95% CI: 0.59, 0.99; p trend = 0.04) with colorectal cancer risk reached statistical significance. The association between total fruits and vegetables and colon cancer (5^{th} vs. 1^{st} quintile HR: 0.69; 95% CI: 0.43, 1.09; p trend = 0.16) and total fruits and both colon (5^{th} vs. 1^{st} quintile HR: 0.76; 95% CI: 0.49, 1.20; p trend = 0.14) and rectal cancers (5^{th} vs. 1^{st} quintile HR: 0.56; 95% CI: 0.33, 0.97; p trend = 0.11) suggest an inverse dose-response effect, but were not significant. In general, the categories of fruit (citrus fruits and watermelon) were inversely associated with colorectal, colon and rectal cancer while the legumes group was the only

vegetable category which showed an inverse association with colorectal, colon and rectal cancer (**Table 2**). The multivariable-adjusted models which excluded the first year of follow-up, in general, yielded similar results, so the remaining analyses utilized data from all years of follow-up (results not shown). After exclusion of participants who reported a substantial increase or decrease in the consumption of fruits and vegetables over the past 5 years, the pattern for the associations remained similar (results not shown).

When fruit and vegetable consumption was analyzed continuously (for a 20 g/day change), a marginally significant inverse linear association was observed between fruits and colon cancer (HR 0.98; $p = 0.06$), fruits and rectal cancer (HR 0.97; $p = 0.06$) and watermelon and rectal cancer (HR 0.96; $p = 0.06$). A significant positive association was observed between allium vegetable consumption and rectal cancer (HR 1.14; $p = 0.04$) (results not shown). Penalized spline models gave no indication for a non-linear association for any of the fruit and vegetable categories (results not shown).

Statistical interactions for the categories of fruits and vegetables were observed with the risk of colon cancer between allium vegetables and BMI (inverse association only for overweight/obese individuals; p interaction = 0.03), citrus fruits and physical activity (inverse association mainly among individuals with no leisure time physical activity; p interaction = 0.02), and green leafy vegetables and physical activity (inverse association only among individuals with at least some leisure time physical activity; p interaction < 0.01). For the risk of rectal cancer, statistical interactions were observed between watermelon and BMI (inverse association only for overweight/obese individuals; p interaction = 0.03), allium vegetables and physical activity (inverse association mainly among individuals with at least some physical activity; p interaction = 0.05) and citrus

fruits and physical activity (inverse association only among individuals with at least some physical activity; p interaction = 0.05) (results not shown). When total fruits and vegetables were stratified by BMI, physical activity and smoking status, fruit consumption showed an inverse association with the risk of rectal cancer, but only among overweight or obese participants (3^{rd} vs. 1^{st} tertile HR: 0.28; 95% CI: 0.13, 0.60). Total fruit and vegetable consumption also appeared to have an inverse association with rectal cancer only among individuals with at least some leisure time physical activity (3^{rd} vs. 1^{st} tertile HR: 0.54; 95% CI: 0.29, 1.02) while total fruits only had an inverse association with colon cancer risk among ever smokers (3^{rd} vs. 1^{st} tertile HR: 0.59; 95% CI: 0.37, 0.95) (**Table 3**).

DISCUSSION

In this prospective cohort study of men in Shanghai, China, we found an inverse association between fruit intake and the risk of colorectal, colon and rectal cancers. There was little evidence for an association between total vegetable intake and colorectal cancer; although an inverse association was observed for the intake of legumes. When data from the first year of follow-up or participants who reported to have a large change in fruit or vegetable intake were excluded, the estimates of the association patterns were largely unchanged. Some statistical interactions were observed between the fruit and vegetable categories with BMI, smoking, and physical activity, but these findings should be interpreted with caution as they could have possibly resulted from multiple comparisons.

A recent meta-analysis, which included 22 publications, all of which were cohort studies, calculated summary relative risk estimates (RR) of 0.92 (95% CI: 0.86, 0.99),

0.91 (95% CI: 0.84, 0.99) and 0.97 (95% CI: 0.86, 1.09) for the association between the highest category versus the lowest category of intake of total fruits and vegetables and colorectal, colon and rectal cancer risk, respectively. These estimates were similar for fruits and vegetables separately. When the data were stratified by the geographic location of the studies, the summary RRs were 1.17 (95% CI: 0.94, 1.45) for total fruits and vegetables, 1.00 (95% CI: 0.79, 1.28) for total fruits, and 1.02 (95% CI: 0.89, 1.18) for total vegetables in Asian studies (13). The null finding of our study for vegetable intake, thus, is in general agreement with findings from these Asian studies (19-22). The meta-analysis also found an indication of a non-linear inverse association between fruit and vegetable intake with colorectal cancer where the risk reduction was strongest for increases from very low levels of fruit and vegetable intake (13). Because our population, like many other Asian populations, consumes fairly high levels of vegetables, with a mean of approximately 344 g/day (inter-quartile range 212.6 – 429.4 g/day), it may explain why we did not find a significant inverse effect in our study, since our study had very few subjects who consumed low levels of vegetables. Additionally, the length of follow-up for this study was also not as long as some of the studies included in the meta-analysis (13).

For the associations between sub-groups of fruit and vegetables and colorectal, colon and rectal cancers, the results from previous studies have been inconsistent. A number of studies did not find any independent associations between cruciferous vegetable intake with colorectal cancer risk (8;11;23-28), however, a recent meta-analysis found a significant inverse association with a pooled relative risk of 0.82 (95% CI: 0.75, 0.90) for the highest versus the lowest category of intake (29). Similarly, a meta-analysis

found that increased garlic consumption, an allium vegetable, significantly decreased the risk of colorectal cancer with a pooled relative risk for the highest versus the lowest category of intake of 0.66 (95% CI: 0.48, 0.91) (30). However, the majority of studies included were of case-control design and therefore the pooled estimate may have been affected by recall bias. And a recent case-control study did not observe a significant association between garlic intake and colorectal cancer risk (8). Similarly, no association between onions or leeks, which are allium vegetables, with the risk of colon or rectal cancer was observed in a prospective cohort study (31). No consistent association has been observed between legumes and green leafy vegetables on the risk of colorectal cancer (8;10;12;27;28); although a few studies have observed an inverse effect for one or both of these vegetable categories (8;11;28). Citrus fruit has also not been strongly associated with the rate of colorectal cancer (8;10;11;27;28). Few studies individually assessed the association between watermelon intake and colorectal cancer, although the association with lycopene, which is found mainly in tomatoes but is also found in watermelon, has been inconsistent (32-34).

Our study is not without limitations. First, all of the fruit and vegetables intakes were assessed using an FFQ which may not be accurate at estimating the actual amount of dietary intake. However, in a validation study, the FFQ tended to be relatively accurate for fruit intake with some overestimation for the intake of vegetables (16) and FFQs are generally useful for ranking intake which was our main analytic technique in this analysis. We excluded participants who had extreme energy intake in order to remove participants who may not have been accurately reporting nutritional intake. Second, this study was underpowered to detect modest associations. However, the analyses treating

fruit and vegetable intake as a continuous variable, which tend to have more power, found similar results as indicated in the categorical analysis. Finally, although we adjusted for a number of confounders, we cannot rule out residual confounding by unmeasured or unadjusted factors.

This study has a number of important strengths. First, the SMHS is a rigorously designed cohort study with high participation and retention rates. Second, all covariates used in our analyses were assessed prior to the development of any cancer, thereby decreasing the potential for misclassification bias. Third, we determined that prevalent cancer was unlikely to have affected the results because after excluding the first and second years of follow-up our results were unchanged. Finally, results of the many secondary analyses that we conducted yielded similar results which suggests that our findings are robust.

In conclusion, we found that fruit consumption was inversely associated with the risk of colorectal cancer while vegetable intake was largely unrelated to colorectal cancer risk. Given that few individuals consumed low levels of vegetables in our and other Asian studies, pooling data from studies within Asian populations may be necessary to clarify the effect of low vegetable intake on colorectal cancer risk.

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Table 1: Baseline characteristics by colorectal cancer case status of the Shanghai Men's Health Study participants (N = 61,274)

	Colorectal cancer cases	Non-cases	P value
Number of subjects	398	60,876	
Total fruits and vegetables (g/day)	491.55 ± 13.14	495.22 ± 1.06	0.78
Total vegetables (g/day)	349.17 ± 9.64	343.56 ± 0.78	0.56
Total fruits (g/day)	142.38 ± 6.32	151.66 ± 0.51	0.14
Cruciferous vegetables (g/day)	111.71 ± 3.75	110.51 ± 0.30	0.75
Allium vegetables (g/day)	17.21 ± 0.87	16.40 ± 0.07	0.35
Legumes (g/day)	40.49 ± 1.63	40.82 ± 0.13	0.84
Green leafy vegetables (g/day)	102.44 ± 3.39	100.28 ± 0.27	0.53
Other vegetables (g/day)	158.85 ± 5.52	155.34 ± 0.45	0.53
Citrus fruits (g/day)	11.36 ± 0.92	12.55 ± 0.07	0.20
Watermelon (g/day)	72.92 ± 3.97	79.13 ± 0.32	0.12
Age (years)	63.21 ± 0.49	55.31 ± 0.04	< 0.01
Educational level (%)			
≤ Elementary school	6.16%	6.62%	0.59
Middle school	32.59%	33.05%	
High school	34.98%	36.97%	
≥ College	26.27%	23.36%	
Annual per capita family income (%)			
< 6,000 yuan	8.79%	12.55%	0.21
6,000 - 11,999 yuan	39.21%	42.69%	
12,000 - 23,999 yuan	40.48%	35.06%	
≥ 24,000 yuan	11.52%	9.70%	
Occupation			
Professional	27.39%	26.53%	0.80
Clerical	22.71%	21.92%	
Manual worker	49.90%	51.55%	
Cigarette smoking (%)			
Never	32.23%	30.42%	0.25
Past	8.95%	10.98%	
Current	58.83%	58.60%	
Alcohol consumption (%)			
Never	63.02%	66.33%	0.72
Past	4.04%	4.42%	
Current	32.94%	29.25%	
BMI (kg/m^2)	24.24 ± 0.15	23.72 ± 0.01	< 0.01
Leisure time physical activity (MET hours/week) Median (Q1, Q3)	0 (0, 17.15)	0 (0, 7.98)	0.62

	Colorectal cancer cases	Non-cases	P value
Total energy intake (Kcal/day)	1945.35 ± 23.87	1906.98 ± 1.93	0.11
Red meat intake (g/day)	62.77 ± 2.17	63.09 ± 0.17	0.88
Total meat intake (g/day)	133.78 ± 3.90	130.76 ± 0.31	0.44
History of diabetes (%)	7.81%	6.26%	0.15
Family history of colorectal cancer (%)	3.40%	2.10%	0.49

For all characteristics except age and physical activity, means ± standard error and percentages were adjusted for age at baseline.

All P values, other than age, were adjusted for age. The P values were calculated using the Cochran-Mantel Haenszel test for a general association for categorical variables and the test of difference in least-squared means for continuous variables except for physical activity which was tested using physical activity ranks in a general linear model due to non-normality.

BMI: Body mass index; MET: metabolic equivalents.

