Research Article

Cancer Epidemiology, Biomarkers & Prevention

Dietary Glucosinolate Intake, Polymorphisms in Selected Biotransformation Enzymes, and Risk of Prostate Cancer

Astrid Steinbrecher[1], Sabine Rohrmann[1], Maria Timofeeva[2], Angela Risch[2], Eugène Jansen[3], and Jakob Linseisen[1,4]

Abstract

A protective role of glucosinolates in prostate cancer development might be mediated by the induction of biotransformation enzymes. These enzymes, enhancing the elimination of carcinogens from the body, are known to be polymorphic. Therefore, we evaluated whether a possible association between glucosinolate intake and prostate cancer risk is modified by polymorphisms in *GSTT1, GSTM1, GSTA1, GSTP1*, or *NOQ1* genes. A case-control study including 248 prostate cancer cases and 492 matched controls was nested in the prospective European Prospective Investigation into Cancer and Nutrition-Heidelberg cohort. At baseline, participants provided dietary and lifestyle data and blood samples, which were used for genotyping and measurement of serum glutathione *S*-transferase-α concentration. Odds ratios and 95% confidence intervals were calculated by conditional logistic regression. We found an inverse association of glucosinolate intake with prostate cancer risk (adjusted odds ratio, 0.72 per 10 mg/d increment; 95% confidence interval, 0.53-0.96). Stratification by genotype showed significantly reduced risks for subjects with wild-type of *NQO1* (C609T) compared with CT or TT carriers ($P_{interaction}$ = 0.04). Those with deletions in both *GSTM1* and *GSTT1* genes combined had a significantly reduced risk with increasing glucosinolate intake ($P_{interaction}$ = 0.01). There was no effect modification of glucosinolate intake and cancer risk by *GSTA1* (G-52A) or *GSTP1* (A313G) genotype, but serum glutathione *S*-transferase-α concentrations were inversely associated with prostate cancer. This study showed that the inverse association between glucosinolate intake and prostate cancer risk was modified by *NQO1* (C609T) and *GSTM1* and *GSTT1* deletion polymorphisms. This information will help to further elucidate the mechanism of action of potentially protective substances *in vivo*. *Cancer Epidemiol Biomarkers Prev; 19(1); 135–43. ©2010 AACR.*

Introduction

Cruciferous vegetables are currently under investigation for their protective role in prostate cancer development. They are a rich source of glucosinolates, which can be broken down to the biologically active components isothiocyanates and indoles. Both compounds show cancer preventive effects in prostate cancer cell lines and animal experiments (1). Among others, a well-established mechanism of action is the induction of detoxification enzymes, such as glutathione *S*-transferases (GST) or NADPH-quinone oxidoreductase (NQO1), in cell-based studies (2, 3). These enzymes play a crucial role for the metabolism and excretion of carcinogens from the body, because they convert them into water-soluble forms, which are readily excreted via urine. A human intervention study, showing that the serum concentration of GST-α could be increased by a diet rich in cruciferous vegetables (4), indicates that the mechanisms found *in vitro* might indeed act *in vivo*.

Thus far, epidemiologic studies on the association of cruciferous vegetable consumption and prostate cancer risk have shown inconsistent results (5). One reason might be that polymorphisms in detoxification genes such as *GSTs* or *NQO1*, which are induced by isothiocyanates, modulate the potential anticarcinogenic effects of these glucosinolate breakdown products. Some polymorphisms in these genes have functional consequences causing the formation of less or no enzymes or enzymes with reduced activity. For the *NQO1* gene, a C609T polymorphism translates into an enzyme that is unstable *in vivo* (6). For *GSTT1* and *GSTM1*, gene deletions exist, which result in a complete lack of the respective protein (7). *GSTA1* has three linked polymorphisms in the promoter region resulting in a lower transcriptional activity (8). In the *GSTP1* gene, the A313G polymorphism leads to decreased enzyme activity (9). To date, little is known in how far the inducing activity of glucosinolate breakdown

