GSTP1 and *GSTA1* Polymorphisms Interact with Cruciferous Vegetable Intake in Colorectal Adenoma Risk

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Abstract

The possible interplay between cruciferous vegetable consumption, functional genetic variations in glutathione *S*-transferases (GST) *M1*, *T1*, *P1*, and *A1*, and colorectal adenomas, was investigated in a Dutch case-control study. The *GSTM1* and *GSTT1* deletion polymorphisms, and the single nucleotide polymorphisms in *GSTP1* (A313G) and in *GSTA1* (C-69T) were assessed among 746 cases who developed colorectal adenomas and 698 endoscopy-based controls without any type of colorectal polyps. High and low cruciferous vegetable consumption was defined based on a median split in the control group. High consumption was slightly positively associated with colorectal adenomas [odds ratio (OR) 1.15; 95% confidence interval, 0.92-1.44]. For *GSTP1*, a positive association with higher cruciferous vegetable intake was only apparent in individuals with the low-activity *GSTP1* genotype (GG genotype, OR 1.94; 95% confidence interval, 1.02-3.69). This interaction was more pronounced in men, with higher age and with higher meat intake. The *GSTA1* polymorphism may have a modifying role as well: the OR for higher intake compared with lower intake was 1.57 (0.93-2.65) for individuals homozygous for the low expression variant (TT genotype). This seemed to be stronger with younger age and higher red meat intake. Cruciferous vegetable consumption and the combined *GSTA1* and *GSTP1* genotypes showed a statistically significant interaction (P = 0.034). The *GSTM1* and *GSTT1* genotypes did not seem to modify the association between cruciferous vegetable intake and colorectal adenomas. In conclusion, *GSTP1* and *GSTA1* genotypes might modulate the association between cruciferous vegetable intake and colorectal adenomas. (Cancer Epidemiol Biomarkers Prev 2005;14(12):2943–51)

Introduction

Diet and other life-style factors are thought to play a major role in the colorectal neoplastic process (1, 2). Sporadic colorectal cancers arise from acquired DNA alterations which progressively facilitate uncontrolled cell growth, are predominantly epithelial, and most are preceded by adenomas (3).

Consumption of vegetables of the family Cruciferae—in Western food patterns consisting mostly of the genus *Brassica*, including cabbage, cauliflower, Brussels sprouts, and broccoli—has been associated with a decreased risk of colorectal adenomas and cancer (4), although not consistently (5-10). Cruciferous vegetables characteristically contain glucosinolates, phytochemicals that are hydrolyzed to the biologically active isothiocyanates (11). Isothiocyanates show several anticancer properties, including induction of phase II biotransformation enzymes such as glutathione *S*-transferases (GST; EC 2.5.1.18; refs. 11, 12). Thus, they may enhance the detoxification and excretion of carcinogens and prevent alterations to the DNA (13). In addition, isothiocyanates are substrates for GSTs (14-16) and thus GSTs contribute to the excretion of isothiocyanates. The involvement of GSTs in isothiocyanate metabolism has led to the hypothesis that, through slower excretion of isothiocyanates from the body in individuals with genetic variants associated with lower GST capacity, isothiocyanates have more opportunity to exert their chemoprotective effects in these individuals (17).

Genetic polymorphisms that result in the constitutively lower or absence of GST enzyme activity or expression are known for most GST isoforms (18). Research has mostly focussed on the *GSTM1* and *GSTT1* deletion polymorphisms (''null'' genotypes). These partial gene deletions result in the absence of the GSTμ1 or GSTθ1 enzyme, and occur in 23 to 62% and 10 to 64% of the worldwide population, respectively (19). A functional polymorphism resulting in a less active enzyme with lower thermal stability is known in the *GSTP1* gene (20). This A to G substitution at nucleotide position 313, changing an isoleucine to a valine amino acid, is present homozygously in 4 to 12% of the population (21). GSTπ is the most abundant GST isoenzyme in the colon (22). A variant haplotype resulting in 3- to 4-fold lower expression has been identified in the *GSTA1* promoter (23), and occurs homozygously in 2 to 14% of the population (24).

Some epidemiologic studies indicate an interplay between crucifer consumption and metabolism by GSTs. *GSTT1*-null subjects were found to have significantly lower urinary excretion of isothiocyanates relative to *GSTT1*-positive subjects in the Singapore Chinese Health Study (25). In this cohort study, a significantly lower risk of colon cancer was observed among individuals without both the *GSTM1* and *GSTT1* genes who had high intakes of cruciferous vegetables, compared with those with low intakes; this was not seen among individuals with one or both genes present (26). In a U.K. case-control study, a significant inverse association between high versus low cruciferous vegetable consumption and colorectal cancer risk was observed among individuals with the *GSTT1*-null genotype as compared to those with one or both alleles present (27). In the U.S., Lin et al. observed a lower risk of colorectal adenomas with higher consumption of cruciferous vegetables (in particular, broccoli), which was statistically significant in individuals who lacked the *GSTM1* gene only (17). The aim of the present case-control analysis was to further explore, in a Dutch population the hypothesis

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that in individuals with lower imputed GST capacity (here, *GSTM1* null, *GSTT1* null, *GSTP1* 313 G, and *GSTA1* −69 T variants), higher cruciferous vegetable consumption is associated with a greater reduction of the risk of colorectal adenomas.