Table 2: Hazard ratios (HRs) for associations between the intakes of various fruits and vegetables and colorectal cancer incidence in the Shanghai Men's Health Study (N = 61,274)

	Colorectal cancer			Colon cancer			Rectal cancer		
	Cases	**HR**	**(95% CI)**	**Cases**	**HR**	**(95% CI)**	**Cases**	**HR**	**(95% CI)**
Total fruits and vegetables									
< 284.34	88	Ref		52	Ref		36	Ref	
284.34-395.10	73	0.82	(0.60, 1.12)	44	0.83	(0.55, 1.24)	29	0.80	(0.49, 1.31)
395.11-510.38	85	0.93	(0.68, 1.26)	50	0.92	(0.61, 1.37)	35	0.94	(0.58, 1.51)
510.39-675.14	79	0.85	(0.61, 1.17)	49	0.89	(0.59, 1.35)	30	0.79	(0.47, 1.32)
≥ 675.15	73	0.71	(0.50, 1.01)	41	0.69	(0.43, 1.09)	32	0.75	(0.44, 1.29)
p trend		0.09			0.16			0.34	
Total vegetables									
< 192.60	82	Ref		52	Ref		30	Ref	
192.60-269.05	83	1.04	(0.76, 1.41)	47	0.94	(0.63, 1.39)	36	1.22	(0.75, 1.98)
269.06-348.84	76	0.97	(0.70, 1.33)	44	0.89	(0.59, 1.34)	32	1.11	(0.67, 1.85)
348.85-466.63	72	0.88	(0.64, 1.23)	43	0.84	(0.55, 1.28)	29	0.97	(0.57, 1.65)
≥ 466.64	85	1.00	(0.72, 1.41)	50	0.95	(0.62, 1.47)	35	1.10	(0.64, 1.89)
p trend		0.83			0.80			0.97	
Total fruits									
< 42.38	87	Ref		49	Ref		38	Ref	
42.38-99.93	82	0.84	(0.62, 1.14)	53	0.96	(0.65, 1.43)	29	0.68	(0.42, 1.11)
99.94-160.41	79	0.80	(0.58, 1.09)	47	0.84	(0.55, 1.27)	32	0.74	(0.45, 1.21)
160.42-239.23	77	0.76	(0.55, 1.05)	41	0.71	(0.46, 1.11)	36	0.82	(0.50, 1.33)
≥ 239.24	73	0.67	(0.48, 0.95)	46	0.76	(0.49, 1.20)	27	0.56	(0.33, 0.97)
p trend		0.03			0.14			0.11	

	Colorectal cancer			Colon cancer			Rectal cancer		
	Cases	**HR**	**(95% CI)**	**Cases**	**HR**	**(95% CI)**	**Cases**	**HR**	**(95% CI)**
Cruciferous vegetables									
< 50.07	73	Ref		41	Ref		32	Ref	
50.07-78.70	89	1.25	(0.92, 1.71)	56	1.40	(0.93, 2.10)	33	1.06	(0.65, 1.73)
78.71-112.99	75	1.04	(0.75, 1.44)	46	1.14	(0.74, 1.74)	29	0.92	(0.55, 1.53)
113.00-159.74	73	1.00	(0.72, 1.39)	40	0.97	(0.63, 1.52)	33	1.03	(0.63, 1.69)
≥ 159.75	88	1.12	(0.81, 1.55)	53	1.20	(0.79, 1.84)	35	1.02	(0.62, 1.68)
p trend		0.93			0.94			0.95	
Allium vegetables									
< 8.29	138	Ref		80	Ref		58	Ref	
8.29-16.40	127	0.97	(0.76, 1.24)	86	1.15	(0.84, 1.56)	41	0.74	(0.49, 1.10)
≥ 16.41	133	0.99	(0.77, 1.27)	70	0.91	(0.65, 1.28)	63	1.09	(0.75, 1.59)
p trend		0.96			0.44			0.40	
Legumes									
< 16.99	88	Ref		50	Ref		38	Ref	
16.99-27.62	90	1.00	(0.75, 1.35)	54	1.07	(0.72, 1.57)	36	0.91	(0.58, 1.45)
27.63-39.36	77	0.85	(0.62, 1.16)	48	0.95	(0.63, 1.42)	29	0.72	(0.44, 1.18)
39.37-58.23	63	0.69	(0.50, 0.96)	35	0.69	(0.44, 1.07)	28	0.70	(0.42, 1.15)
≥ 58.24	80	0.82	(0.59, 1.13)	49	0.92	(0.60, 1.40)	31	0.69	(0.42, 1.14)
p trend		0.10			0.39			0.13	
Green leafy vegetables									
< 44.97	83	Ref		49	Ref		34	Ref	
44.97-71.41	87	1.09	(0.81, 1.47)	50	1.06	(0.71, 1.58)	37	1.14	(0.71, 1.82)
71.42-101.83	74	0.95	(0.69, 1.30)	47	1.03	(0.69, 1.54)	27	0.84	(0.50, 1.40)
101.84-144.54	67	0.85	(0.61, 1.18)	40	0.87	(0.57, 1.32)	27	0.83	(0.50, 1.39)
≥ 144.55	87	1.05	(0.77, 1.43)	50	1.03	(0.68, 1.55)	37	1.08	(0.67, 1.76)
p trend		0.90			0.85			0.98	

	Colorectal cancer			Colon cancer			Rectal cancer		
	Cases	**HR**	**(95% CI)**	**Cases**	**HR**	**(95% CI)**	**Cases**	**HR**	**(95% CI)**
Other vegetables									
< 72.33	79	Ref		49	Ref		30	Ref	
72.33-109.85	84	1.12	(0.82, 1.53)	51	1.10	(0.74, 1.64)	33	1.15	(0.70, 1.89)
109.86-152.23	85	1.13	(0.83, 1.55)	43	0.93	(0.61, 1.41)	42	1.45	(0.90, 2.35)
152.24-220.32	65	0.87	(0.61, 1.22)	41	0.89	(0.58, 1.37)	24	0.83	(0.47, 1.44)
≥ 220.33	85	1.05	(0.75, 1.47)	52	1.07	(0.69, 1.64)	33	1.03	(0.60, 1.77)
p trend		0.79			0.94			0.63	
Citrus fruits									
< 2.70	136	Ref		82	Ref		54	Ref	
2.70-12.60	125	0.86	(0.67, 1.11)	68	0.78	(0.56, 1.09)	57	0.99	(0.67, 1.45)
≥ 12.61	137	0.82	(0.64, 1.06)	86	0.86	(0.62, 1.19)	51	0.76	(0.51, 1.14)
p trend		0.19			0.62			0.14	
Watermelon									
< 33.33	146	Ref		88	Ref		58	Ref	
33.33-93.32	125	0.91	(0.71, 1.17)	72	0.87	(0.63, 1.21)	53	0.97	(0.66, 1.42)
≥ 93.33	127	0.77	(0.59, 0.99)	76	0.76	(0.55, 1.06)	51	0.77	(0.51, 1.15)
p trend		0.04			0.11			0.17	

All models were adjusted for age, total energy intake, red meat intake, total meat intake, education, income, occupation, smoking status, alcohol consumption, BMI, physical activity, history of diabetes mellitus, and family history of colorectal cancer.

Quantile cut-points are presented in g/day.

Table 3: Stratified hazard ratios (HRs) by BMI, physical activity and smoking for associations between fruits and vegetables with colon and rectal cancer incidence in the Shanghai Men's Health Study (N = 61,274)

	Colon cancer				Rectal cancer			
	Overweight/Obese N = 20,273		Under/Normal weight N = 41,001		Overweight/Obese N = 20,273		Under/Normal weight N = 41,001	
	HR	**(95% CI)**	**HR**	**(95% CI)**	**HR**	**(95% CI)**	**HR**	**(95% CI)**
Total fruits and vegetables		*p interaction* = 0.58				*p interaction* = 0.27		
< 358.76	Ref		Ref		Ref		Ref	
358.76-556.41	0.85	(0.53, 1.38)	1.10	(0.72, 1.69)	0.55	(0.29, 1.05)	1.07	(0.65, 1.76)
≥ 556.42	0.89	(0.53, 1.48)	0.85	(0.52, 1.40)	0.41	(0.20, 0.81)	1.39	(0.82, 2.35)
p trend	0.69		0.48		0.01		0.21	
Total vegetables		*p interaction* = 0.25				*p interaction* = 0.59		
< 244.93	Ref		Ref		Ref		Ref	
244.93-380.34	0.79	(0.49, 1.29)	1.14	(0.75, 1.73)	0.71	(0.37, 1.37)	1.27	(0.79, 2.03)
≥ 380.35	0.99	(0.61, 1.60)	0.79	(0.48, 1.28)	0.60	(0.30, 1.18)	1.11	(0.65, 1.89)
p trend	0.94		0.29		0.16		0.75	
Total fruits		*p interaction* = 0.32				*p interaction* < 0.01		
< 80.79	Ref		Ref		Ref		Ref	
80.79-183.55	1.19	(0.74, 1.92)	0.71	(0.46, 1.10)	0.78	(0.43, 1.42)	1.06	(0.63, 1.78)
≥ 183.56	0.85	(0.50, 1.44)	0.70	(0.44, 1.13)	0.28	(0.13, 0.60)	1.72	(1.03, 2.89)
p trend	0.42		0.16		< 0.01		0.03	

	Colon cancer				Rectal cancer			
	No physical activity N = 39,502		At least some physical activity N = 21,772		No physical activity N = 39,502		At least some physical activity N = 21,772	
	HR	**(95% CI)**	**HR**	**(95% CI)**	**HR**	**(95% CI)**	**HR**	**(95% CI)**
Total fruits and vegetables		*p interaction* = 0.40			**Total fruits and vegetables**		*p interaction* = 0.09	
< 358.76	Ref		Ref		< 358.76	Ref	Ref	
358.76-556.41	1.13	(0.73, 1.74)	0.88	(0.55, 1.40)	358.76-556.41	1.00	(0.58, 1.72)	0.70	(0.40, 1.23)
≥ 556.42	0.81	(0.48, 1.37)	0.92	(0.56, 1.51)	≥ 556.42	1.37	(0.78, 2.40)	0.54	(0.29, 1.02)
p trend	0.42		0.81		*p trend*	0.24		0.06	
Total vegetables		*p interaction* = 0.02			**Total vegetables**		*p interaction* = 0.18	
< 244.93	Ref		Ref		< 244.93	Ref	Ref	
244.93-380.34	1.31	(0.86, 1.99)	0.69	(0.43, 1.12)	244.93-380.34	1.27	(0.75, 2.15)	0.83	(0.48, 1.44)
≥ 380.35	0.79	(0.47, 1.33)	0.95	(0.59, 1.51)	≥ 380.35	1.28	(0.72, 2.25)	0.60	(0.32, 1.11)
p trend	0.35		0.98		*p trend*	0.43		0.10	
Total fruits		*p interaction* = 0.20			**Total fruits**		*p interaction* = 0.62	
< 80.79	Ref		Ref		< 80.79	Ref	Ref	
80.79-183.55	0.72	(0.46, 1.15)	1.13	(0.71, 1.78)	80.79-183.55	1.03	(0.60, 1.75)	0.81	(0.45, 1.45)
≥ 183.56	0.81	(0.50, 1.32)	0.72	(0.43, 1.21)	≥ 183.56	1.10	(0.63, 1.92)	0.75	(0.41, 1.39)
p trend	0.43		0.13		*p trend*	0.74		0.39	

	Colon cancer					Rectal cancer			
	Ever smoked N = 42,645		Never smoked N = 18,629			Ever smoked N = 42,645		Never smoked N = 18,629	
	HR	**(95% CI)**	**HR**	**(95% CI)**		**HR**	**(95% CI)**	**HR**	**(95% CI)**
Total fruits and vegetables		*p interaction* = 0.20			**Total fruits and vegetables**		*p interaction* = 0.29		
< 358.76	Ref		Ref		< 358.76	Ref		Ref	
358.76-556.41	0.90	(0.61, 1.34)	1.20	(0.70, 2.07)	358.76-556.41	0.71	(0.45, 1.13)	1.43	(0.63, 3.26)
≥ 556.42	0.61	(0.38, 0.99)	1.42	(0.81, 2.49)	≥ 556.42	0.79	(0.48, 1.28)	1.38	(0.58, 3.26)
p trend	0.04		0.23		*p trend*	0.36		0.56	
Total vegetables		*p interaction* = 0.68			**Total vegetables**		*p interaction* = 0.27		
< 244.93	Ref		Ref		< 244.93	Ref		Ref	
244.93-380.34	0.99	(0.66, 1.49)	0.97	(0.58, 1.61)	244.93-380.34	1.15	(0.74, 1.78)	0.74	(0.34, 1.62)
≥ 380.35	0.79	(0.50, 1.25)	1.06	(0.63, 1.80)	≥ 380.35	0.81	(0.48, 1.34)	1.08	(0.51, 2.28)
p trend	0.29		0.79		*p trend*	0.36		0.69	
Total fruits		*p interaction* = 0.23			**Total fruits**		*p interaction* = 0.74		
< 80.79	Ref		Ref		< 80.79	Ref		Ref	
80.79-183.55	0.97	(0.66, 1.44)	0.81	(0.47, 1.38)	80.79-183.55	0.86	(0.55, 1.35)	1.20	(0.52, 2.80)
≥ 183.56	0.59	(0.37, 0.95)	0.94	(0.54, 1.63)	≥ 183.56	0.85	(0.52, 1.37)	1.18	(0.50, 2.80)
p trend	0.03		0.99		*p trend*	0.51		0.78	

BMI stratified models were adjusted for age, total energy intake, red meat intake, total meat intake, education, income, occupation, smoking status, alcohol consumption, BMI, physical activity, family history of colorectal cancer, and history of diabetes mellitus.

Physical activity stratified models adjusted for age, total energy intake, red meat intake, total meat intake, education, income, occupation, smoking status, alcohol consumption, BMI, family history of colorectal cancer, and history of diabetes mellitus. The at least some physical activity model additionally adjusted for physical activity (continuous).

Models stratified by smoking adjusted for age, total energy intake, red meat intake, total meat intake, education, income, occupation, alcohol consumption, BMI, physical activity, family history of colorectal cancer, and history of diabetes mellitus. The ever smoker models additionally adjusted for current smoking.

Quantile cut-points are presented in g/day.