Authors' Affiliations: Divisions of [1]Cancer Epidemiology and [2]Epigenomics and Cancer Risk Factors, German Cancer Research Center, Heidelberg, Germany; [3]Laboratory for Health Protection Research, National Institute for Public Health and the Environment, Bilthoven, the Netherlands; and [4]Institute of Epidemiology, Helmholtz Zentrum München, Neuherberg, Germany

Corresponding Author: Jakob Linseisen, Institute of Epidemiology, Helmholtz Zentrum München, Ingolstädter Landstr. 1, D-85764 Neuherberg, Germany. Phone: 49-89-3187-3202; Fax: 49-89-3187-2951. E-mail: j.linseisen@helmholtz-muenchen.de

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products on the expression of GSTs and NQO1 is modified by these functional polymorphisms.

However, more complexity is added to this topic because isothiocyanates are not only inducers but also substrates for GSTs. Thus, lack or decreased activity of a *GST* gene might lead to higher or prolonged accumulation of isothiocyanates within the body, which in turn could increase protection against carcinogens by inducing other detoxification enzymes or other anticarcinogenic mechanisms (5).

Recently, we were able to show significant inverse associations between the intake of glucosinolates and the risk of prostate cancer in male participants of the European Prospective Investigation into Cancer and Nutrition (EPIC)-Heidelberg cohort (10). Here, we examined in a case-control study nested within the EPIC-Heidelberg cohort whether the abovementioned polymorphisms in *GST* and *NQO* genes modulate this association. Furthermore, we investigated the relationship between measured serum GST-α concentration and the intake of glucosinolates and prostate cancer because GST-α concentration might be useful as biomarker for glucosinolate exposure.

Materials and Methods

Study Population

This analysis is based on data from the EPIC-Heidelberg study, an ongoing prospective cohort study assessing the association between dietary, lifestyle, and metabolic factors and the risk of cancer. From 1994 to 1998, a random sample of the general population of Heidelberg, Germany, was invited and 11,928 men (ages 40-64 years) and 13,612 women (ages 35-64 years) agreed to participate (11). At baseline, dietary, lifestyle, medical, and socioeconomic data were collected via self-administered questionnaires and personal interview; anthropometric measures were taken by trained personnel. Additionally, 95.8% of the participants provided blood samples at baseline, which were fractionated into serum, plasma, buffy coat, and erythrocytes and subsequently stored in liquid nitrogen at −196°C. All participants gave written informed consent and the study was approved by the ethics committee of the Heidelberg Medical School.

Follow-up questionnaires were mailed to the participants every 2 to 3 years to assess information on health status. Participation rates of the completed three follow-ups were >90%. Self-reported cases of prostate cancer were verified based on medical reports by the study physician. Additionally, death certificates of deceased participants were checked for prostate cancer as underlying cause of death

For the present study, a nested case-control approach was used based on all male EPIC-Heidelberg participants with blood samples available and free of prevalent cancer (except nonmelanoma skin cancer) at baseline. All incident prostate cancer cases (C61, C63.8, and C63.9; *International Classification of Diseases for Oncology, Second Edition*) diagnosed by end of February 2007 were selected. Following an incidence density sampling protocol, two controls were matched per case by age (5-year age groups) and time of recruitment (6-month intervals). The final study population comprised 248 cases and 492 controls.

Laboratory Analyses

Serum samples of the study participants were used to determine GST-α concentration by enzyme immunoassay with Biotrin HEPKIT-α following the protocol of the manufacturer. Serum concentrations measured reflect GSTA1 and GSTA2 concentrations and will be termed GST-α throughout the article. Intraday and interday coefficients of variation were 4.9% and 5.8%.