Materials and Methods

Population. The study design and population have been previously described (28, 29). Cases and controls were recruited among those undergoing endoscopy in 10 endoscopy outpatient clinics in the Netherlands between June 1997 and October 2002. Participants were informed of the study by endoscopy staff at the time of endoscopy or by mail at 3-month intervals using endoscopy reports of all patients who had undergone endoscopy. All received the same information package. Eligibility criteria were: Caucasian, Dutch speaking, ages 18 to 75 years at time of endoscopy, no hereditary colorectal cancer syndromes, no chronic inflammatory bowel disease, no history of colorectal cancer and no (partial) bowel resection. Cases had at least one histologically confirmed colorectal adenoma ever in their lives. Suitability of this case definition was confirmed by analyses restricted to cases who were first diagnosed at endoscopy of inclusion, yielding similar conclusions. Controls had no medical history of any type of polyp, confirmed by full colonoscopy (75%) or sigmoidoscopy combined with X-ray (10%). Fifteen percent of the subjects did not have full visualization of the colon, i.e., they had a sigmoidoscopy without X-ray or colonoscopy where the cecum was not reached.

The study was approved by the Medical Review Boards of all participating hospitals and of Wageningen University. The overall response rate was estimated to be 55%, varying from 35% to 90% among outpatient clinics. The total study population consisted of 1,477 participants (768 cases and 709 controls). All gave their written informed consent.

Diet and Other Lifestyle Factors. Dietary intake was assessed by a self-administered food frequency questionnaire developed for the Dutch European Prospective Investigation into Cancer and Nutrition cohort and processed using the Dutch food composition table (30). Cruciferous vegetable consumption was calculated as the sum of raw and cooked crucifers. For cooked crucifers, the frequency question was phrased ''how often do you habitually consume Brussels sprouts, cauliflower, broccoli and (other) cabbage,'' which covers the most commonly consumed cruciferous vegetables in the Netherlands. The frequency of cooked vegetable consumption could be indicated per day, week, month, or year. If the sum of frequencies for the individual cooked vegetables was not equal to the question on total cooked vegetable consumption, the frequencies for individual vegetables were corrected proportionally (30). Raw cabbage was measured as 1 of 10 types of raw vegetables, and frequency of consumption could be indicated as always/most of the time, often, sometimes or seldom/never. Color photographs were included for 21 food items; cooked and raw crucifer portion size were estimated from a photograph containing cooked red cabbage and raw mixed salad, respectively. Frequencies and portion sizes were multiplied to obtain the amount (in grams) for each food item. The food frequency questionnaire referred to habitual intake in the year preceding endoscopy or bowel problems. General life-style factors and disease-related issues, such as physical activity, smoking, self-reported family history of cancer, and dietary changes due to bowel problems were assessed by a self-administered questionnaire. Both food frequency questionnaire and general questionnaire were handed at the time of endoscopy or sent within 3 months after endoscopy. Endoscopy-related and other medical information was abstracted from the patient's medical record.

Genotyping. EDTA-treated whole blood was collected and stored at −20°C until DNA extraction (QIAamp 96 DNA blood kit, Qiagen, Inc.). An average DNA concentration of ~20 ng/μL was used for genotyping analyses. DNA was stored at 4°C, in random order in 8 × 12 array banks. Samples were interspersed with water controls to check for cross-contamination. Laboratory staff was blinded for case-control status.

The *GSTM1* and *GSTT1* deletion polymorphisms were determined using a general multiplex PCR amplification method (31). A β-globin gene fragment was present as a positive control. The *GSTP1* A313G polymorphism was assessed by PCR amplification followed by RFLP according to Harries et al. (32). The *GSTA1* C-69T polymorphism was determined by PCR-RFLP according to Coles et al. (23). They reported this single nucleotide polymorphism to be fully linked (in 55 liver samples) to a G to A substitution at position −52 in the promoter region (23), which has been shown to be functional in cell studies (33). In a subsample of 93 individuals, we determined the nucleotide sequence of the relevant promoter region by strand termination sequencing, and also found that the C-69T single nucleotide polymorphism and the presumed functional G-52A single nucleotide polymorphism were in 100% linkage disequilibrium. Some modifications to the Coles' PCR-RFLP protocol were made; Denaturing, annealing, and elongation times were set at 30, 30, and 45 seconds, respectively, and annealing temperature at 61°C. In the RFLP, the *Eam*1104I restriction enzyme was used (Fermentas, Inc.). Positive controls were included in the genotyping analyses. *GSTP1* and *GSTA1* genotypes were in Hardy-Weinberg equilibrium among both controls and cases, as tested by a χ^2 test. To test reproducibility, one row out of each DNA bank (8% of the population) was genotyped in duplicate; reproducibility was 100%.

Data Analysis. Eighteen participants (11 cases and 7 controls) were excluded because blood was unavailable or the amount of DNA was insufficient, and therefore all polymorphism results were absent. Fifteen patients (11 cases and 4 controls) were excluded because of incomplete data on vegetable consumption. This resulted in a final population of 746 cases and 698 controls.

Cruciferous vegetable intakes were dichotomized by a median split based on the distribution in the control group. Conventional logistic regression was used to calculate odds ratios (OR) and 95% confidence intervals (CI), using a single reference group (low GST capacity variant and low crucifer intake).

Evaluated as possible confounders of the relation between median split cruciferous vegetable intake and colorectal adenomas were: consumption of fruit, vegetables minus crucifers, and meat; intake of energy, folate, calcium, dietary fiber, vitamin C, coffee, alcohol, and saturated fat; physical activity, use of nonsteroidal anti-inflammatory drugs, smoking, education, hormone replacement therapy, body mass index, abdominal problems, defecation problems, and self-reported family history of colorectal cancer. Folate intake, age (four categories) and sex changed the β-estimate by 35%, 20%, and 20%, respectively, and were included in the model. Energy intake did not affect the estimates, but was included for comparability with other studies.