FACTORS ASSOCIATED WITH URINARY ISOTHIOCYANATE LEVELS IN CHINESE WOMEN AND MEN

by

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ABSTRACT

Purpose: Isothiocyanate (ITC) levels in the urine are a biomarker of cruciferous vegetable intake and also reflect levels of glutathione *S*-transferase (*GST*) enzymatic activity. We assessed these and other determinants of urinary ITC level in a population of over 4,000 people in Shanghai, China.

Methods: This study included participants in the Shanghai Women's Health Study (3,589 women) and the Shanghai Men's Health Study (1,015 men) whose urinary ITC levels had been previously ascertained. Urinary ITC was assessed using high-performance liquid chromatography. Usual dietary intake of cruciferous vegetables was assessed using a validated food frequency questionnaire and total dietary ITC was calculated. Recent cruciferous vegetable intake was also determined. *GST* genotypes were assessed using duplex real-time quantitative polymerase chain reaction assays. Spearman correlations were calculated between the covariates and urinary ITC levels and linear regression analyses were used to calculate the mean urinary ITC according to *GST* genotype.

Results: The median value for urinary ITC was 1.61 nmol/mg creatinine. Self-reported usual cruciferous vegetable intake was weakly correlated with urinary ITC level in men ($r_s = 0.1733$) and in women ($r_s = 0.0988$), while self-reported recent cruciferous vegetable intake was more strongly correlated with urinary ITC ($r_s = 0.2400$ for number of times consumed cruciferous vegetables in 24 hours before urine collection in men and women combined). Overall, the *GST* genotypes were not associated with urinary ITC level, but significant differences according to genotype were observed among current smokers and participants who provided an afternoon urine sample.

Conclusions: In this population with relatively high cruciferous vegetable consumption, self-reported intake was only weakly correlated with ITC level. *GST* genotypes, in addition to other factors, may be important in ITC metabolism and excretion, but other factors appear to explain more variation in urinary levels of ITC.

INTRODUCTION

Cruciferous vegetables, which include bok choy, broccoli and turnips, have in common the presence of glucosinolates. When cruciferous vegetables are consumed, these glucosinolates are converted to isothiocyanate (ITC) (1, 2). ITC is thought to prevent cancer through the inhibition of phase-I enzymes (carcinogen activating) and the activation of phase-II enzymes (carcinogen detoxifying) (2). Glutathione *S*-transferases (GSTs) are phase II enzymes which are induced by and catalyze the conjugation of ITCs for excretion in the urine (3, 4). Interactions of cruciferous vegetable consumption and/or urinary ITC levels with polymorphisms in the *GST* genes that encode these enzymes, have therefore been evaluated in numerous studies on the risk of cancers of the breast, colorectum, lung, and stomach (5-11). Deletions of the *GST* genes are associated with altered enzymatic GST activity (12, 13).

Cruciferous vegetable consumption is typically assessed using a dietary recall instrument such as a food frequency questionnaire (FFQ). However, the data on cruciferous vegetables derived from FFQs have several limitations, including recall errors (14). There are additional limitations when calculating dietary ITC values, which combine FFQ data with laboratory data on the ITC content of the specific vegetables. Because of the limitations of dietary recall data, a biomarker of cruciferous vegetable intake, such as urinary ITC, has been used to quantify this exposure. However, ITC is excreted rapidly after cruciferous vegetable consumption with all ITC metabolites eliminated within 48 hours (15). Therefore, urinary ITC level reflects only very recent intake, whereas a FFQ assesses usual intake over a longer period of time.

Although the intake of cruciferous vegetables and *GST* gene polymorphisms are likely the most important factors determining urinary ITC excretion, factors such as age, gender, body mass index (BMI), the intake of other nutrients, and kidney function have all been associated with urinary excretion of metabolites (16-18). Therefore, in order to evaluate the extent to which individual factors affect levels of urinary ITC, our goal was to identify all factors, including self-reported cruciferous vegetable intake and *GST* gene variants, that are associated with urinary ITC levels using data from the Shanghai Women's Health Study (SWHS) and Shanghai Men's Health Study (SMHS).

METHODS

Source population

The SWHS and SMHS are prospective, population-based cohort studies in Shanghai, China, with details of the study designs have been published previously (19, 20). In brief, for the SWHS, 74,941 women living in Shanghai aged 40 to 70 years old were recruited from 1996 to 2000. For the SMHS, 61,483 men aged 40 to 74 years old with no cancer history were recruited from 2002 to 2006. The participation rates for the SWHS and SMHS were 92.7% and 74.1%, respectively. Trained interviewers administered the baseline surveys and obtained anthropometric measurements and biological samples (spot urine, blood and/or buccal cells). Buccal cell samples were requested only if the participant was unwilling to provide a blood sample. After collection, biological samples were transported in Styrofoam boxes with ice packs at 0-4°C and then placed into long term storage at -70°C. Follow-up interviews were conducted in 2000 to 2002 for the SWHS and in 2004 to 2008 for the SMHS with participation rates at the second interview of 99.8% and 97.6%, respectively. For both cohorts, the interviews were ap-

proved by all relevant Institutional Review Boards and informed consent was obtained from all participants.

Nested case-control study participants

Data was available from three nested case-control studies within the SWHS and one within the SMHS which were conducted to assess the association between urinary ITC and cancer. The methods for the studies of colorectal cancer and lung cancer in the SWHS have been published previously (7, 8). We also included ITC data from nested case-control studies of breast cancer in the SWHS and colorectal cancer in the SMHS. In summary, from the SWHS, 430 breast cancer cases diagnosed before February 2007, 328 colorectal cancer cases diagnosed before January 2006, and 232 lung cancer cases diagnosed before February 2003, were selected for urinary ITC ascertainment. From the SMHS, 341 colorectal cancer cases diagnosed before January 2011 were selected. In both cohorts, incidence density sampling was used to select controls that were free of cancer at the time of the case's cancer diagnosis. The controls were matched to cases on sex, age at baseline (± 2 years), date (± 30 days) and time (morning or afternoon) of urine collection, interval since last meal (± 2 hours), menopausal status (pre- or post-; women only), and antibiotic use in the past week (yes or no). For the female colorectal and lung cancer cases, 4 controls were selected for each case. For each of the breast cancer cases, 2-4 controls were selected, and for each of the male colorectal cancer cases, 2 controls were selected. Within the SWHS, some controls were used in more than one of the nested case-control studies (shared controls) and some of these controls developed cancer after sampling. The nested case-control study of colorectal cancer in the SMHS did not use shared controls, but controls could also have become cases after sampling. Therefore, for

this analysis, we considered participants who ever developed cancer during follow-up (through December 31, 2010) to be cases and those who did not to be non-cases. For the present analysis, we combined data from all four nested case-control studies with 3,603 women and 1,020 men. Cancer status of all participants was defined as whether cancer had been diagnosed on or before December 31, 2010.

Assessment of cruciferous vegetable intake and dietary ITC

Usual dietary intake over the past 12 months was assessed at baseline and follow-up using a validated food frequency questionnaire (FFQ) in both the SWHS and SMHS. The FFQ captured about 86% and 89% of the average food intake in the population for the SWHS and SMHS, respectively, and was tested for validity and reliability (21, 22). The FFQ assessed how often (daily, weekly, monthly, yearly or never) the participant consumed a specific food or food group. If the participant had consumed that food or food group, she or he was then asked the amount consumed for that time period. The average amounts of each food group were calculated by summing the intake for each component food. Nutrient intake was calculated using the Chinese Food Composition Tables (23).

Consumption of commonly consumed cruciferous vegetables, such as Chinese greens, green cabbage, Chinese cabbage/bok choy cabbage, cauliflower and white turnip/radish, was assessed in the FFQs for both the SWHS and SMHS. Their intake was combined to create the total cruciferous vegetable variable and was also used to calculate the average intake of dietary ITC by linking the reported consumption of each cruciferous vegetable to data on ITC content of those cruciferous vegetables in Asia (24, 25). We calculated these intake variables both using only baseline data and by averaging the base-

line and follow-up responses. Among participants who were alive and cancer-free at follow-up, and who provided follow-up questionnaire data (N = 3,989; 86.6%), the baseline and follow-up responses for cruciferous vegetable intake and total energy intake were averaged, while baseline values were used for the other participants (N = 615; 13.4%).

Participants were asked about recent consumption of green cabbage, Chinese cabbage and cauliflower at the baseline urine sample collection. The question assessed the total number of times that these foods were consumed over the last week, the total number of times that these foods were consumed over the past 24 hours, and how many days or hours ago the participant had last consumed these foods. The participants who reported to not have consumed these cruciferous vegetables over the last week were categorized to have last consumed cruciferous vegetables 8 days ago.

Measurement of urinary ITC

High-performance liquid chromatography was used to determine total urinary ITC and ITC metabolites as previously described in detail (8, 26). For these assays, laboratory staff was blinded to the case-control status of the samples and all urine samples and standards were assayed in triplicate for the SWHS and in duplicate for the SMHS. In each laboratory run, representative standards (three in SWHS and two in SMHS) and a reagent blank were included. A standard curve was created weekly using data from samples of *N*-acetyl-L-cysteine conjugates of phenethyl ITC (0.2-25 mmol/L) in urine obtained from subjects on a controlled diet. The average of the ITC measurements for each participant was calculated and used for analysis. If the standard deviation of the mean was greater than 10%, the individual ITC values were checked and the sample was reanalyzed as necessary. To control for variability between batches, all samples for each set of cases

and controls were included in the same analytic run. The limit of detection for urinary ITC was 0.1 μmol/L. For undetectable ITC levels, the value was set to 0.1 μmol/L divided by the square root of two. All ITC levels were adjusted for urine creatinine level and reported as nmol/mg creatinine.

For the SWHS, the urinary ITC analysis was conducted in 3 batches, where batch 1 was completed in July of 2006, batch 2 in August of 2007, and batch 3 in August of 2008. The SMHS analysis was completed in August of 2012 and was completed in one batch. Time between sample collection and sample processing was calculated in order to adjust for length of urine storage time. The within batch and between batch coefficients of variation were 15.1% and 13.7%, respectively.

GST genotyping

DNA was extracted from blood (86.1%) and buccal cell (13.9%) samples. For both the SWHS and SMHS, the copy numbers (0, 1, or 2 gene copies) of the *GSTM1* and *GSTT1* genes were assessed using duplex real-time quantitative polymerase chain reaction (PCR)-based assays using the methods described in the NCI SNP500 project with modifications (27). The sequences used in the assay design were obtained from GenBank (*GSTM1*, NM_000561 and *GSTT1*, NM_000853). The real-time PCR were conducted in a 384-well plate in ABI PRISM 7900 Sequence Detection Systems (Applied Biosystems, Foster City, CA). The laboratory staff was blinded to the case-control status of the samples. Coriell DNA samples containing 0, 1 or 2 copies of the *GSTM1* and *GSTT1* genes were included for internal quality control. The concordance rate for quality control samples, including water, Coriell DNA and blinded DNA samples was 100%. *GSTM1* genotypes were within Hardy-Weinberg (H-W) equilibrium among the non-cases from the

SWHS (p = 0.1143) and SMHS (p = 0.1897). *GSTT1* genotypes were also within H-W equilibrium among the non-cases from the SWHS (p = 0.6924) and SMHS (p = 0.8361).

GSTM1 and *GSTT1* were categorized as *GSTM1*-null or *GSTT1*-null versus carrier (0 versus 1 or 2 copies). A combined category of *GSTM1* and *GSTT1* was also included with three groups: *GSTM1*-null and *GSTT1*-null, one null and one carrier genotype, and both carrier.

Other covariates of interest

Additional variables that were available for study included several demographic, dietary, behavioral and medical factors assessed in the baseline questionnaire. Demographic variables of interest were age and education level. Participants with missing data for education (4 women and 19 men) were set as the most common educational category, high school education. Each participant's BMI was calculated from the interviewer-measured height and weight from the baseline visit. Behavioral characteristics, assessed at baseline, included cigarette smoking, alcohol consumption, tea consumption, ginseng intake, amount of exercise per week (MET hours/day), and menopausal status for women. The two women with missing menopausal status were considered pre-menopausal because they were younger than the median age of menopause in our sample of 49.5 years. We considered self-reported prevalent conditions (including pulmonary TB, chronic bronchitis, asthma, chronic gastritis, chronic hepatitis, gallstones, diabetes, high blood pressure, coronary heart disease, stroke, and polyps) as well as previous surgical interventions (including gastrectomy and cholecystectomy) reported at the baseline interview. Participants were not specifically asked about prevalent kidney disorders, but were able to list additional prevalent conditions or diseases, which were categorized using

ICD-9 codes. Because urinary ITC levels may be altered by diminished kidney function, we created categories for chronic kidney disease (ICD-9 403, 404, and 585), nephritis (ICD-9 580-589) other urinary disorders (ICD-9 590-599), and for a combined "any kidney disorder" (ICD-9 403, 404, 580-599).