Genomic DNA was extracted from buffy coat with FlexiGene kit (Qiagen) in accordance with the manufacturer's instructions. DNA was stored at 4°C until use. To determine deletions of the *GSTM1* and *GSTT1* genes, a semiquantitative genotyping assay on the LightCycler 480 (Roche) was used, with multiplexing of both genes using albumin as reference gene and internal control to confirm amplification (12). This method allows for the distinction of homozygous, heterozygous, and noncarriers. Determination was done in triplicate and a SD of >10% led to repeated analysis. Five percent of the samples were repeated for quality-control reasons and concordance of the assigned genotypes was >95%. Genotyping for polymorphisms of the genes *NQO1* (C609T, rs1800566), *GSTA1* (G-52A, rs3957357), and *GSTP1* (A313G, rs1695) were done as multiplex on the MassArray system (Sequenom) applying the iPLEX method and matrix-assisted laser desorption/ionization—time-of-flight mass spectrometry for analyte detection. The analysis was carried out by Bioglobe. All duplicated samples (quality-control repeats of 8% of the samples) to verify interexperimental reproducibility and accuracy delivered concordant genotype results.

All laboratory analyses were carried out with the laboratory personnel blinded to the case-control status.

Dietary and Lifestyle Data

Habitual diet during the previous year was assessed at baseline by validated self-administered semiquantitative food frequency questionnaires. Participants filled in portion size and consumption frequency of 145 food items and the average daily food consumption for each participant was calculated. The nutrient intake for each participant was computed by linking food consumption data to the German Food Code and Nutrient Data Base BLS II.3 and a database on glucosinolate content, established to assess glucosinolate intake in EPIC-Heidelberg. This database covered the amount of 26 individual glucosinolates in 18 different vegetables and condiments (13). Information on lifestyle and sociodemographic characteristics was assessed at study entry by questionnaires and personal interview.

Statistical Analysis

Baseline characteristics of the study population are given as mean and SD or percentages by case-control status. Median and interquartile range for dietary intake data and geometric mean and 95% confidence intervals (95% CI) for serum GST-α concentration were computed (skewed distribution).

Genotype frequencies of the selected gene polymorphisms are presented and the χ^2 test was used to check for Hardy-Weinberg equilibrium. Main effects of the polymorphisms on the risk of prostate cancer were computed by conditional logistic regression estimating odds ratios (OR) and 95% CI, with the most frequent variant being the reference category. The analysis was stratified by case set. Due to small numbers in some genotype categories, we also combined the heterozygote and homozygote (mutant) categories. In case of *GSTM1*, where the wild-type (both copies of the gene present) was rare, we combined wild-type and heterozygote genotype. Furthermore, we combined *GSTM1* and *GSTT1* genotypes by counting the number of deleted alleles and grouping into zero to one, two, and three to four deleted alleles.

Main effects of glucosinolate intake on prostate cancer risk were calculated by conditional logistic regression stratified by case set. Adjustments were made for possible confounding factors that were family history of prostate cancer in first-degree relatives (yes/no), smoking status (never/former/current), educational attainment (no or primary school/secondary or technical school/university degree), body mass index (BMI; continuously), intake of vegetables (continuously), and total energy intake (continuously in kJ/d). Simultaneous adjustment for family history of prostate cancer and intake of vegetables changed the estimate for glucosinolate intake by ~10% and is thus presented here; none of the other variables altered the OR meaningfully.

To evaluate potential effect modification of the association between glucosinolate intake and prostate cancer risk by genotype, we calculated OR (95% CI) of prostate cancer for the continuous glucosinolate intake variable stratified by genotype with unconditional logistic regression adjusting for the matching variables (time of recruitment and 5-year age group). We repeated this analysis adjusting additionally for family history of prostate cancer and vegetable intake. Tests for interaction were computed based on the likelihood ratio statistics comparing the conditional logistic regression model with and without interaction terms (of genotype and continuous glucosinolate intake variable).

Serum GST-α concentration showed a right skewed distribution; therefore, analyses were done on the log-transformed variable. To examine factors associated with GST-α serum levels, we restricted analysis to controls with GST-α concentrations within the range covered by the internal standard (<0.25 and >200 μg/L), leaving 482 control subjects for the analyses. Spearman correlation coefficients for GST-α concentration and the continuous variables BMI, glucosinolate intake, vegetable intake, fruit intake, age, and energy intake were computed. Geometric mean GST-α concentrations adjusted for matching factors over categories of smoking status, educational attainment, alcohol intake, or GST genotype were calculated by generalized linear models. Finally, we assessed the effect of potentially influencing factors on GST-α concentration by multivariate linear regression.