The P value for interaction was calculated by χ^2 test comparing the −2 log-likelihood values of the models with and without crucifer-by-genotype interaction term(s). $P < 0.05$ was considered statistically significant. Genotypes were considered individually and in combinations; grouping rationales were: correspondence with the literature (*GSTM1/T1*), high colonic gene expression (*GSTP1/T1*), high hepatic gene expression (*GSTA1/M1*), and posterior combination of the most promising separate genotypes (*GSTP1/A1*).

The crucifer-by-genotype interaction was further studied in strata of age (≤ and >55 years), sex, smoking (ever and never), and meat consumption (fresh red and processed, ≤ and > median intake). Stratifying rationales were: indication from the literature for a possible modification by age (34) and sex (35), and our hypothesis that individuals with high exposure to (pro)carcinogens from meat and cigarettes, and low activity GST variants, may benefit most from high cruciferous vegetable intake. For comparison, the cruciferous vegetable median split based on population controls was maintained. Sex-specific median cutoff points yielded similar estimates as population-based medians; to enable comparison, we present the latter. Statistical analyses were done using SAS software, version 9.1 (SAS Institute, Cary, NC).

Results

Table 1 shows the characteristics of the study population. The control group included more women than the case group. Cases were older and more often smokers. Controls more often had defecation problems or other abdominal complaints than cases. Cases, on the other hand, more often experienced rectal bleeding and more often had a history of endoscopy and came for surveillance. Cases consumed more cruciferous vegetables and total vegetables than controls, and had a higher alcohol intake. The distribution of GST variants did not differ between cases and controls.

In Table 2, the associations between cruciferous vegetable intake, GST polymorphisms and colorectal adenomas are presented. Consumption of cruciferous vegetables was slightly positively associated with colorectal adenomas. A positive association with higher cruciferous vegetable intake was more obvious for the *GSTP1* low activity genotype (GG) and *GSTA1* low expression genotype (TT). The combination of *GSTP1* and *GSTA1* genotypes showed a statistically significant interaction with cruciferous vegetable consumption; adenoma risk was higher with high cruciferous vegetable intake for subjects with one or both of the low capacity genotypes (*GSTP1* GG or *GSTA1* TT), but not for subjects with both high-capacity variants (*GSTP1* AA and *GSTA1* CC). In the low crucifer group, the high-capacity variants appeared to have a higher adenoma risk than the low-capacity variants. *GSTM1* and *GSTT1* deletion polymorphisms did not seem to affect the association between cruciferous vegetable intake and colorectal adenomas.

When, instead of a median split, we used tertiles of cruciferous vegetable intake, the high crucifer intake × low *GSTP1* activity (GG) variant and high crucifer intake × low *GSTA1* expression variant combinations still had the highest adenoma risk: OR, 1.52 (95% CI, 0.70-3.28; P for interaction, 0.081), and 2.27 (95% CI, 1.21-4.24; P for interaction, 0.081), respectively. Cruciferous vegetable consumption expressed as a continuous variable showed significant interaction with the *GSTA1* variants ($P = 0.030$).

About half ($n = 394$) of all cases did not have a history of colorectal polyps. Analyses restricted to these newly

Table 1. Population characteristics

Characteristics		Cases	Controls
		$n = 746$	$n = 698$
General			
Sex	% female, g/wk etc.	46.5	61.8
Age (mean ± SD)	years	59.0 ± 10.1	51.5 ± 13.6
Smoking status	% ever	66.8	55.3
Body mass index (mean ± SD)		26.1 ± 3.9	25.5 ± 4.1
Physical activity	% low	38.2	33.2
Regular nonsteroidal anti-inflammatory drug use (≥12/y)	% yes	26.7	29.7
Education	% low*	35.9	33.0
Medical (% yes)			
Family history of colorectal cancer		23.2	20.0
Abdominal complaints		34.0	59.0
Defecation problems		25.7	43.4
Blood loss per anum		30.6	18.9
Surveillance/follow-up		48.0	6.1
Dietary intake, median (10-90th percentile)			
Energy	kJ/d	8,394 (5,889-11,800)	8,113 (5,452-11,808)
Cruciferous vegetables	g/wk	137 (41-322)	129 (32-289)
	freq/wk	0.9 (0.3-1.9)	0.8 (0.2-1.7)
Total vegetables†	g/d	114 (69-180)	109 (68-169)
	freq/wk	7.0 (4.6-11.5)	7.0 (4.7-11.0)
Total fruit	g/d	127 (18-367)	125 (27-350)
	freq/wk	7.0 (1.0-21.0)	7.0 (1.5-21.0)
Folate	μg/d	194 (139-272)	185 (131-260)
Dietary fiber	g/d	23 (15-32)	23 (15-32)
Total meat	g/d	107 (40-177)	102 (35-172)
	freq/wk	14.5 (5.9-31.0)	14.0 (5.4-29.9)
Fresh red meat	g/d	60 (16-100)	53 (13-96)
	freq/wk	4.3 (1.7-6.0)	4.2 (1.6-6.1)
Processed meat	g/d	30 (6-75)	29 (5-73)
	freq/wk	9.2 (1.03-25.4)	8.4 (1.09-24.1)
Alcohol	g/d	9 (0-42)	4 (0-31)
Coffee	cups/d	4 (2-7)	4 (0-8)
Genotypes (%)			
GSTM1	present/null	48.6/51.4	45.9/54.1
GSTT1	present/null	82.0/18.0	81.8/18.2
GSTP1	AA/AG/GG	42.5/45.3/12.2	42.8/45.3/11.9
GSTA1	CC/CT/TT	33.8/47.9/18.3	34.8/47.1/18.1

*Primary school or lower vocational training only.
†Definition of total vegetables includes nonstarch legumes (string beans and peas) and excludes potatoes and vegetable juice.