Statistical analysis

From the SWHS, we excluded 4 women who had cancer prior to the baseline interview, 4 women with extreme reported total energy intake (< 500 or > 3,500 kcal/day), 5 women with missing data for both *GST* genes, and 1 woman with missing BMI data. After these exclusions 3,589 women (1,071 cases and 2,518 non-cases) remained for analysis. From the SMHS, we excluded 1 man with extreme reported total energy intake (< 500 or > 4,200 kcal/day) and 4 men with missing data on both *GST* genes which left 1,015 men (350 cases and 665 non-cases) for analysis.

Descriptive statistics of the two cohorts were calculated for the population and differences were calculated using the Pearson chi-square statistic for categorical variables and the Wilcoxon rank-sum test for continuous variables. Spearman correlations (r_s) were calculated for all measured covariates, including self-reported factors, matching variables, and incident cancer during follow-up, with urinary ITC levels adjusted for batch effects. Spearman correlations were also calculated for self-reported usual cruciferous vegetable and dietary ITC intake and recent cruciferous vegetable intake with urinary ITC levels adjusted for batch and total energy intake and then additionally for other previously identified statistically significant correlations. The strength of the correlations was assessed between the self-reported baseline measures and the average baseline and follow-up measures. The effect of trimming 5% from each tail of the distribution of uri-

nary ITC and self-reported cruciferous vegetable intakes were also evaluated. We evaluated the shape of the curve expressing the relationship between usual cruciferous vegetable intake and urinary ITC using a restricted cubic spline function with three knots.

Urinary ITC levels, usual cruciferous vegetable intake and dietary ITC were categorized into quintiles by sex, and the kappa statistic was calculated for agreement between urinary ITC and the self-reported measures of intake for categorizing study participants. Linear regression models were created to determine the association between the *GST* gene variants and smoking history with urinary ITC levels. Adjustment for additional significant correlations and self-reported cruciferous vegetable intake were compared with models that adjusted only for batch. Urinary ITC was natural log transformed to approximate normality for the linear regression models and the β estimate and 95% confidence intervals (CIs) were back-transformed to the linear scale after regression. The shape of the association between usual cruciferous vegetable intake and urinary ITC by *GST* genotype was evaluated with a restricted cubic spline model. Effect modification of the association between *GST* gene variants and urinary ITC by smoking status and morning versus afternoon urine sample collection was evaluated using stratified linear regression models. A linear prediction model of the natural log of ITC was created using backwards selection. Variables with a p value less than 0.10 remained in the model. Statistically significant factors and the model R^2 were presented. SAS 9.3 was used for all analyses and a 2-sided p value less than 0.05 was considered statistically significant except in the backwards selection model which used a cut-point of 0.10.

RESULTS

Women and men from the SWHS and SMHS differed on several baseline characteristics including age, education, smoking history, alcohol and tea consumption, family history of cancer, BMI, and leisure time physical activity ($p < 0.01$). The participants reported significantly different intakes of cruciferous vegetables with women having a median cruciferous vegetable intake of 82.5 g/day and men having a median intake of 90.9 g/day ($p < 0.0001$). However, the median values for urinary ITC were not statistically different for women and men with 1.7 and 1.5 nmol/mg creatinine, respectively ($p = 0.2953$; Table 1). When data from women and men were combined, urinary ITC levels ranged from undetectable to 602.6 nmol/mg creatinine with a median level of 1.61 nmol/mg creatinine.

In general, strong correlations were not observed between the baseline sociodemographic, behavioral, or physical characteristics with urinary ITC (Supplementary Table 1). A weak, inverse correlation with urinary ITC was observed for smoking and alcohol consumption. Never smokers had a geometric mean urinary ITC level of 1.59 (95% CI: 1.52, 1.66) nmol/mg creatinine while current smokers had a geometric mean level of 1.40 (95% CI: 1.22, 1.61) nmol/mg creatinine after adjustment for batch. Participants who had never consumed alcohol had a geometric mean urinary ITC level of 1.57 (95% CI: 1.50, 1.64) nmol/mg creatinine and those who had ever consumed alcohol had a level of 1.36 (95% CI: 1.17, 1.57) nmol/mg creatinine. Strong correlations were generally not observed between baseline prevalent conditions and prior surgeries, and no correlation was observed between any of the kidney disorders (chronic kidney disease, nephritis or other urinary disorders) with urinary ITC. Weak correlations were observed for a history

of chronic gastritis and a previous gastrectomy with urinary ITC. Participants who had ever had gastritis or a gastrectomy had geometric mean levels of urinary ITC of 1.70 (95% CI: 1.56, 1.86) and 2.46 (95% CI: 1.68, 3.59) nmol/mg creatinine, respectively. Statistically significant correlations were observed between some of the matching variables and urinary ITC levels, including providing a blood sample and a morning urine sample, but no significant correlations were observed between the number of hours since last meal, antibiotic use or incident cancer during follow-up.

In women and men, urinary ITC was significantly, but weakly, correlated with baseline usual cruciferous vegetable intake ($r_s = 0.1149$, $p < 0.0001$) and dietary ITC calculated from self-reported intake ($r_s = 0.1172$, $p < 0.0001$) after adjustment for batch and total energy intake. The correlations were stronger in men ($r_s = 0.1733$) than women ($r_s = 0.0988$). No interaction was observed between cruciferous vegetable intake and sex on the association with urinary ITC ($p = 0.7845$), and no interaction was observed among women between cruciferous vegetable intake and batch on the association with urinary ITC ($p = 0.3897$). Among the individual cruciferous vegetables, the strongest correlations were observed for white radish/turnip ($r_s = 0.1104$, $p < 0.0001$), green cabbage ($r_s = 0.0975$, $p < 0.0001$), and Chinese greens ($r_s = 0.0921$, $p < 0.0001$) when the data were combined for women and men. Stronger correlations were observed between the measures of recent cruciferous vegetable intake. For men and women combined, the Spearman correlations were 0.2591 ($p < 0.0001$) for the number of times cruciferous vegetables were consumed in the past week, 0.2400 ($p < 0.0001$) for the number of times cruciferous vegetables were consumed in the past 24 hours and -0.2877 ($p < 0.0001$) for the number of hours since the last intake of cruciferous vegetables (Table 2). Usual cru-

ciferous vegetable consumption was generally not correlated with the measures of recent intake (results not shown). Additional adjustment for other significant covariates, including morning or afternoon urine sample collection and smoking, did not materially alter the correlations (Table 2). Similarly, the correlations between the average reported intake of cruciferous vegetables from the baseline and follow-up FFQs did not increase the strength of the correlation and correlations without adjustment for total energy intake were similar (results not shown). Among only non-cases, the correlation between cruciferous vegetable intake and urinary ITC levels was also similar ($r_s = 0.1260$, $p < 0.0001$) after adjustment for batch and total energy intake. When 5% of each tail of the urinary ITC distribution was removed, the correlations remained similar. When 5% of each tail of the reported cruciferous vegetable consumption distribution was removed, the correlations were generally similar, although the correlation between Chinese cabbage/bok choy cabbage was no longer statistically significant ($r_s = 0.0214$, $p = 0.1684$). Usual cruciferous vegetable intake showed a strong non-linear association with the log of urinary ITC ($p_{non\text{-}linearity} = 0.0008$; Figure 1). Poor agreement (kappa < 0.20) was observed between quintiles of urinary ITC and quintiles of cruciferous vegetable intake and between quintiles of urinary ITC and quintiles of dietary ITC (results not shown).

The geometric mean levels of urinary ITC by *GST* genotype are presented in Table 3. In general, the mean level of urinary ITC was lower among participants with the null genotype; however, the observed differences were not statistically significant. Adjustment for significant baseline covariates did not alter the associations and the inclusion of self-reported cruciferous vegetable intake in the model did not materially change the results. When geometric mean levels of urinary ITC were calculated by *GST* genotype

among participants who did not take antibiotics in the past week, results were similar (results not shown). Smokers, both current and past, tended to have lower urinary ITC levels with geometric mean urinary ITC levels of 1.59 (95% CI: 1.52, 1.66), 1.29 (95% CI: 1.04, 1.59) and 1.40 (95% CI: 1.22, 1.61) nmol/mg creatinine for never, past and current smokers, respectively after adjustment for batch ($p = 0.1020$). Non-linear associations were detected between cruciferous vegetable intake and log urinary ITC level among all *GST* genotypes except for participants with the null genotype for both *GSTM1* and *GSTT1* and for participants with both copies of both *GSTM1* and *GSTT1* (results not shown).

When the analyses were stratified by smoking status, current smokers with the carrier genotype for both the *GSTM1* and *GSTT1* genes had a significantly higher urinary level of ITC than the other combinations with a geometric mean of 1.91 (95% CI: 1.52, 2.42) nmol/mg creatinine ($p = 0.0200$). Current smokers with the carrier genotype of the *GSTM1* gene also had a slightly higher urinary output of ITC with a geometric mean of 1.66 (95% CI: 1.40, 1.96) nmol/mg creatinine ($p = 0.0861$). However, the *GSTM1* gene did not appear to have an effect in non-smokers or previous smokers and the *GSTT1* gene did not appear to directly modify urinary ITC output among any of the smoking categories (Table 4). Additional adjustment for age did not materially alter the estimates. When stratified by sex and smoking status, among smokers, both women and men having two copies of both the *GSTM1* and *GSTT1* gene had higher geometric mean urinary ITC levels (ITC_{women} 2.78, 95% CI: 1.64, 4.70 nmol/mg creatinine; ITC_{men} 1.73, 95% CI: 1.34, 2.25 nmol/mg creatinine) than the other genotypes, but the difference by genotype was statistically significant only among women ($p_{women} = 0.0108$; p_{men} 0.2895). Timing

of the urine sample collection may have modified the association between the *GST* genes and urinary ITC as a difference in urinary ITC level was detected for the *GSTM1* gene ($p = 0.0467$) and the combination *GSTM1*/*GSTT1* gene category ($p = 0.0325$) only among participants who provided a urine sample in the afternoon (Table 5). When the association between the *GST* genes and cruciferous vegetable consumption were stratified by morning or afternoon sample collection, no differences in cruciferous vegetable consumption were observed (results not shown). When the urinary ITC level was calculated by *GST* genotype among current smokers with an afternoon urine sample, the urinary ITC levels varied significantly within the combination *GSTM1*/*GSTT1* gene category ($p = 0.0443$) with the highest urinary ITC levels among participants with 2 copies of both the *GSTM1* and *GSTT1* genes with a geometric mean level of urinary ITC of 2.13 (95% CI: 1.50, 3.02) nmol/mg creatinine (results not shown).

The final model to predict the log of urinary ITC level selected using backwards selection included previous gastrectomy ($p = 0.0995$), leisure time physical activity ($p = 0.0887$), history of diabetes ($p = 0.0681$), history of chronic gastritis ($p = 0.0680$), ever consuming ginseng ($p = 0.0547$), history of high blood pressure ($p = 0.0418$), history of coronary heart disease ($p = 0.0317$), number of times consumed cruciferous vegetables in the past 24 hours ($p < 0.0001$), time of sample collection ($p < 0.0001$), blood or buccal cell sample ($p < 0.0001$), usual cruciferous vegetable intake ($p < 0.0001$), sample batch ($p < 0.0001$), and time since last intake of cruciferous vegetables ($p < 0.0001$). However, this model was able to predict only 11.7% ($R^2 = 0.117$) of the variation in urinary ITC (results not shown).

DISCUSSION

In this study of Chinese men and women, self-reported intake of cruciferous vegetables was correlated with urinary ITC levels from a spot urine sample; however the observed correlations were generally weak. The strongest correlations were observed between self-reported recent cruciferous vegetable intake and urinary ITC. When the data were categorized into quintiles, there was poor agreement between the quintiles of self-reported cruciferous vegetable intake and urinary ITC. Overall, urinary ITC did not appear to be related to *GST* gene polymorphisms, but when the data were stratified by smoking status, some differences by genotype were observed among current smokers. Additionally, when stratified by time of urine sample collection, among participants who provided afternoon urine sample, those with the *GSTM1*-null genotype had lower urinary ITC output than the carrier genotype. The linear prediction model was able to explain only a small proportion of the variation in urinary ITC levels.