Associations between GST-α concentration and prostate cancer risk were evaluated by conditional logistic regression stratified by case set. We excluded participants with GST-α concentrations outside the range covered by the internal standard; if a case was excluded, the two corresponding controls (in the case set) were also omitted, leaving 715 participants of which 243 were cases. The analysis was repeatedly adjusted for the potential confounding factors BMI, alcohol intake categories, and additional glucosinolate intake. Analyses stratified by genotype were computed by unconditional logistic regression adjusting for matching factors. All analyses were done with SAS 9.1 (SAS Institute).

Results

Baseline description of the study population is given in Table 1. Cases and controls did not differ with respect to age or BMI. Cases tended to be never smokers more often and were less likely to have the highest educational attainment. Additionally, 7.3% of cases in comparison with 1.8% of controls reported a history of prostate cancer in first-degree relatives. Median intake of glucosinolates was lower in cases than in controls; similarly, cases had a lower geometric mean serum GST-α concentration than controls.

The genotype frequencies of the *NQO1* and *GST* genes are depicted in Table 2. For the *NQO1* and the *GSTM1* gene, Hardy-Weinberg equilibrium was not reached in controls. None of the genes was associated with the risk of total prostate cancer. The intake of glucosinolates was significantly inversely associated with the risk of prostate cancer (OR, 0.79 per 10 mg/d increment; 95% CI, 0.63-0.99). Adjustment for family history of prostate cancer and vegetable intake strengthened this association (OR, 0.72; 95% CI, 0.53-0.96).

Analysis of the association of glucosinolate intake by genotype strata showed a significantly reduced OR for participants who are homozygote for the wild-type of the *NQO1* polymorphism (OR, 0.72; 95% CI, 0.55-0.95) but not for the heterozygote or homozygote mutants (OR, 1.02; 95% CI, 0.76-1.38; Table 3). The test of interaction was statistically significant with $P = 0.04$. In participants with the double deletion of the *GSTM1* gene, the intake of glucosinolates was associated with an OR (95% CI) for prostate cancer of 0.74 (0.55-0.99); however, the test for interaction contrasted to those with at least one present allele did not reach statistical significance ($P = 0.15$). Similarly, the OR in the deletion strata of the *GSTT1* gene was reduced (OR, 0.78; 95% CI, 0.61-1.01), but again the interaction test was not significant. When

Table 1. Baseline characteristics of cases and controls in the EPIC-Heidelberg nested case-control study (n = 740)

	Cases (n = 248)	Controls (n = 492)
	Mean ± SD	**Mean ± SD**
Age (y)	58.1 ± 4.8	58.1 ± 4.8
BMI (kg/m^2)	27.3 ± 3.6	27.3 ± 3.4
	***n* (%)**	***n* (%)**
Smoking status		
Never	99 (39.9)	166 (33.7)
Former	112 (45.2)	241 (49.0)
Current	37 (14.9)	85 (17.3)
Educational attainment		
No/primary school	89 (35.9)	158 (32.1)
Secondary/technical school	82 (33.1)	153 (31.1)
University	77 (31.1)	181 (36.8)
Positive family history of prostate cancer	18 (7.3)	9 (1.8)
	Median (interquartile range)	**Median (interquartile range)**
Energy (kJ/d)	8,433 (7,251-9,922)	8,657 (7,206-10,455)
Glucosinolates (mg/d)	7.7 (5.2-11.5)	9.2 (5.8-13.4)
Vegetables (g/d)	100.1 (78.7-129.3)	102.1 (79.5-139.6)
Serum GST-α concentration* (μg/L)	3.39 (2.98-3.85)	4.30 (3.93-4.71)