Table 2. Interplay between cruciferous vegetable intake (g/wk), GST genotypes, and the risk of colorectal adenomas

Crucifer intake (g/wk)*	*n* (cases/controls)		Age + sex-adjusted OR (95% CI), *P* for interaction†		Multiple adjusted‡ OR (95% CI), *P* for interaction†	
	≤129	>129	≤129	>129	≤129	>129
All genotypes	336/349	410/349	1	1.22 (0.98-1.52)	1	1.15 (0.92-1.44)
Individual genotypes§						
GSTM1						
Null	168/185	213/192	1	1.18 (0.87-1.59)	1	1.12 (0.83-1.53)
Present	166/163	194/157	1.09 (0.79-1.49)	1.39 (1.01-1.89)	1.09 (0.80-1.50)	1.30 (0.94-1.79)
			0.72		0.81	
GSTT1						
Null	62/61	71/66	1	1.13 (0.68-1.88)	1	1.08 (0.64-1.80)
Present	272/287	336/283	0.97 (0.64-1.46)	1.20 (0.80-1.80)	0.99 (0.65-1.49)	1.15 (0.76-1.73)
			0.75		0.79	
GSTP1						
GG‖	34/43	56/40	1	2.06 (1.09-3.89)	1	1.94 (1.02-3.69)
AG	151/169	183/147	1.22 (0.72-2.07)	1.61 (0.95-2.71)	1.22 (0.72-2.06)	1.50 (0.89-2.55)
AA	148/137	166/162	1.43 (0.84-2.43)	1.39 (0.82-2.34)	1.40 (0.82-2.39)	1.30 (0.76-2.20)
			0.10		0.11	
GSTA1						
TT‖	53/63	82/63	1	1.67 (1.00-2.81)	1	1.57 (0.93-2.65)
CT	166/169	187/160	1.26 (0.81-1.97)	1.42 (0.91-2.21)	1.24 (0.79-1.95)	1.32 (0.84-2.06)
CC	113/117	136/126	1.19 (0.75-1.91)	1.39 (0.87-2.21)	1.17 (0.73-1.88)	1.30 (0.82-2.08)
			0.42		0.43¶	
Genotype combinations						
GSTM1/T1						
Both null	30/33	32/36	1	1.15 (0.56-2.35)	1	1.11 (0.54-2.29)
M1 or T1 null	170/180	220/186	1.18 (0.67-2.07)	1.37 (0.79-2.39)	1.21 (0.69-2.12)	1.33 (0.76-2.32)
Both present	134/135	155/127	1.16 (0.66-2.06)	1.53 (0.86-2.71)	1.20 (0.67-2.11)	1.46 (0.82-2.59)
			0.86		0.91	
GSTP1/A1						
P1 GG and/or A1 TT	81/100	129/94	1	1.87 (1.23-2.84)	1	1.76 (1.16-2.69)
Remaining genotypes	196/211	216/196	1.23 (0.85-1.77)	1.37 (0.95-1.99)	1.22 (0.84-1.77)	1.28 (0.88-1.87)
P1 AA and A1 CC	54/38	60/59	1.71 (1.00-2.92)	1.33 (0.81-2.16)	1.69 (0.99-2.88)	1.24 (0.76-2.03)
			0.033		0.034	
GSTA1/M1						
A1 CT/TT and M1 null	118/127	143/125	1	1.18 (0.82-1.70)	1	1.12 (0.78-1.62)
A1 CC and M1 null	49/58	66/67	0.93 (0.58-1.50)	1.05 (0.67-1.64)	0.94 (0.58-1.51)	1.03 (0.66-1.60)
A1 CT/TT and M1 present	100/104	124/98	1.03 (0.70-1.52)	1.38 (0.94-2.02)	1.04 (0.70-1.54)	1.30 (0.88-1.91)
A1CC and M1 present	64/59	69/59	1.11 (0.70-1.74)	1.33 (0.85-2.09)	1.10 (0.70-1.74)	1.25 (0.79-1.97)
			0.96		0.98	
GSTP1/T1						
P1 AG/GG and T1 null	35/35	45/34	1	1.36 (0.69-2.68)	1	1.26 (0.64-2.49)
P1 AA and T1 null	27/26	26/32	1.03 (0.49-2.18)	0.91 (0.44-1.89)	0.99 (0.46-2.09)	0.87 (0.41-1.81)
P1 AG/GG and T1 present	149/176	194/153	0.88 (0.51-1.52)	1.30 (0.76-2.22)	0.89 (0.51-1.53)	1.23 (0.71-2.11)
P1 AA and T1 present	120/111	137/130	1.10 (0.63-1.93)	1.08 (0.62-1.88)	1.09 (0.62-1.92)	1.01 (0.58-1.76)
			0.34		0.37	

*Mean cruciferous vegetable intake was 73.1 ± 36.3 and 70.3 ± 36.6 g/wk for cases and controls, respectively, in the lower-than-median intake group; and 242.5 ± 108.4 and 230.3 ± 109.2 g/wk, respectively, in the higher-than-median intake group.
†Calculated as the LR test comparing the models with and without crucifer-by-genotype interaction terms.
‡Adjusted for age (in four categories: <45, <55, <65, <75 y), sex, energy intake (kJ/d) and folate intake (g/d).
§Numbers do not always add up to numbers under ''All genotypes'' due to missing genotype data (M1,T1: 6; P1: 8; A1: 9).
‖Least active/expressed variant(s).
¶When cruciferous vegetable intake is considered as a continuous variable, crucifer × genotype interaction is significant ($P = 0.030$).