In a previous study of post-menopausal women in the United States, a relatively weak correlation (Pearson correlation = 0.22) was observed between cruciferous vegetable intake from a FFQ and urinary dithiocarbamate, another biomarker of cruciferous vegetable intake (28). This observed correlation was stronger than the correlation with usual intake in our study; however the study assessed cruciferous vegetable intake only during the week prior to a cruciferous vegetable intervention and the correlation in our study for recent intake was similar to this finding. Another study among a Chinese population in Singapore noted statistically significant associations between consumption of cruciferous vegetables and urinary ITC ($p = 0.0004$) and between dietary ITC and urinary ITC ($p = 0.0003$), but did not report the strength of the associations (24). The observed

correlations in our study were all statistically significant ($p < 0.0001$), but the correlations were weak. Relatively weak correlations ($r_s = 0.16$, $p < 0.01$) were observed in a population of female controls from the Shanghai Breast Cancer Study between both usual cruciferous vegetable intake and dietary ITC with urinary levels of ITC (29).

Feeding studies have shown that urinary ITC is a useful biomarker of dietary exposure to ITC with a high correlation between cruciferous vegetable dose and urinary output of ITC with a Spearman correlation of 0.93 for a 24 hour urine collection (15, 26). However, urinary ITC levels reflect recent cruciferous vegetable intake such that after consumption, urinary ITC has a peak excretion between two and six hours with low to no presence after 24 to 48 hours (15). In contrast, the cruciferous vegetable intake measure from the FFQ that we utilized assessed usual intake over the past year. Therefore, it is possible that the weak correlation could be partially due to the difference in the relevant time period of exposure assessed. And when we considered the recent intake of cruciferous vegetables using crude measure of intakes, number of times of intake over the past week or 24 hours, the correlations were strengthened. However, recent cruciferous vegetable intake was largely unrelated to usual cruciferous vegetable consumption, but both were correlated with urinary ITC, which could indicate that these measures assess a different dietary construct. Another cause of the weak correlation between the usual intake of cruciferous vegetables and urinary ITC could be due to differences in the timing of the urine sample collection since participants with a morning sample were unlikely to have recently consumed cruciferous vegetables. The weak correlations could also be related to measurement error in the cruciferous vegetable intake data from the FFQ. In general, FFQ data are prone to dietary recall errors (14), do not typically cover every cruciferous

vegetable that produces ITC (30), and do not account for variability in actual ITC exposure affected by cooking methods (31) and storage conditions (32). In the calculation of dietary ITC, an additional source of measurement error could be due to the fact that the actual ITC levels in the consumed vegetables may be different than what was calculated in the laboratory, although we used ITC data from vegetables in Asia (24, 25). It is also possible that some of the variation between cruciferous vegetable intake and urinary ITC was affected by chronic conditions, such as gastrointestinal disease, since in the linear prediction model both a history of chronic gastritis (N = 938; 20.4%) and previous gastrectomy (N = 52; 1.1%) were statistically associated with urinary ITC. Previous research has found that gut microflora affects metabolism of cruciferous vegetables (1, 33) which may link these gastrointestinal conditions to urinary ITC excretion. However, all chronic condition data was collected by self-report and it is beyond the scope of this study to consider this potential mechanism. Future research could consider how these and other conditions affect gut microflora and subsequently levels of urinary ITC, and if these changes may affect the potential chemopreventive abilities of ITC.

Previous research has been inconsistent on the effect of the *GSTM1* and *GSTT1* genes on the urinary output of ITC, however it has been hypothesized that individuals with a null genotype of *GST* would not excrete ITC as rapidly due to lower levels of GST enzyme activity that would prolong exposure to ITC (34). One study found that urinary ITC was higher among participants with the *GSTM1* null genotype (35), another found that urinary ITC was slightly higher among participants with the *GSTT1* null genotype (29), while another study reported no difference by genotype (36). We found no indication for a difference by genotype in our entire sample, but *GSTM1* and the combination of

GSTM1 and *GSTT1* genotypes appeared to be associated with urinary ITC levels among current smokers. Smoking is a GST inducer and in our sample, urinary ITC level was lower for the smokers with a null genotype for *GSTT1* or for both *GSTM1* and *GSTT1*. In a previous feeding study, at baseline, nine cigarette smokers consuming a diet excluding cruciferous vegetables had urinary levels of dithiocarbamate ranging from 0 to 7.6 μmol/8 hours whereas non-smoking participants had a baseline urinary range of 0.01 to 0.91 μmol/8 hours (37). Smoking may therefore be related to ITC excretion, although in our study, current smokers had lower levels of urinary ITC than never smokers. The *GSTM1* and the combination of *GSTM1* and *GSTT1* genotypes also were associated with urinary ITC levels for participants who provided an afternoon urine sample, suggesting that these genotypes may be relevant to ITC excretion rates when exposure levels are higher, since afternoon samples are presumably collected sooner following consumption of cruciferous vegetables than morning samples would be.

This study has a number of important strengths including the relatively large sample size, high response rate and the population-based study design of the parent study. Although there are limitations to the self-reported measures of cruciferous vegetable intake and dietary ITC, the FFQ assessed a different time of exposure than urinary ITC and both FFQs had relatively high validity and reliability measures (21, 22). We also included a crude measure of recent intake for comparison. The urinary ITC measurement had limitations as only a spot urine sample was provided. However, it was found that individual variation in urinary ITC over time was low in the Shanghai Women's Health Study, suggesting that a spot urine sample in this population represents regular cruciferous vegetable intake (7). Residual confounding of the association could be an issue espe-

cially since none of the measured covariates were strongly associated with urinary ITC. For example, we did not assess other *GST* genes, such as *GSTP1* or *GSTA1*, or other potential metabolizing genes on the urinary excretion of ITC. Finally, laboratory errors in the assessment of urinary ITC and the *GST* gene variants could occur, however quality control procedures probably minimized errors and any bias would likely be non-differential.

In conclusion, self-reported intake of cruciferous vegetables in this population having relatively high cruciferous vegetable consumption was only moderately correlated with urinary ITC levels. *GST* gene variants, in particular *GSTM1*, may be important in ITC metabolism and excretion among current smokers and shortly after cruciferous vegetable intake. Gastrointestinal conditions may play a role in the metabolism and excretion of ITC. Future research on urinary ITC and health risk should take into consideration the influence of genotype, smoking, time of biological sample collection and upper gastrointestinal diseases.

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Table 1: Demographic characteristics of the nested case-control studies of urinary ITC in the Shanghai Women's and Men's Health Studies

	Shanghai Women's Health Study	Shanghai Men's Health Study	p value
Number of participants	3,590	1,015	
Age (years)	59.5	65.6	< 0.0001
Educational level (%)			
≤ Elementary school	35.7%	14.0%	< 0.0001
Middle school	29.8%	32.3%	
High school	22.8%	27.0%	
≥ College	11.8%	26.7%	
Cigarette smoking (%)			
Never	96.6%	38.4%	< 0.0001
Past	0.6%	18.2%	
Current	2.9%	43.3%	
Alcohol consumption (%)			
Never	97.3%	68.4%	< 0.0001
Ever	2.7%	31.6%	
Tea consumption (%)			
Never	74.3%	37.8%	< 0.0001
Ever	25.7%	62.2%	
Ginseng intake (%)			
Never	66.1%	63.7%	0.1580
Ever	33.9%	36.3%	
Family history of cancer (%)			
Yes	26.8%	31.9%	0.0013
No	73.2%	68.1%	
BMI (kg/m^2)	24.3	23.9	< 0.0001
Leisure time physical activity (MET hours/day)	0.0	0.3	< 0.0001
Total energy intake (Kcal/day)	1630.6	1826.9	< 0.0001
Cruciferous vegetable intake (g/day)	82.5	90.9	< 0.0001
Dietary ITC (μmol/day)	7.0	7.9	< 0.0001
Urinary ITC (nmol/mg creatinine)	1.7	1.5	0.2953

Note: Median values are presented for continuous variables. Statistical differences were tested using the Pearson chi-square test for categorical variables and the Wilcoxon rank-sum test for continuous variables.

Table 2: Spearman correlations between reported dietary intakes and urinary ITC levels

	Women Model 1		Men Model 1		Women and men Model 1		Women and men Model 2	
	r_s	p value	r_s	p value	r_s	p value	r_s	p value
Cruciferous vegetables (g/day)	0.0988	<.0001	0.1733	<.0001	0.1149	<.0001	0.1100	<.0001
Greens, Chinese greens (g/day)	0.0808	<.0001	0.1320	<.0001	0.0921	<.0001	0.0880	<.0001
Green cabbage (g/day)	0.0970	<.0001	0.1011	0.0013	0.0975	<.0001	0.0940	<.0001
Chinese cabbage, bok choy cabbage (g/day)	0.0382	0.0222	0.0682	0.0299	0.0432	0.0034	0.0447	0.0024
Cauliflower (g/day)	0.0506	0.0025	0.0977	0.0018	0.0607	<.0001	0.0555	0.0002
White radish/turnip (g/day)	0.0882	<.0001	0.1903	<.0001	0.1104	<.0001	0.1079	<.0001
Dietary ITC (µmol/day)	0.1055	<.0001	0.1617	<.0001	0.1172	<.0001	0.1119	<.0001
Cruciferous vegetables (times/last week)	0.2530	<.0001	0.2839	<.0001	0.2591	<.0001	0.2563	<.0001
Cruciferous vegetables (times/24 hours)	0.2416	<.0001	0.2365	<.0001	0.2400	<.0001	0.2390	<.0001
Hours since last cruciferous vegetable intake	-0.2839	<.0001	-0.3048	<.0001	-0.2877	<.0001	-0.2832	<.0001

Model 1 adjusted for batch effects and total energy intake.

Model 2 adjusted for batch effects, total energy intake, age and factors significantly associated with urinary ITC including time between sample collection and sample processing, providing a blood or buccal cell sample, morning or afternoon urine sample collection, smoking status, alcohol consumption, history of chronic gastritis, and previous gastrectomy.

Table 3: Geometric mean urinary ITC levels by *GST* copy number

		Model 1			Model 2			Model 3	
	N	**Mean**	**(95% CI)**	**p value**	**Mean**	**(95% CI)**	**p value**	**Mean**	**(95% CI)**	**p value**
GSTM1										
Null	2,667	1.51	(1.43, 1.59)	0.1199	1.51	(1.44, 1.60)	0.1627	1.51	(1.44, 1.60)	0.1766
Carrier	1,895	1.61	(1.51, 1.71)		1.60	(1.51, 1.71)		1.60	(1.51, 1.70)	
GSTT1										
Null	2,282	1.53	(1.44, 1.62)	0.5192	1.53	(1.44, 1.62)	0.5229	1.53	(1.44, 1.62)	0.5217
Carrier	2,311	1.57	(1.48, 1.66)		1.57	(1.48, 1.66)		1.57	(1.48, 1.66)	
GSTM1/GSTT1										
Both null	1,294	1.52	(1.41, 1.64)	0.1449	1.53	(1.42, 1.65)	0.1710	1.53	(1.42, 1.65)	0.1887
One null and one carrier	2,331	1.51	(1.43, 1.60)		1.51	(1.43, 1.60)		1.52	(1.43, 1.60)	
Both carrier	925	1.68	(1.53, 1.84)		1.67	(1.53, 1.83)		1.67	(1.53, 1.82)	

Model 1 adjusted for batch effects.

Model 2 adjusted for batch effects, age and factors significantly associated with urinary ITC including time between sample collection and sample processing, providing a blood or buccal cell sample, morning or afternoon urine sample collection, smoking status, alcohol consumption, history of chronic gastritis, and previous gastrectomy.

Model 3 included all covariates from Model 2 plus cruciferous vegetable and total energy intake.