*Geometric mean and (95% CI) adjusted by matching factors of 243 cases and 482 controls (those with GST-α concentrations outside the range covered by the internal standard or with missing values were excluded).

we combined *GSTM1* and *GSTT1* genotypes by counting the number of deleted alleles, we found a significant inverse association of glucosinolate intake and prostate cancer for participants with three or more deletions (OR, 0.61; 95% CI, 0.43-0.87) but not for those with two deletions (OR, 1.21; 95% CI, 0.90-1.64) or one or no deletion (OR, 0.88; 95% CI, 0.58-1.32). The test for interaction of *GSTM1/GSTT1* genotype and glucosinolate intake was highly significant (P = 0.007). Repeating this analysis with adjustment for vegetable intake and family history of prostate cancer strengthened the associations.

The geometric mean (95% CI) serum GST-α concentration in healthy controls was 4.31 μg/L (3.92-4.73). The correlation coefficient for GST-α concentration was 0.23 (P < 0.01) with BMI and -0.10 (P = 0.03) with glucosinolate intake. Age, total energy intake, vegetable intake, fruit intake, and alcohol intake were not correlated with GST-α concentration; however, when we categorized alcohol into intake groups, we found a U-shaped relation with lowest GST-α concentration for the intake group of 20.0 to 29.9 g/d and higher values for intake ≥60.0 or <5.0 g/d (Table 4). Mean GST-α concentrations did not differ over categories of smoking status, educational attainment, or *GSTA1, GSTP1, GSTP1*, or *GSTM1* genotype. In multivariate linear regression analysis adjusting for smoking status, education, vegetable intake, fruit intake, alcohol intake, age, and matching factors, glucosinolate intake was significantly inversely associated with serum GST-α (per 1 mg increment of glucosinolate intake log GST-α was reduced by 0.03 units; P = 0.0005) and BMI (per 1 unit increase log GST-α increased by 0.07 units; P < 0.0001). This regression model explained 15% of the variance (adjusted R^2 = 11%).

Serum GST-α concentrations were inversely correlated with prostate cancer risk. Participants in the highest tertile of GST-α had an OR (95% CI) of 0.54 (0.37-0.80; Table 5). Adjusting for BMI, alcohol, or glucosinolate intake did not change the estimates. After excluding cases diagnosed within the first 2 years of follow-up, risk estimates were slightly lower (OR, 0.49; 95% CI, 0.32-0.76) in the highest tertile of GST-α.

Discussion

This study showed inverse associations between dietary glucosinolate intake and risk of prostate cancer, especially in particular genotypes of the *GSTM1, GSTT1*, and *NQO1* genes. Subjects with deletions of both *GSTM1* and *GSTT1* genes had a significantly reduced risk with increasing glucosinolate intake. Furthermore, men homozygous for the wild-type of *NQO1* C609T had a significantly reduced risk of developing prostate cancer with increasing glucosinolate intake. We found no effect modifications of polymorphisms in *GSTA1* and *GSTP1* genes.

GST-α serum concentrations were inversely associated with glucosinolate intake. Furthermore, GST-α concentrations were inversely related to prostate cancer risk.

The findings of this study might help to understand the biological actions of dietary glucosinolates *in vivo*. Some prospective epidemiologic studies suggest an inverse association between prostate cancer and intake of glucosinolates, which is often assessed using the consumption of cruciferous vegetables as proxy (14-16). Analyses of the male EPIC-Heidelberg cohort showed significant inverse associations between directly quantified glucosinolate intake and prostate cancer risk (10), which we confirmed in the present case-control study nested within the EPIC-Heidelberg cohort. Experimental studies in prostate cancer and other cell lines point to several distinct cancer chemopreventive mechanisms of glucosinolate breakdown products, of which the induction of detoxification enzymes, especially by isothiocyanates, is well established (3). It is likely that genetic variation in these detoxification enzymes modifies the potential effects of isothiocyanates, in a way that carriers of a polymorphism that leads to an altered function of the respective protein might experience different effects of glucosinolate intake than noncarriers with respect to cancer risk (17). Thus, we chose GSTs and NQO1 because these enzymes play an important role in the elimination of potential carcinogens and the selected polymorphisms are known to result in reduced or missing enzyme activity.