diagnosed cases yielded similar conclusions, with more pronounced results for *GSTP1* and *GSTA1* genotypes; for *GSTP1*, the OR for high crucifer intake × *GSTP1* GG-genotype was 2.90 (95% CI, 1.32-6.39; *P* for interaction, 0.029); for *GSTA1*, the OR for high crucifer intake × *GSTA1* TT genotype was 1.92 (1.03-3.58; *P* for interaction, 0.17); and for the combined *GSTA1* and *GSTP1* genotypes; *P* for interaction was 0.0023, with an OR of 2.31 (95% CI, 1.40-3.84) for the high intake × low GST capacity combination. Median intake of cruciferous vegetables among incident cases was slightly, but not statistically significantly, lower than intake among prevalent cases (143 versus 135 g/wk; $P = 0.48$).

In 15% of our controls, a full visualization of the colon was not accomplished. However, restriction to controls with full visualization ($n = 591$) yielded similar results as analyses including all controls (data not shown).

Further evaluation in strata of age and sex showed differences in colorectal adenoma risk (Table 3). *GSTP1* genotypes and cruciferous vegetable intake showed a statistically significant interaction in the higher, but not in the lower age category. The *GSTA1* genotype showed a near-significant interaction with cruciferous vegetable medians in the lower age category only. In men, but not in women, there was a statistically significant interaction between the *GSTP1* genotype and cruciferous vegetable intake. Noteworthy in this respect is the lower prevalence of the *GSTP1* GG genotype in men: 10.2% (cases 10.6%, controls 9.7%) versus 13.6% for women (cases 14.0%, controls 13.3%; $P = 0.054$). The overall colorectal adenoma risk (without genotype stratification) with higher cruciferous vegetable consumption seemed higher in men. In individuals with a positive family history of colorectal cancer, prevalence of the *GSTA1* low expression genotype (*GSTA1* TT) was significantly lower than in individuals without a positive family history of cancer (13.5% versus 19.1%; $P = 0.027$). The overall colorectal adenoma risk with higher cruciferous vegetable consumption was higher in individuals with a positive family history: OR, 1.83 (1.12-3.01). Unfortunately, power was too low for

gene-environment analyses in separate strata of family history.

In strata of possible environmental exposure to carcinogens (Table 4), the higher risk with higher cruciferous vegetable intake associated with the low activity *GSTP1* variant seemed more pronounced in subgroups of higher (red and processed) meat consumption, and possibly in nonsmokers. For *GSTA1*, results seemed more pronounced in the higher red meat subgroup.

Discussion

In this endoscopy-based case-control study of colorectal adenomas, the *GSTP1* A313G and *GSTA1* C-69T single nucleotide polymorphisms appear to modify the association between cruciferous vegetable consumption and colorectal adenoma risk, although with results that contradict our hypothesis that low-capacity GST genotypes benefit (most) from higher consumption: compared with the low crucifer consumption × low GST capacity variant combination, the highest adenoma risk was observed in the high crucifer consumption × low GST capacity variant combination. There was no indication for an interplay between cruciferous vegetable consumption and *GSTM1* or *GSTT1* deletion polymorphisms in this population.

Our study has strengths and weaknesses. The response rate was ~55%, and varied rather widely by clinic, depending on local recruitment factors. However, selection procedures were identical for cases and controls, reducing the possibility for differential selection bias. Unfortunately, we do not have sufficient data on patients not participating in the study to further evaluate the possible selection bias due to nonresponse. Our control group underwent endoscopy, mostly because of bowel problems, and as such, may not be fully representative of the average individual without adenomas in the population. Therefore, risk estimates cannot be extrapolated to the general population inadvertently. However, adenomas are fairly prevalent in the population and often do not give symptoms (36). Thus, the fact that our control status was defined by endoscopy can be seen as an advantage. Full visualization of the colon was achieved for most controls and restriction of analyses to this group did not change the results. Combined with the fact that adenomas were histologically confirmed, case-control misclassification is unlikely. Bowel complaints may lead to a change in food habits. Only few participants, however, indicated an increase in vegetable intake (16 cases and 18 controls) or a decrease in cabbage intake (8 cases and 4 controls) specifically. Exclusion of these participants from analyses did not change our conclusion. Excluding those with bowel problems weakened results for *GSTP1*, strengthened results for *GSTA1*, and yielded similar results for the *GSTP1/A1* combination, although statistical significance was lost due to small numbers.

Our case group consisted of new as well as previously diagnosed cases. Restriction of analyses to new cases did not change our conclusions and thus information bias due to differences in the recall of dietary habits and/or time span of adenoma diagnosis and related surveillance is likely to have been limited. Information on reproducibility and validity of

Table 3. Interplay between cruciferous vegetable intake (g/wk), *GSTP1* and *GSTA1* genotype, and the risk of colorectal adenomas in subgroups of age and sex