Table 4: Geometric mean urinary ITC levels by *GST* copy number stratified by smoking status

	Never smokers				Past smokers				Current smokers			
	N	**Mean**	**(95% CI)**	**p value**	**N**	**Mean**	**(95% CI)**	**p value**	**N**	**Mean**	**(95% CI)**	**p value**
GSTM1												
Null	2,242	1.54	(1.45, 1.63)	0.2965	123	1.32	(1.04, 1.68)	0.7802	302	1.36	(1.18, 1.58)	0.0861
Carrier	1,600	1.62	(1.51, 1.73)		75	1.39	(1.02, 1.89)		220	1.66	(1.40, 1.96)	
GSTT1												
Null	1,932	1.55	(1.45, 1.65)	0.4455	100	1.42	(1.09, 1.84)	0.5476	250	1.44	(1.23, 1.69)	0.8300
Carrier	1,918	1.60	(1.50, 1.71)		103	1.26	(0.97, 1.64)		290	1.48	(1.28, 1.71)	
GSTM1/GSTT1												
Both null	1,106	1.53	(1.41, 1.67)	0.3502	52	1.26	(0.87, 1.82)	0.5243	136	1.55	(1.25, 1.92)	0.0200
One null and one carrier	1,949	1.55	(1.46, 1.65)		113	1.43	(1.12, 1.84)		269	1.29	(1.11, 1.51)	
Both carrier	780	1.68	(1.52, 1.85)		31	1.06	(0.66, 1.71)		114	1.91	(1.52, 2.42)	

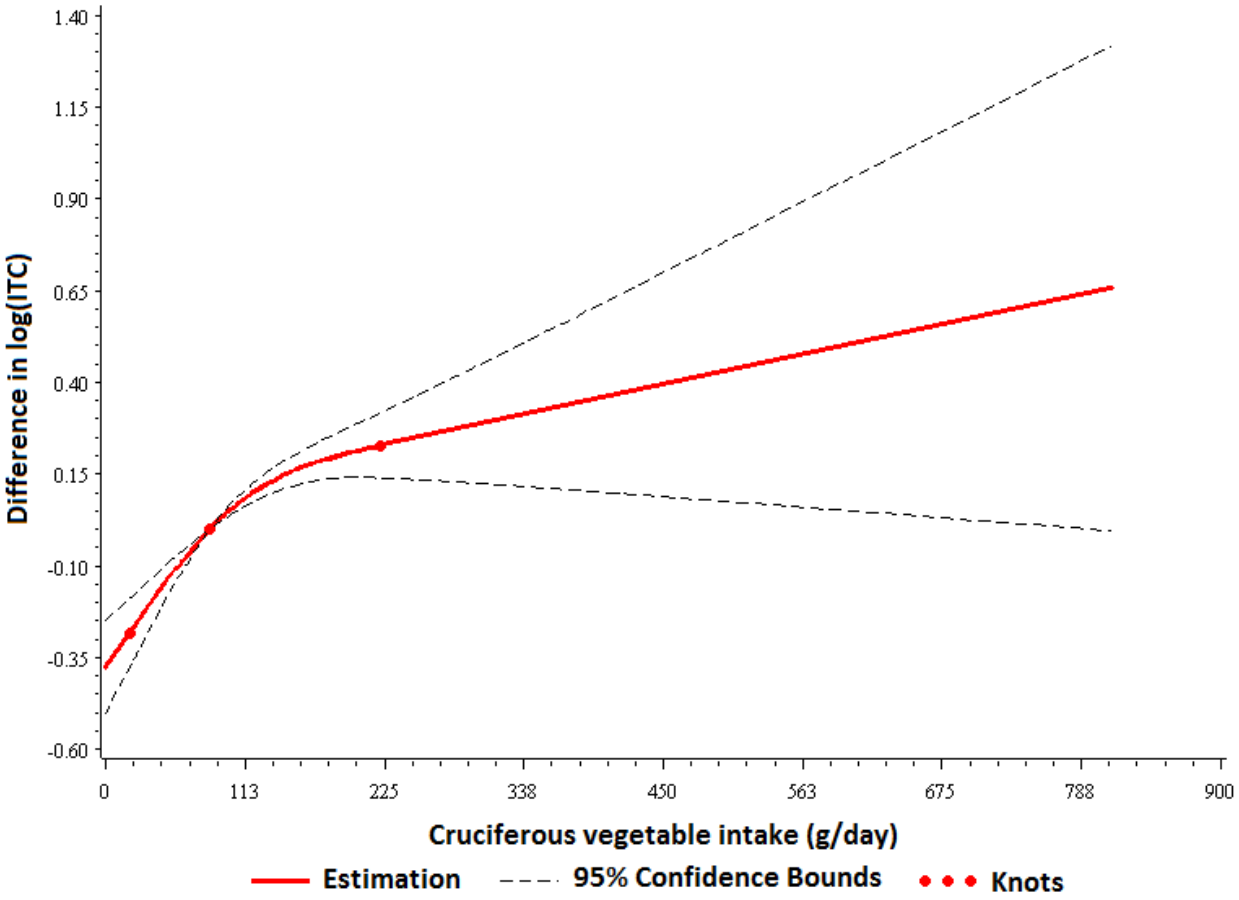
All models adjusted for sex and batch effects.

Table 5: Geometric mean urinary ITC levels by *GST* copy number stratified by morning or afternoon urine sample collection

	Morning urine sample				Afternoon urine sample			
	N	**Mean**	**(95% CI)**	**p value**	**N**	**Mean**	**(95% CI)**	**p value**
GSTM1								
Null	1,188	1.42	(1.32, 1.54)	0.9217	1,479	1.58	(1.47, 1.70)	0.0429
Carrier	857	1.43	(1.31, 1.57)		1,038	1.77	(1.63, 1.93)	
GSTT1								
Null	1,011	1.43	(1.31, 1.55)	0.9405	1,271	1.61	(1.49, 1.74)	0.4098
Carrier	1,045	1.43	(1.32, 1.56)		1,266	1.69	(1.56, 1.83)	
GSTM1/GSTT1								
Both null	586	1.43	(1.28, 1.60)	0.9737	708	1.60	(1.45, 1.78)	0.0325
One null and one carrier	1,014	1.42	(1.30, 1.54)		1,317	1.59	(1.48, 1.72)	
Both carrier	440	1.44	(1.27, 1.64)		485	1.93	(1.70, 2.18)	

All models adjusted for sex and batch effects.

Figure 1: The association between cruciferous vegetable intake and the log of urinary isothiocyanate using restricted cubic splines with 3 knots



Supplemental Table 1: Correlations between baseline demographic and lifestyle characteristics, prevalent conditions and matching variables with urinary ITC levels among women and men

	r_s [A]	p value
Age (years)	-0.0257	0.0819
Educational level[B]	0.0209	0.1560
Smoking history[C]	-0.0332	0.0244
Ever drink alcohol	-0.0332	0.0245
Ever drink tea	0.0079	0.5940
Ever eat ginseng	-0.0261	0.0770
Family history of cancer	0.0155	0.2921
BMI (kg/m^2)	0.0048	0.7454
Leisure time PA (MET hours/week)	-0.0284	0.0542
Baseline total energy intake (Kcal/day)	0.0276	0.0612
Menopause (men considered post-menopausal)	-0.0014	0.9245
History of pulmonary TB	-0.0088	0.5518
History of chronic bronchitis	-0.0104	0.4812
History of asthma	0.0088	0.5517
History of chronic gastritis	0.0342	0.0202
History of chronic hepatitis	0.0121	0.4139
History of gallstones	-0.0127	0.3910
History of diabetes	0.0250	0.0904
History of high blood pressure	0.0180	0.2224
History of CHD	-0.0141	0.3394
History of stroke	-0.0252	0.0869
History of polyps	0.0091	0.5363
Previous gastrectomy	0.0319	0.0303
Previous cholecystectomy	-0.0055	0.7077
History of chronic kidney disease	-0.0222	0.1320
History of nephritis	-0.0081	0.5808
History of other urinary disorders	0.0200	0.1740
History of any urinary disorders	0.0151	0.3066
Time between sample collection and pro-cessing	0.0386	0.0088
Sample type (blood-1, buccal cell-0)	0.0710	<.0001
Morning sample	-0.0468	0.0015
Hours since last meal	0.0109	0.4590
Antibiotic use	0.0209	0.1556
Incident cancer during follow-up	0.0121	0.4106

A: Adjusted for batch.

B: Education level was coded as ≤ elementary school (1), middle school (2), high school (3) and ≥ college (4).

C: Smoking history treated as never smoker (1), past smoker (2), and current smoker (3).

Note: All yes/no style variables treated as no (0) and yes (1).

CRUCIFEROUS VEGETABLES, GLUTATHIONE *S*-TRANSFERASE POLYMORPHISMS AND THE RISK OF COLORECTAL CANCER AMONG CHINESE MEN

by

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ABSTRACT

Purpose: The association between cruciferous vegetable intake and colorectal cancer (CRC) has been inconsistent in previous studies, which has been hypothesized to be related to *GST* gene polymorphisms. Therefore, we aimed to prospectively assess the association between cruciferous vegetable intake and CRC risk and to evaluate effect modification by *GST* gene polymorphisms.

Methods: Using incidence density sampling, CRC cases (N = 340) diagnosed prior to December 31, 2010 within the Shanghai Men's Health Study were matched to non-cases (N = 673) on age, date of sample collection, time of sample collection, time after last meal, recent antibiotic use and the availability of the required biospecimen. Cruciferous vegetable intake was assessed using a food frequency questionnaire completed at baseline, and by isothiocyanate (ITC) levels from baseline spot urine samples. Both measures of intake were categorized in tertiles based on the distribution in the controls. *GSTM1* and *GSTT1* copy numbers were determined using real-time quantitative polymerase chain reaction assays and were categorized as null (0 copies) versus carrier (1 or 2 copies). Conditional logistic regression was used to calculate the odds ratios (ORs) and 95% confidence intervals (95% CIs) for the association between cruciferous vegetable intake and *GST* gene variants with colorectal cancer. Statistical interactions were evaluated using logistic regression with adjustment for matching variables, and stratified ORs were presented for each *GST* gene category.

Results: CRC risk was not associated with cruciferous vegetable intake, whether measured by self-report or by urinary ITC, nor with *GST* gene variants. No statistical interactions were detected between cruciferous vegetable intake and *GST* gene variants on the

odds of CRC. Stratifying by timing of urine sample collection (morning versus afternoon) and excluding CRC cases diagnosed in the first two years of follow-up did not materially alter the results.

Conclusions: This study provides no evidence supporting the involvement of cruciferous vegetable intake in the development of CRC in Chinese men.

INTRODUCTION

Cruciferous vegetables, including bok choy, broccoli, cauliflower, and turnips, have long been studied for their potential protective effects against cancer development. The preventive benefit has been proposed to be related to the presence of isothiocyanate (ITC), a compound derived from cruciferous vegetables, which has been observed to reduce oxidative stress, induce differentiation and decrease inflammation (1, 2). However, the results from epidemiologic studies that focus on the association between cruciferous vegetable consumption and the risk of colorectal cancer have typically been weak or null (3-9). Similarly, studies that considered the association between levels of urinary ITC and colorectal cancer had varying results (10-12).

ITC induces GST activity and then is metabolized by glutathione *S*-transferase (GST) enzymes for elimination from the body (13). Individuals with a homozygous deletion of both copies of the *GSTM1* or *GSTT1* gene do not produce the GSTM1 or GSTT1 enzyme, respectively (13). The absence of these enzymes could lead to decreased activity of GST and lengthened exposure to ITC which could increase the anti-carcinogenic effects of ITC. Previous epidemiological research has suggested that *GST* gene polymorphisms may interact with cruciferous vegetable intake or urinary ITC to modify the risk of colorectal cancer, but the evidence is not entirely consistent (3, 5-7, 10, 12, 14, 15).

We, therefore, evaluated the association between cruciferous vegetable consumption, both estimated by self-report and by urinary ITC, with colorectal cancer risk and to estimate the potential interaction between cruciferous vegetable intake and *GST* gene polymorphisms on the risk of colorectal cancer using data from the Shanghai Men's Health Study (SMHS).

METHODS

Source population

The overall design and methodology for the SMHS has been previously described in detail (16). Briefly, the SMHS is a prospective, population-based cohort study in Shanghai, China where men aged 40 to 74 years without a history of cancer were recruited between March 2002 and June 2006. A total of 61,483 men gave informed consent resulting in an overall participation rate of 74.1%. At enrollment, participants were asked to provide a spot urine sample and a blood sample. A buccal cell sample was requested from participants unwilling to provide a blood sample. Biological samples were transported in Styrofoam boxes with ice packs at about 0-4°C and then put into long-term storage at -70°C. The SMHS received approval from the Institutional Review Board at Vanderbilt University and the Shanghai Cancer Institute.

Colorectal cancer case ascertainment and control selection

Annual record linkage with the population-based Shanghai Cancer Registry and the Shanghai Municipal Vital Statistics Unit was conducted to identify incident cancer cases and decedents, respectively. Participants identified as incident cancer cases were verified through home visits and medical charts were obtained to document detailed diagnostic information. Participants diagnosed with primary incident colorectal cancer as identified in the Shanghai Cancer Registry prior to December 31, 2010 formed the case group for this study. Colorectal cancer was defined as a primary tumor having ICD-9 code 153 (malignant neoplasm of colon) or 154 (malignant neoplasm of rectum, rectosigmoid junction, and anus).

Control subjects were identified from the SMHS using incidence density sampling with a 2:1 control to case selection ratio. Controls were matched to cases on age (+/- 2 years), date of sample collection (+/- 30 days), time of sample collection (morning or afternoon), time after last meal (+/- 2 hours), recent antibiotic use (yes or no) and the availability of the required biospecimen. Because biological samples from SMHS participants were limited and because case-control studies within this cohort test for different biomarkers, subjects of previous case-control studies were excluded from selection.

Assessment of cruciferous vegetable intake

Usual dietary intake over the past 12 months was assessed at baseline using a validated food frequency questionnaire (FFQ). The FFQ captured about 89% of the average food intake in the SMHS population and was previously tested for validity and reliability (17, 18). The FFQ assessed how often (daily, weekly, monthly, yearly or never) the participant consumed a specific food or food group and the amount consumed for that time period. The average amounts of each food group were calculated by summing the intake for each component food. Nutrient intake was calculated using the Chinese Food Composition Tables (19).