GSTs are phase II enzymes, catalyzing the conjugation of reduced glutathione to a variety of electrophilic compounds, rendering them more water-soluble. However, isothiocyanates are not only inducers but also substrates of GSTs and the catalyzed conjugation with glutathione is the first step in eliminating isothiocyanates from the body (18). For *GSTT1* and *GSTM1*, deletion polymorphism are described that result in a complete lack of the respective

Table 2. OR (95% CI) for prostate cancer associated with genetic polymorphisms in *GST* and *NQO1* genes in the EPIC-Heidelberg nested case-control study (n = 740)

Genotype	All	Cases (%)	Controls (%)	OR* (95% CI)
GSTM1				
pres/pres	66	19 (7.7)	47 (9.6)	1.00
pres/del	277	103 (41.5)	174 (35.4)	1.43 (0.80-2.55)
del/del	396	126 (50.8)	270 (55.0)	1.15 (0.65-2.04)
HWE†	0.08		0.02	
GSTT1				
pres/pres	251	88 (35.5)	163 (33.1)	1.00
pres/del	368	116 (46.8)	252 (51.2)	0.87 (0.62-1.22)
del/del	121	44 (17.7)	77 (15.7)	1.08 (0.69-1.69)
HWE†	0.47		0.21	
GSTM1/GSTT1				
0 or 1 del	144	54 (21.8)	90 (18.3)	1.00
2 del	298	92 (37.1)	206 (42.0)	0.76 (0.51-1.15)
3 or 4 del	297	102 (41.1)	195 (39.7)	0.89 (0.59-1.35)
GSTP1 (A313G)				
AA	341	125 (50.4)	216 (43.9)	1.00
AG	323	95 (38.3)	228 (46.3)	0.72 (0.52-1.01)
GG	76	28 (11.3)	48 (9.8)	1.00 (0.60-1.69)
HWE†	0.97		0.28	
GSTA1 (G-52A)				
GG	216	68 (27.4)	148 (30.1)	1.00
GA	379	132 (53.2)	247 (50.2)	1.16 (0.81-1.64)
AA	145	48 (19.4)	97 (19.7)	1.08 (0.68-1.70)
HWE†	0.36		0.74	
NQO1 (C609T)				
CC	496	163 (65.7)	333 (67.7)	1.00
CT	213	80 (32.3)	133 (27.0)	1.25 (0.89-1.75)
TT	31	5 (2.0)	26 (5.3)	0.41 (0.16-1.06)
HWE†	0.19		0.01	

Abbreviation: HWE, Hardy-Weinberg equilibrium.

*Conditional logistic regression stratified by case set.

†P value of χ^2 test.

Table 3. OR (95% CI) for association of glucosinolate intake and prostate cancer in strata of genetic polymorphisms in the EPIC-Heidelberg nested case-control study (n = 740)