Crucifer intake (g/wk)*	*n* (cases/controls)		OR (95% CI)†, *P* for interaction‡		*n* (cases/controls)		OR (95% CI)†, *P* for interaction‡	
	≤129	>129	≤129	>129	≤129	>129	≤129	>129
Age			≤55 y§				>55 y§	
All genotypes	119/205	134/195	1	1.14 (0.82-1.59)	217/144	276/154	1	1.17 (0.86-1.58)
GSTP1								
GG‖	11/21	15/27	1	1.10 (0.41-2.94)	23/22	41/13	1	3.22 (1.35-7.69)
AG	51/104	59/76	0.93 (0.41-2.09)	1.38 (0.61-3.15)	100/65	124/71	1.50 (0.76-2.93)	1.61 (0.83-3.14)
AA	55/80	57/92	1.26 (0.56-2.86)	1.13 (0.50-2.56)	93/57	109/70	1.54 (0.78-3.05)	1.45 (0.74-2.84)
			0.34				0.040	
GSTA1								
TT‖	14/39	27/35	1	2.31 (1.03-5.18)	39/24	55/28	1	1.22 (0.61-2.45)
CT	63/95	55/91	2.00 (0.99-4.04)	1.58 (0.77-3.22)	103/74	132/69	0.90 (0.50-1.65)	1.19 (0.65-2.17)
CC	41/71	49/69	1.58 (0.76-3.29)	2.12 (1.02-4.40)	72/46	87/57	0.98 (0.52-1.85)	0.95 (0.51-1.77)
			0.060				0.67	
Sex			Male				Female	
All genotypes	190/145	209/122	1	1.24 (0.89-1.72)	146/204	201/227	1	1.05 (0.77-1.44)
GSTP1								
GG‖	18/19	24/7	1	3.75 (1.25-11.2)	16/24	32/33	1	1.33 (0.57-3.08)
AG	80/73	101/54	1.25 (0.59-2.62)	1.99 (0.94-4.22)	71/96	82/93	1.16 (0.55-2.44)	1.14 (0.54-2.39)
AA	91/53	82/61	1.93 (0.91-4.09)	1.45 (0.68-3.06)	57/84	84/101	1.04 (0.49-2.22)	1.12 (0.54-2.35)
			0.0095				0.82	
GSTA1								
TT‖	35/29	38/21	1	1.37 (0.65-2.92)	18/34	44/42	1	1.72 (0.81-3.64)
CT	83/63	103/61	1.06 (0.57-1.95)	1.38 (0.75-2.54)	83/106	84/99	1.49 (0.76-2.90)	1.29 (0.66-2.54)
CC	70/53	66/40	1.16 (0.62-2.18)	1.25 (0.65-2.41)	43/64	70/86	1.19 (0.58-2.45)	1.35 (0.68-2.70)
			0.83				0.28	

*Mean cruciferous vegetable intake was 73.1 ± 36.3 and 70.3 ± 36.6 g/wk for cases and controls, respectively, in the lower-than-median intake group; and 242.5 ± 108.4 and 230.3 ± 109.2 g/wk, respectively, in the higher-than-median intake group.
†Adjusted for age (in 4 categories: <45, <55, <65, <75 y), sex, energy intake (kJ/d) and folate intake (g/d), if applicable.
‡Calculated as the LR test comparing the models with and without crucifer-by-genotype interaction terms.
§Age in the lower and higher age category was 44.1 ± 8.8 and 64.7 ± 5.6 years, respectively.
‖Least active/expressed variant(s).

Table 4. Interplay between cruciferous vegetable intake (g/wk), *GSTP1* and *GSTA1* genotype, and the risk of colorectal adenomas in subgroups of meat consumption and smoking

Crucifer intake (g/wk)*	*n* (cases/controls)		OR (95% CI)†, *P* for interaction‡		*n* (cases/controls)		OR (95% CI)†, *P* for interaction‡	
	≤129	>129	≤129	>129	≤129	>129	≤129	>129
Red meat (g/d)§			≤53				>53	
All genotypes	161/173	161/176	1	0.93 (0.66-1.30)	175/176	249/173	1	1.36 (1.00-1.86)
GSTP1								
GG‖	17/19	26/24	1	1.44 (0.58-3.61)	17/24	30/16	1	2.67 (1.07-6.69)
AG	65/85	68/77	1.05 (0.48-2.28)	0.97 (0.44-2.11)	86/84	115/70	1.39 (0.67-2.89)	2.04 (0.98-4.22)
AA	78/69	65/75	1.33 (0.61-2.89)	1.08 (0.49-2.38)	70/68	101/87	1.34 (0.63-2.82)	1.48 (0.72-3.04)
			0.56				0.22	
GSTA1								
TT‖	29/30	28/33	1	1.05 (0.48-2.28)	24/33	54/30	1	2.36 (1.14-4.87)
CT	86/87	64/76	1.12 (0.59-2.12)	0.78 (0.40-1.51)	80/82	123/84	1.41 (0.74-2.67)	2.01 (1.07-3.77)
CC	46/56	67/67	0.85 (0.43-1.70)	1.04 (0.53-2.02)	67/61	69/59	1.66 (0.86-3.22)	1.66 (0.86-3.24)
			0.30				0.16	
Processed meat (g/d)¶			≤29				>29	
All genotypes	156/160	189/189	1	0.96 (0.69-1.33)	180/189	221/160	1	137 (1.00-1.88)
GSTP1								
GG‖	19/18	28/24	1	1.23 (0.50-3.03)	15/25	28/16	1	3.25 (1.27-8.30)
AG	73/68	79/89	1.11 (0.51-2.40)	0.81 (0.38-1.75)	78/101	104/58	1.42 (0.68-2.98)	2.87 (1.35-6.10)
AA	62/74	79/76	0.87 (0.40-1.89)	1.03 (0.48-2.22)	86/63	87/86	2.24 (1.05-4.75)	1.66 (0.79-3.49)
			0.32				0.0016	
GSTA1								
TT‖	23/33	42/36	1	1.85 (0.88-3.89)	30/30	40/27	1	1.41 (0.67-2.98)
CT	82/79	81/87	1.69 (0.88-3.25)	1.30 (0.68-2.51)	84/89	106/74	0.94 (0.50-1.75)	1.36 (0.72-2.54)
CC	48/48	63/66	1.47 (0.72-2.97)	1.38 (0.70-2.72)	65/69	73/60	1.00 (0.53-1.91)	1.27 (0.66-2.43)
			0.14				0.92	
Smoking			Never				Ever	
All genotypes	102/146	146/165	1	1.19 (0.82-1.73)	234/202	264/184	1	1.16 (0.87-1.55)
GSTP1								
GG‖	12/18	24/15	1	3.32 (1.16-9.53)	22/25	32/25	1	1.44 (0.64-3.26)
AG	48/76	54/72	1.14 (0.48-2.71)	1.17 (0.49-2.78)	103/93	129/75	1.28 (0.65-2.50)	1.79 (0.91-3.50)
AA	40/53	66/78	1.27 (0.52-3.09)	1.34 (0.57-3.15)	108/84	100/84	1.46 (0.74-2.85)	1.30 (0.66-2.57)
			0.12**				0.29	
GSTA1								
TT‖	14/24	31/30	1	2.16 (0.88-5.30)	39/39	51/33	1	1.37 (0.71-2.64)
CT	57/71	65/80	1.45 (0.64-3.29)	1.25 (0.56-2.75)	109/98	122/80	1.16 (0.67-2.01)	1.45 (0.81-2.53)
CC	29/52	48/55	0.96 (0.41-2.27)	1.45 (0.64-3.32)	84/65	88/71	1.32 (0.74-2.35)	1.26 (0.71-2.23)
			0.14				0.59	