The FFQ included the following cruciferous vegetables: greens/Chinese greens, green cabbage, Chinese cabbage/bok choy cabbage, cauliflower, and white turnip were considered cruciferous vegetables. Total self-reported cruciferous vegetable intake was categorized into tertiles based on the distribution of intake within the controls.

Measurement of urinary ITC

High-performance liquid chromatography (HPLC) was used to determine total urinary ITC and ITC metabolites from baseline spot urine samples. This method has been

previously described in detail (20, 21). Laboratory staff was blinded to the case status of the samples. For each laboratory run, two representative standards and a reagent blank were included. Each week, a standard curve was created using data from samples of *N*-acetyl-L-cysteine conjugates of phenethyl ITC (0.2-25 mmol/L) in urine obtained from subjects on a controlled diet. All urine samples and standards were assayed in duplicate and the average ITC level for each participant was used for analysis. If the standard deviation of the mean was greater than 10%, the individual ITC values were checked and the sample was reanalyzed if necessary. To control for laboratory variability, all samples for each matched set of cases and controls were included in the same analytic run. The limit of detection for this HPLC method was 0.1 µmol/L, so for undetectable ITC levels, the ITC value was set to 0.1 µmol/L divided by the square root of two. The laboratory coefficient of variation for ITC was 4.3%. Urinary creatinine was measured using Jaffe' alkaline picrate procedure (22). All ITC levels were adjusted for urine volume by urine creatinine level and reported as nmol/mg creatinine. Urinary ITC level was categorized into tertiles based on the distribution within the controls.

GST genotyping

DNA was extracted from blood (86.0%) and buccal cell (14.0%) samples. The *GSTM1* and *GSTT1* gene copy numbers (0, 1 or 2) were determined using duplex real-time quantitative polymerase chain reaction (PCR)-based assays with the methods described in the NCI SNP500 project including modifications (23). The sequences for the assay design were obtained from GenBank (*GSTM1*, NM_000561 and *GSTT1*, NM_000853) and a 384-well plate in ABI PRISM 7900 Sequence Detection Systems was used (Applied Biosystems, Foster City, CA). The laboratory staff was blinded to the case

status of the samples and Coriell DNA samples containing 0, 1 or 2 copies of the *GSTM1* and *GSTT1* genes were included for internal quality control. The concordance rate for quality control samples, including water, Coriell DNA and blinded DNA samples was 100%. *GSTM1* and *GSTT1* genotypes were within Hardy-Weinberg (H-W) equilibrium among the controls ($p = 0.25$ and $p = 0.69$, respectively).

GSTM1 and *GSTT1* were categorized by copy number (0, 1 or 2 gene copies) and *GSTM1*-null or *GSTT1*-null versus carrier (0 versus 1 or 2 copies). A combined category of *GSTM1* and *GSTT1* was created with *GSTM1*-null and *GSTT1*-null, one null and one carrier, and both carrier.

Other covariates of interest

Additional covariates for analysis included a number of demographic, dietary, behavioral and medical factors from the baseline questionnaire. Demographic variables of interest were age, education level, occupation, and annual per capita family income. Participants with data missing on education (N = 19), income (N = 1) or occupation (N = 1) were assigned to the most common categories, middle school education, 6,000 – 11,999 yuan annual per capita family income, and occupation in manual work. Each participant's body mass index (BMI) was calculated from the interviewer-measured height and weight at the baseline visit. Behavioral characteristics from the baseline questionnaire were cigarette smoking, alcohol consumption, and amount of leisure time physical activity (MET hours/day). We also determined history of diabetes and family history of cancer from the baseline questionnaire. The two participants missing data on family history of cancer were classified as having no family history of cancer. Dietary characteristics of interest were red meat, total meat and total energy intake as derived from the FFQ.

Statistical analysis

Data from 341 cases and 679 matched controls were available for analysis. We excluded one control with extreme energy intake (total energy intake < 500 or > 4,200 kcal per day), one case and three controls with missing data on both *GSTM1* and *GSTT1* copy number, and the two controls for the excluded case which left 340 cases and 673 matched controls for analysis. Seven cases had a selection ratio of 1:1 and 333 cases had a selection ratio of 2:1.

Descriptive statistics were calculated for cases and controls and tested using the Wald test from conditional logistic regression. Odds ratios (ORs) and 95% confidence intervals (CIs) were applied to evaluate the association between cruciferous vegetable intake, urinary ITC, and the *GST* gene variants with incident colorectal cancer case status using conditional logistic regression. Models were created with adjustment for potential confounders including age, BMI, leisure time physical activity, total energy intake, red meat intake, total meat intake, education, income, occupation, smoking, alcohol consumption and family history of cancer. Tests for trend were calculated by including the tertile as a continuous variable. Restricted cubic spline models with 3 knots placed at 10%, 50% and 90% with adjustment for confounders and matching variables were created for a 20 g/day change in cruciferous vegetable intake or a 1 nmol/mg creatinine change in urinary ITC. We created stratified models by *GST* gene variants by breaking the matched pairs and adjusting for matching variables and confounders in unconditional logistic regression models. Statistical interactions between tertiles of cruciferous vegetable intake and urinary ITC with *GST* gene variants were tested using the likelihood ratio test. Sensitivity analyses were also conducted. In a previous analysis (Vogtmann et al,

unpublished), we found that *GST* gene variants had an association with urinary ITC only in afternoon urine samples; therefore we investigated the interaction between urinary ITC and *GST* genes with colorectal cancer separately for morning and afternoon urine samples. We also assessed the effect of excluding participants who reported recently taking antibiotics. And in order to determine whether undiagnosed colorectal cancer may have affected the association estimates, we re-conducted the analyses after excluding the colorectal cancer cases diagnosed within two years of baseline, as well as their matched controls. SAS 9.3 was utilized for analyses and two-sided p-values are presented.

RESULTS

Descriptive statistics of the colorectal cancer cases and matched controls are presented in Table 1. In general, cases were similar to controls, including for self-reported cruciferous vegetable intake, urinary ITC, age, educational level, occupation, cigarette smoking history, alcohol consumption, leisure time physical activity, red meat and total meat intake, history of diabetes and a family history of colorectal cancer (all $p > 0.1$). Compared to controls, cases were more likely to have a higher income (9.1% versus 7.0% with annual per capital family income of $\geq$ 24,000 yuan; $p = 0.0619$), have a higher BMI (24.4 versus 23.8 kg/m^2; $p = 0.0082$) and have a family history of cancer (37.9% versus 28.8%; $p = 0.0032$).

Self-reported cruciferous vegetable intake was not associated with colorectal cancer with an adjusted OR of 0.85 (95% CI: 0.60, 1.22) for the 2nd tertile and 1.06 (95% CI: 0.76, 1.50) for the 3rd tertile compared to the lowest tertile of intake ($p_{trend} = 0.6679$). Similarly, urinary ITC levels were also unrelated to colorectal cancer with an adjusted OR of 1.28 (95% CI: 0.90, 1.81) and 1.12 (95% CI: 0.79, 1.60) for the 2nd and the 3rd ter-

tiles, respectively, compared with the 1st tertile (p_{trend} = 0.5379) (Table 2). The restricted cubic spline models did not show a significant non-linear association between cruciferous vegetable intake or urinary ITC and colorectal cancer (results not shown). *GSTM1* and *GSTT1* genotype also did not appear to have an association with colorectal cancer. Compared to the null genotype, the adjusted OR of colorectal cancer for the *GSTM1* carrier genotype was 1.04 (95% CI: 0.78, 1.38). For the *GSTT1* gene, the adjusted OR for colorectal cancer was 0.84 (95% CI: 0.63, 1.11) for the carrier versus the null genotype. And the combination category of *GSTM1* and *GSTT1* was not significantly associated with the odds of colorectal cancer (Table 3).

Statistical interactions were not observed between self-reported cruciferous vegetable intake or urinary ITC, and the *GST* gene variants on the odds of colorectal cancer (Table 4). Similarly, no statistical interactions or trends were observed when cruciferous vegetable intake and urinary ITC were analyzed as continuous variables (results not shown). When the analyses for interaction with urinary ITC were conducted separately for morning or afternoon urine samples, no statistical interactions were observed for the morning urine samples, but interactions were observed for the *GSTT1* gene ($p_{interaction}$ = 0.0248) and the *GSTM1*/*GSTT1* combination ($p_{interaction}$ = 0.0919) in the afternoon urine samples. However, within each genotype of the afternoon urine samples, the only trend observed was a positive association between urinary ITC and colorectal cancer among participants with a null genotype for both *GSTM1* and *GSTT1* with an adjusted OR of 0.91 (95% CI: 0.37, 2.24) for the 2nd tertile and 2.24 (95% CI: 0.94, 5.31) for the 3rd tertile of urinary ITC compared to the 1st tertile (p_{trend} = 0.0636). When participants who recently took antibiotics were excluded from analysis, a nearly statistically significant

interaction was observed between urinary ITC and the combined *GSTM1*/*GSTT1* category ($p_{interaction}$ = 0.0674) although no trends between urinary ITC tertile and the odds of colorectal cancer were observed within each strata (results not shown). When the cases diagnosed within 2 years of baseline and their matched controls were excluded, the results remained similar (results not shown).

DISCUSSION

From this study of colorectal cancer cases and matched controls nested in the SMHS, we did not find an association between colorectal cancer and cruciferous vegetable intake, either from a FFQ or using urinary level of ITC, a biomarker of intake. We also did not find an association between *GSTM1* or *GSTT1* polymorphisms and colorectal cancer risk, and no interaction between cruciferous vegetable intake or urinary ITC with either *GST* gene variant on the odds of colorectal cancer. The findings were generally similar within the sensitivity analyses.

Previous studies investigating the association between self-reported cruciferous vegetable intake and colorectal cancer have been mixed, although a recent meta-analysis reported a pooled risk ratio (RR) of 0.82 (95% CI: 0.75, 0.90) for the highest category of cruciferous vegetable intake compared to the lowest among men and women combined. The association was weaker for prospective cohort studies (RR 0.93; 95% CI: 0.87, 1.00) suggesting that the association may have been influenced by the case-control design and possibly related to recall bias (24). Unlike self-reported cruciferous vegetable consumption, urinary ITC measurements are unrelated to dietary recall, but reflect more recent cruciferous vegetable consumption since ITC metabolites have been found to be eliminated within 48 hours of cruciferous vegetable intake (25). Studies on the association

between ITC and colorectal cancer have also been variable. One study, also among men in Shanghai, demonstrated an inverse association between ITC and colorectal cancer among cases whose urine samples were collected at least 5 years prior to diagnosis (2nd through 4th versus 1st quartile; OR 0.70; 95% CI: 0.49, 0.99) (11). In our study, when we excluded cases that were diagnosed within two years of baseline, we did not observe an association between urinary ITC and colorectal cancer. Similar to our findings, a study among women in Shanghai did not find an independent association between ITC and CRC (12). A case-control study in the United States found an inverse association between detectable ITC and the incidence of colorectal cancer (OR 0.59; 95% CI: 0.36, 0.98) among men and women combined, but when the study was analyzed by the amount of ITC detected, the inverse association no longer remained significant (10).

In general, most studies have not observed an independent association between *GSTM1* or *GSTT1* and the risk of colorectal cancer (5, 10, 12, 14, 15, 26). One case-control study in the United States did find an inverse association between having 0 or 1 copies of the *GSTM1* gene versus 2 copies and left-sided advanced colorectal adenoma (OR = 0.6; 95% CI = 0.4, 0.9) among women and men combined, but no independent association was observed with the *GSTT1* gene (23).

The literature on the interaction between cruciferous vegetable intake and *GST* gene variants on the risk of colorectal cancer risk has been inconsistent. For instance, in a case-control study in Singapore among women and men, an inverse association was observed between dietary ITC intake and colorectal cancer among participants with the null genotype for both the *GSTM1* and *GSTT1* genes (high dietary ITC versus low OR 0.43; 95% CI: 0.20, 0.96), but when the genotypes were considered individually, no inverse

associations were observed (5). Similarly, in a case-control study within the Shanghai Women's Health Study (SWHS), the strongest inverse association between urinary ITC and colorectal cancer was observed among women with both *GSTM1* and *GSTT1* null genotypes (3^{rd} versus 1^{st} tertile OR 0.51; 95% CI: 0.27, 0.95), but individually, an inverse association was observed among women with the *GSTM1* null genotype, but not the *GSTT1* null genotype (12). Other studies have found no indication of an interaction between cruciferous vegetables and *GST* genotypes, or interactions with *GSTM1* or *GSTT1* only (7, 10, 14, 15). We did not detect any interaction between cruciferous vegetable intake or urinary ITC level with either *GST* gene variant. The lack of association observed in this study within the SMHS compared to the SWHS could be related to gender differences or the prevalence of other risk factors, such as smoking. Future research could consider the potential interaction between smoking status and *GST* gene polymorphisms.