Genotype	Cases/controls	Crude OR* (95% CI)	$P_{interaction}$	Adjusted OR† (95% CI)	$P_{interaction}$
		per 10 mg/d increment of glucosinolate intake			
GSTM1					
pres/pres	19/47	0.98 (0.52-1.83)		1.24 (0.51-3.03)	
pres/del	103/174	0.95 (0.70-1.28)		0.82 (0.55-1.22)	
del/del	126/270	0.74 (0.55-0.99)	0.38	0.67 (0.45-0.98)	0.30
≥1del	229/444	0.82 (0.67-1.02)	0.57	0.74 (0.56-0.97)	0.84
≥1pres	122/221	0.96 (0.73-1.25)	0.15	0.92 (0.65-1.29)	0.12
GSTT1					
pres/pres	88/163	0.99 (0.71-1.38)		0.89 (0.57-1.38)	
pres/del	116/252	0.78 (0.59-1.04)		0.70 (0.48-1.02)	
del/del	44/77	0.75 (0.42-1.36)	0.53	0.78 (0.38-1.58)	0.73
≥1del	160/329	0.78 (0.61-1.01)	0.25	0.74 (0.54-1.02)	0.42
GSTM1/GSTT1					
0 or 1 del	54/90	0.88 (0.58-1.32)		0.85 (0.50-1.42)	
2 del	92/206	1.21 (0.90-1.64)		1.14 (0.75-1.72)	
3 or 4 del	102/195	0.61 (0.43-0.87)	0.01	0.56 (0.35-0.87)	0.01
GSTP1					
AA	125/216	0.82 (0.61-1.09)		0.87 (0.61-1.26)	
AG	95/228	0.85 (0.61-1.18)		0.68 (0.44-1.05)	
GG	28/48	1.06 (0.59-1.90)	0.62	0.82 (0.38-1.81)	0.64
≥1G	123/276	0.89 (0.68-1.18)	0.63	0.73 (0.50-1.05)	0.81
GSTA1					
GG	68/148	0.73 (0.48-1.12)		0.63 (0.38-1.05)	
GA	132/247	0.83 (0.63-1.10)		0.89 (0.62-1.29)	
AA	48/97	0.93 (0.62-1.40)	0.85	0.80 (0.44-1.46)	0.81
≥1A	180/344	0.86 (0.69-1.08)	0.81	0.87 (0.64-1.17)	0.90
NQO1					
CC	163/333	0.72 (0.55-0.95)		0.64 (0.44-0.91)	
CT	80/133	1.04 (0.77-1.42)		1.04 (0.69-1.55)	
TT	5/26	0.81 (0.24-2.78)	0.11	1.94 (0.03-127.27)	0.22
≥1T	85/159	1.02 (0.76-1.38)	0.04	0.98 (0.66-1.46)	0.10

NOTE: $P_{interaction}$ for test of interaction between glucosinolate intake and genotype.
*OR calculated by unconditional logistic regression adjusted for matching variables (time of recruitment and 5-year age group).
†Additionally adjusted for family history of prostate cancer and vegetable intake.

protein (7). The frequencies of homozygous deletions for *GSTT1* and *GSTM1* are 20% and 53%, respectively, in Caucasian populations (19), which corresponds well to those found in our study. Our genotyping method allowed for distinguish carriers of one or two alleles of both *GSTM1* and *GSTT1* in contrast to most other studies. We found a significant inverse association between glucosinolate intake and prostate cancer risk for individuals with deletions in *GSTM1* and *GSTT1*. Our results are in contrast to the study by Joseph et al. (20) who reported a significantly reduced risk of prostate cancer for individuals with a high (versus low) broccoli intake only in subjects with the *GSTM1* present (homozygote present or heterozygote combined) genotype. The authors hypothesized that the inducing effects of isothiocyanates on GSTs may be more important than their role in metabolism and elimination of isothiocyanates itself. Our results support the hypothesis that individuals with deletion of *GSTM1* or *GSTT1* genes have reduced elimination of isothiocyanates with a subsequently prolonged circulation within the body and thus greater chemoprevention by induction of other detoxification enzymes or via other mechanisms (17).

With respect to lung cancer, similarly diverse results on effect modification by GST genotype exist. Whereas some studies from Asia have shown protective effects of cruciferous vegetables/glucosinolates/isothiocyanates in individuals with the *GSTT1* or *GSTM1* deleted genotype (21, 22), other studies conducted in the United States found effects in individuals with *GSTT1* or *GSTM1* present genotype (23, 24). Gasper et al. (25) hypothesized that, in the United States, broccoli is the