*Mean cruciferous vegetable intake was 73.1 ± 36.3 and 70.3 ± 36.6 g/wk for cases and controls, respectively, in the lower-than-median intake group; and 242.5 ± 108.4 and 230.3 ± 109.2 g/wk, respectively, in the higher-than-median intake group.
†Adjusted for age (in four categories: <45, <55, <65, <75), sex, energy intake, and folate intake.
‡Calculated as the LR test comparing the models with and without crucifer-by-genotype interaction terms.
§Fresh red meat consumption defined as sum of pork, beef and unclassified (non-organ) meats; consumption in the lower and higher category was 28.7 ± 15.2 and 82.1 ± 21.2 g/d, respectively.
‖Least active/expressed variant(s).
¶Processed meat defined as preserved and ready-to-eat meat products; consumption in the lower and higher category was 13.7 ± 8.2 and 57.8 ± 32.7 g/d, respectively.
**Lemeshow goodness-of-fit test ($P = 0.049$).

our cruciferous vegetable measurement is not available, and we cannot rule out some degree of exposure misclassification. For total vegetables, the reproducibility was 0.76 and 0.65 for men and women, respectively, and relative validity was 0.38 and 0.31 for men and women, respectively, which is in the range of estimates that others find for food frequency questionnaires (30). Genotype misclassification is assumed to be low, as measurement and scoring was blind, negative and positive controls were included and duplos were assessed with excellent reproducibility. The genotypes are sufficiently prevalent and the study population sufficiently large to render power adequate for most of the analyses.

Our study has many strengths, yet the risk of selection and information bias remains a point of attention in case-control studies. Bias cannot be ruled out in the explanation of our finding of a slightly higher adenoma risk with higher cruciferous vegetable consumption. However, we add to an existing literature, of both case-control and cohort studies, in which increased risks, significant and nonsignificant, of colorectal adenomas or cancer with higher intakes of cruciferous vegetables have been reported (5-8, 10). Unlike these studies, we also incorporated genetic polymorphisms in our design.

We hypothesized that inherited decreased GST capacity, which presumably results in a longer biological half-life of phytochemicals from cruciferous vegetables, may confer increased chemopreventive potential when these vegetables are consumed at higher levels. Other studies preceded ours: for the *GSTM1* and/or *GSTT1* deletion genotypes, lower colorectal adenoma (17) and cancer (26, 27, 34) risks were observed with higher cruciferous vegetable intake. In the case-control study by Lin et al., however, the observed

decreased risk of colorectal adenomas with higher cruciferous vegetable intake in *GSTM1*-null subjects did not apply to all types of crucifers (i.e., not to cabbage, cauliflower, or Brussels sprouts), but was restricted to broccoli (17). We were unable to separate crucifer type, but we generated toplists of cruciferous vegetables in the 1998 Dutch National Food Consumption Survey (37) and found that broccoli contributed only ~8% to the total amount of crucifers by weight. The cruciferous vegetable contributing most in this population was cauliflower (~30%). Interestingly, in some of the aforementioned other studies reporting increased risk, risk appeared to be higher for cauliflower or cabbage (5, 7), or cauliflower and cabbage contributed 97% to cruciferous vegetable intake (8). Cruciferous vegetables differ in their composition of glucosinolates (38) and different (patterns of) consumption may be one reason why we were unable to reproduce the findings by Lin et al. in our population (17). Lin et al. also investigated the *GSTT1* genotype (39), but without convincing results, whereas the interplay between cruciferous vegetables and GST genotypes reported by Turner et al. was limited to *GSTT1* and could not be seen for *GSTM1* (27). The differential isothiocyanate excretion levels among individuals with different *GSTT1* genotypes found by Seow et al. was not replicated by Fowke et al., and both found urinary isothiocyanate levels unaffected by *GSTM1* genotype (25, 40). The use of genotype assays that are able to differentiate between the presence of one or two alleles (41) may help in creating a more consistent view concerning the modifying effects by *GSTM1* and *GSTT1* genotypes.