Our study has a number of strengths. All of the self-reported data were obtained prior to diagnosis of cancer and therefore any misreporting of dietary intake should be unrelated to case status. In addition, the blinding of laboratory staff during ITC sample processing and genotyping should decrease any potential bias within the laboratory. Some limitations should also be noted. First, there are limitations to the self-reported measures of cruciferous vegetable intake, however we also analyzed our data according to the ITC biomarker status, and found similar results. Second, laboratory errors in the assessment of urinary ITC and the *GST* gene variants could be an issue, however these errors would most likely be non-differential. Third, the use of a spot urine sample has limitations, however, a study within the SWHS found that spot urine samples collected four times over a 12 month period were strongly correlated with an intra-class correlation

coefficient of 0.50 (12), so the spot urine sample may still estimate typical cruciferous vegetable consumption. Fourth, our study was likely underpowered for the interaction analyses, although when we analyzed the joint effect of the tertiles of cruciferous vegetable consumption or urinary ITC with the *GST* gene variants (results not shown), results remained similar. Finally, although we have carefully adjusted for multiple potential confounders, residual confounding may still be present.

In conclusion, in this prospective study of middle aged and elderly Chinese men, we did not find that cruciferous vegetable intake or urinary level of ITC was significantly associated with the risk of colorectal cancer. Further, there was no indication of an interaction between cruciferous vegetable intake and *GST* gene variants on the risk of colorectal cancer. Future research could consider the potential non-linear effect of cruciferous vegetables in a population with lower intake and also the effect of the microbiome on the association between cruciferous vegetable consumption and colorectal cancer.

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Table 1: Baseline characteristics of the colorectal cancer cases and matched controls from the Shanghai Men's Health Study

	Colorectal cancer cases	Matched controls	p value
Number of subjects	340	673	
Age			
< 55 years	22.4%	21.4%	0.2268
55 - 59 years	10.9%	11.6%	
60 - 64 years	15.6%	14.6%	
65 - 69 years	23.5%	23.5%	
≥ 70 years	27.6%	29.0%	
Educational level (%)			
≤ Elementary school	12.4%	14.9%	0.5259
Middle school	33.8%	31.6%	
High school	25.9%	27.6%	
≥ College	27.9%	25.9%	
Annual per capita family income (%)			
< 6,000 yuan	5.3%	10.0%	0.0619
6,000 - 11,999 yuan	48.5%	45.5%	
12,000 - 23,999 yuan	37.1%	37.6%	
≥ 24,000 yuan	9.1%	7.0%	
Occupation			
Professional	35.6%	37.4%	0.4421
Clerical	20.6%	17.1%	
Manual worker	43.8%	45.5%	
Cigarette smoking (%)			
Never	37.1%	38.9%	0.1830
Past	16.2%	19.3%	
Current	46.8%	41.8%	
Alcohol consumption (%)			
Never	68.2%	68.4%	0.9219
Ever	31.8%	31.6%	
BMI (kg/m^2)	24.4 ± 3.4	23.8 ± 3.1	0.0082
Leisure time PA (MET hours/week)	1.7 ± 2.7	1.7 ± 2.7	0.6846
Total energy intake (Kcal/day)	1913.7 ± 440.6	1849.1 ± 446.4	0.0301
Red meat intake (g/day)	57.5 ± 36.4	55.7 ± 42.3	0.4996
Total meat intake (g/day)	122.6 ± 69.3	115.7 ± 75.8	0.1337
History of diabetes (%)	11.2%	9.2%	0.3234
Family history of cancer (%)	37.9%	28.8%	0.0032

Continuous variables are presented as mean ± standard deviation.

Differences between cases and controls were tested using the Wald test from the conditional logistic regression model.

Table 2: Association between cruciferous vegetable consumption, isothiocyanate and colorectal cancer for cases and matched controls

	Unadjusted		Adjusted	
	OR	**(95% CI)**	**OR**	**(95% CI)**
Cruciferous vegetable consumption				
Tertile 1	1.00		1.00	
Tertile 2	0.88	(0.64, 1.23)	0.85	(0.60, 1.22)
Tertile 3	1.14	(0.83, 1.55)	1.06	(0.76, 1.50)
p trend	0.3897		0.6679	
Increase of 20 g/day of cruciferous vegetable intake	1.01	(0.98, 1.04)	1.00	(0.97, 1.04)
Urinary ITC				
Tertile 1	1.00		1.00	
Tertile 2	1.17	(0.85, 1.62)	1.28	(0.90, 1.81)
Tertile 3	1.09	(0.78, 1.52)	1.12	(0.79, 1.60)
p trend	0.6119		0.5379	
Increase of 1 nmol/mg creatinine of urinary ITC	1.00	(0.97, 1.02)	0.99	(0.97, 1.01)

Adjusted models included age, BMI, leisure time physical activity, total energy intake, red meat intake, total meat intake, education, income, occupation, smoking, alcohol consumption and family history of cancer.

Table 3: Association between glutathione *S*-transferase gene variants and colorectal cancer

	Unadjusted		Adjusted	
	OR	**(95% CI)**	**OR**	**(95% CI)**
GSTM1				
Null	1.00		1.00	
Carrier	0.99	(0.76, 1.30)	1.04	(0.78, 1.38)
GSTT1				
Null	1.00		1.00	
Carrier	0.86	(0.66, 1.12)	0.84	(0.63, 1.11)
GSTM1/GSTT1				
Both null	1.00		1.00	
One null/One carrier	0.75	(0.55, 1.03)	0.76	(0.54, 1.06)
Both carrier	0.80	(0.55, 1.18)	0.83	(0.55, 1.24)
p trend	0.1977		0.2793	

Adjusted models included age, BMI, leisure time physical activity, total energy intake, red meat intake, total meat intake, education, income, occupation, smoking, alcohol consumption and family history of cancer.

Table 4: Evaluation of associations between cruciferous vegetable consumption, isothiocyanate, and colorectal cancer by glutathione *S*-transferase genotype

		Tertile of cruciferous vegetable consumption							Tertile of ITC						
		1	2		3				1	2		3			
	N		OR	(95% CI)	OR	(95% CI)	p trend	p int		OR	(95% CI)	OR	(95% CI)	p trend	p int
GSTM1															
Null	580	1.00	0.67	(0.42, 1.07)	1.04	(0.66, 1.63)	0.7792	0.2867	1.00	1.13	(0.72, 1.77)	1.06	(0.68, 1.65)	0.8111	0.9546
Carrier	393	1.00	1.04	(0.59, 1.84)	1.28	(0.73, 2.23)	0.3786		1.00	1.27	(0.72, 2.24)	1.11	(0.63, 1.94)	0.7629	
GSTT1															
Null	491	1.00	0.78	(0.48, 1.28)	1.11	(0.68, 1.81)	0.6831	0.8807	1.00	0.80	(0.49, 1.30)	1.01	(0.63, 1.63)	0.9392	0.1061
Carrier	514	1.00	0.90	(0.54, 1.50)	1.11	(0.67, 1.82)	0.6457		1.00	1.50	(0.92, 2.43)	0.95	(0.57, 1.59)	0.8302	
GSTM1/GSTT1															
Both null	275	1.00	0.43	(0.21, 0.87)	0.81	(0.42, 1.57)	0.6302	0.4721	1.00	0.98	(0.50, 1.90)	1.44	(0.76, 2.73)	0.2531	0.1375
One null/One carrier	495	1.00	0.97	(0.59, 1.61)	1.42	(0.86, 2.36)	0.1746		1.00	1.08	(0.67, 1.74)	0.71	(0.43, 1.17)	0.1919	
Both carrier	195	1.00	1.16	(0.45, 2.96)	0.95	(0.41, 2.24)	0.8496		1.00	2.45	(0.99, 6.04)	1.79	(0.74, 4.34)	0.2342	

Unconditional logistic regression models (other than overall model)

All models adjusted for age, BMI, leisure time physical activity, total energy intake, red meat intake, total meat intake, education, income, occupation, smoking, alcohol consumption, family history of cancer, and matching variables including sample type (blood/buccal cell), collection time (morning/afternoon), time (hours) between last meal and sample collection, recent antibiotic use, and time (days) between sample collection and assays for ITC.

SUMMARY CONCLUSIONS

Colorectal cancer is a significant global concern with an estimated 1,234,108 cases and 609,051 deaths worldwide in 2008. Of these cases and deaths, approximately 18% occurred in China (1). An increase in colorectal cancer incidence has been observed in China and is hypothesized to be at least partially related to increased exposure to the Western diet and lifestyle, which includes a decreased consumption of fruits and vegetables. Fruits and vegetables have long been studied for their preventive effect on the risk of colorectal cancer, but findings from these studies have been inconsistent. Cruciferous vegetables, in particular, may prevent colorectal cancer through effects of isothiocyanate (ITC), a byproduct of cruciferous vegetables. However, epidemiological studies considering the association between cruciferous vegetable intake, assessed either by a dietary recall method or by urinary ITC, and colorectal cancer have not yielded consistent results. Some of the variability in these findings may be due to different intakes of cruciferous vegetables between populations or variations in genetic polymorphisms between individuals, especially glutathione *S*-transferase (*GST*) gene polymorphisms. Therefore, this dissertation sought to address the association between cruciferous vegetable consumption and colorectal cancer risk while taking into account variations in the *GST* gene.

First, in order to evaluate the association between self-reported cruciferous vegetable intake and colorectal cancer, we used prospective cohort data from the Shanghai Men's Health Study (SMHS) and also considered the association between other vegetables and fruits with colorectal cancer. In this study, we found an inverse association be-

tween fruit intake and the risks of colorectal, colon and rectal cancers. There was little evidence for an association between total vegetable intake and colorectal cancer; although an inverse association was observed for the intake of legumes. No association was observed between cruciferous vegetable intake and the risks of colorectal, colon or rectal cancer. When data from the first year of follow-up of participants who reported a large change in fruit or vegetable intake were excluded, the estimates of the association were largely unchanged. Some statistical interactions were observed between the fruit and vegetable categories with BMI, smoking, and physical activity, but these findings should be interpreted with caution.

Since we did not observe an association between self-reported cruciferous vegetable consumption and colorectal cancer, we next evaluated the correlations between self-reported cruciferous vegetable consumption and a biomarker of intake, urinary ITC, using nested case-control data from the Shanghai Women's and Men's Health Studies. We also assessed the effect of *GST* gene variants on the levels of urinary ITC. In this study, self-reported intake of cruciferous vegetables was weakly correlated with urinary ITC levels from a spot urine sample and the strongest correlations were observed between recent cruciferous vegetable intake and urinary ITC compared to usual intake of cruciferous vegetables. Overall, urinary ITC did not appear to be related to *GST* gene polymorphisms, but when the data were stratified by smoking status, some differences by genotype were observed among current smokers. When the data were stratified by time of urine sample collection, among participants who provided an afternoon urine sample, those with the *GSTM1*-null genotype had lower urinary ITC output than the carrier genotype.

After the evaluation of the correlations between self-reported cruciferous vegetable intake and urinary ITC, we then used this nested case-control data to assess the association with colorectal cancer and to evaluate the potential interaction between both measures of intake and *GST* gene variants on the risk of colorectal cancer in the SMHS. In this study, we did not find an association between colorectal cancer and cruciferous vegetable intake, either from self-report or from urinary level of ITC, a biomarker of intake. We also did not find an association between *GSTM1* or *GSTT1* polymorphisms and colorectal cancer risk, and did not find an interaction between cruciferous vegetable intake or urinary ITC with either *GST* gene variant on the odds of colorectal cancer. The observed findings were similar within the sensitivity analyses.

In conclusion, in these three prospective studies, we did not observe that cruciferous vegetable intake, as assessed by self-report or by urinary level of ITC, was significantly associated with the risk of colorectal cancer. Further, there was no indication of an interaction between cruciferous vegetable intake and *GST* gene variants on the risk of colorectal cancer among men in Shanghai, China.

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THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Institutional Review Board for Human Use

DATE: October 19, 2011

MEMORANDUM

TO: Emily J. Vogtmann
Principal Investigator

FROM: Cari Oliver
Assistant Director, UAB OIRB

RE: Request for Determination—Human Subjects Research
IRB Protocol #N110927001 – Association Between Cruciferous Vegetable Intake and Colorectal Cancer Incidence

A member of the Office of the IRB has reviewed your application for Designation of Not Human Subjects Research for above referenced proposal.

The reviewer has determined that this proposal is **not** subject to FDA regulations and is **not** Human Subjects Research. Note that any changes to the project should be resubmitted to the Office of the IRB for determination.

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