For the *GSTP1* A313G polymorphism, Fowke et al. found a significant positive trend between cruciferous vegetable intake and fasting first-morning urinary isothiocyanate excretion in the high activity *GSTP1* AA genotype (40). No association between urinary excretion of total isothiocyanate and the *GSTP1* A313G genotype was observed by Seow et al. (25), however, nor did they find an interplay between *GSTP1* genotype, cruciferous vegetable intake and colorectal cancer among Singapore Chinese (26). It must be noted that the frequency of the *GSTP1* 313G allele among Asian subjects is low (3.5-5%), resulting in low power, and this may explain the lack of association. The results of our study do not support the hypothesis that imputed decreased GSTπ or GSTα capacity in combination with high crucifer intake protects against colorectal adenoma, but they may support an interaction between cruciferous vegetable consumption and these genotypes. Both the *GSTP1* low-activity variant (GG genotype) and the *GSTA1* low-expression variant (TT genotype) appeared to increase the risk of colorectal adenomas with higher cruciferous vegetable intake. This higher adenoma risk with a low capacity GST variant could directly reflect the slower processing of genotoxic compounds associated with cruciferous vegetable intake. The genotoxicity of glucosinolate breakdown products is supported by bacterial and cell assays (42, 43). In humans, however, it is believed that a variety of mechanisms operate to prevent the genotoxic effects observed *in vitro* and that toxicity may therefore occur only at exposure doses exceeding human intake 100-fold (44). However, this does suggest that there is a certain dependency on a well functioning biotransformation system. An indirect possibility for a higher adenoma risk, that finds some support in human studies, is the prolonged stimulation of phase I enzymes, notably *CYP1A2*, by glucosinolate-derived (indole) isothiocyanates that are metabolized by GSTs (45-47). If this is not accompanied by sufficient conjugating activity, this might result in a net increased metabolic activation of (pro)carcinogens. In individuals carrying the two most active variants of the *GSTA1* and *GSTP1* gene homozygously, there is no indication of higher adenoma risk with higher crucifer intake, possibly suggesting that sufficient detoxification is provided by these high-capacity variants. There does seem to be a genotype effect in the low cruciferous vegetable group.

GSTs have overlapping substrate specificities (18), and the isoforms may interact. Ketterer and coworkers suggested a link between GSTα and GSTμ1: GSTα sequesters isothiocyanate conjugates and converts them slowly back to the active isothiocyanate (15, 48), but only in the *GSTM1*-null individual; in the *GSTM1*-positive individual, excretion is favored. It is possible then, that a higher availability of GSTα, as partly determined by *GSTA1* C-69T genotype, might result in a higher amount of conservation, especially in *GSTM1*-null individuals. We found no evidence for an interplay between the *GSTM1* and *GSTA1* genotypes in our study. GSTμ1 and GSTα are expressed at a low level in the colon (22), but are highly expressed in the liver (49). They are relevant for the colon because glucosinolate metabolites are also delivered to the colonic crypts via the colonic blood supply, after absorption from the small intestine and passage through the liver (50). The combined genotypes of the genes most expressed in the colon, i.e., *GSTP1* and *GSTT1*, also did not show a clear effect on the association between colorectal adenomas and crucifer intake.

GSTs have dual roles in relation to glucosinolates: apart from their metabolizing roles, a number of GSTs, e.g., *GSTP1* and *GSTA1*, seem to be inducible by glucosinolate breakdown products (35, 51). Although in the *GSTA1* gene, no electrophile-responsive element or xenobiotic-responsive element has been found, other transcription factor binding sites have been identified. The differential expression of the *GSTA1* genotypes defined by the C-69T substitution has been attributed to alteration of the binding of the ubiquitously expressed transcription factor Sp1 (33). The glucosinolate-derived indole isothiocyanate indole-3-carbinol may influence this binding, demonstrating the complicated nature of the GST-glucosinolate relation (52).

Our subgroup analyses showed differences in subgroups of age, sex, meat consumption, and smoking, which we may not be able to fully disentangle from one another. They may be due to chance. Age-dependent decreasing glutathione availability may play a role (53). Generation and sex may influence dietary habits. Also, GST responses to cruciferous vegetable diets may differ between men and women (35). *N*-acetoxy 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine, one of the most important (pro)carcinogens in cooked meat, is metabolized by GSTα and to a lesser extent by GSTπ (54), and thus subjects with low-capacity variants may be less equipped to detoxify (55, 56). If slower processing of glucosinolate-derived compounds leads to prolonged phase I activation, then subjects with low-capacity GST variants and higher exposure to (meat) carcinogens may be burdened even more by these carcinogens. Similarly, GSTπ is involved in the detoxification of polycyclic aromatic hydrocarbons present in cigarette smoke and a potential by-product of meat processing. Slattery et al. also reported a modifying role for smoking and suggested that smoking may influence the balance of phase I and II biotransformation enzymes (34), but how this applies to our results is not clear.

We conclude that genetic variation in the *GSTP1* and the *GSTA1* gene may modulate the relation between cruciferous vegetables and colorectal adenomas. In this population, the advantage of hypothetical prolonged stimulation of phase II enzymes may not outweigh the disadvantage of lower GST enzyme capacity. Further research may explore the specific effects of the different cruciferous vegetable types. Phenotyping of phase I enzymes (e.g., *CYP*s) in relation to phase II GST polymorphisms or proteins may shed more light on the balance between phase I bioactivation and phase II detoxification of (pro)carcinogens. Also, genetic variation in GST inducibility (i.e., in regulatory sequences) may be taken into account.

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