major source of glucosinolate intake; hence, sulforaphane is the most prevalent isothiocyanate in the diet. In contrast, Asians consume more Chinese cabbage and other forms of *Brassica rapa*; thus, the major isothiocyanates ingested are 3-butenyl and 4-pentenyl isothiocyanates. The speed of enzyme-catalyzed conjugation reactions is different for distinct isothiocyanates (26), which in turn may account for the contrasting results of the aforementioned studies. Interestingly, a study by Brennan et al. (27), conducted in a Caucasian population from Europe, found protective effects in *GSTM1* and *GSTT1* null subjects. In our study, sinigrin (2-propenyl isothiocyanate) was the major glucosinolate in the diet, which supports the hypothesis of Gasper et al. (25). However, the second most abundant glucosinolate was glucoraphanin (sulforaphane). The intake of both glucosinolates is highly correlated in our study population; to elucidate distinct effects of single glucosinolates, we would need higher variation in the intake or intake combination of individual glucosinolates.

Table 4. Serum GST-α concentration in healthy controls (n = 482) in categories of potential influencing factors in the EPIC-Heidelberg nested case-control study

	n	Geometric mean (95% CI)	*P*
Smoking status			
Never	161	4.21 (3.58-4.95)	
Former	237	4.18 (3.66-4.78)	
Current	84	4.90 (3.91-6.14)	
			0.47
Educational attainment			
None/primary	156	4.10 (3.48-4.84)	
Secondary/technical	148	4.13 (3.49-4.89)	
University	178	4.65 (3.98-5.44)	
			0.47
Alcohol intake (g/d)			
<5.0	93	4,27 (3.46-5.28)	
5.0-19.9	157	4.22 (3.58-4.97)	
20.0-29.9	74	3.18 (2.51-4.03)	
30.0-59.9	106	4.75 (3.90-5.80)	
≥60	52	5.85 (4.41-7.77)	
			0.02
GSTM1			
pres/pres	47	4.51 (3.34-6.10)	
pres/del	173	4.50 (3.85-5.26)	
del/del	261	4.15 (3.65-4.71)	
			0.69
GSTT1			
pres/pres	160	4.06 (3.45-4.78)	
pres/del	245	4.13 (3.63-4.71)	
del/del	77	5.54 (4.39-7.00)	
			0.07
GSTP1			
AA	211	4.17 (3.62-4.81)	
AG	224	4.28 (3.73-4.91)	
GG	47	5.12 (3.79-6.91)	
			0.48
GSTA1			
GG	144	4.47 (3.77-5.31)	
GA	242	4.27 (3.74-4.88)	
AA	96	4.15 (3.37-5.13)	
			0.85

NOTE: *P* value for difference of adjusted means.

We found no effect modification of glucosinolate intake and prostate cancer risk by *GSTP1* A313G or *GSTA1* G-52A. The reported genotype frequencies of *GSTP1* A313G (19) and of *GSTA1* G-52A (8) for Caucasian populations are in accordance with the frequencies observed in our study.

NQO1 is a cytosolic enzyme catalyzing the reduction of quinones. A C609T base change leads to a mutant enzyme that has <4% of the activity of the wild-type protein and that is unstable *in vivo* (6). Sulforaphane has been shown to be a potent inducer of *NQO1* transcription in human prostate cells (2). In line with these findings, the results of our study have shown a reduced risk of prostate cancer with increasing glucosinolate intake in subjects with *NQO1* wild-type in contrast to those with the homozygous mutant or heterozygous genotype ($P_{interaction}$ = 0.04).

In this study, we focused on polymorphisms in genes that are induced by isothiocyanates; however, indoles play an important role in chemoprevention as well. Especially, the interplay of both glucosinolate breakdown products, isothiocyanates and indoles, on the expression of detoxification enzymes seems an important mechanisms impacting during the phase of tumor initiation (3). Still, an important point to consider is the bioavailability and tissue distribution of glucosinolate breakdown products within the body, which is a basic prerequisite for anticarcinogenic mechanisms at the respective site.

The mean serum GST-α concentration of our healthy participants was in line with those reported for males in other studies ranging from 2.8 to 3.5 μg/L plasma (28, 29). Serum GST-α concentration did not vary over *GSTA1* G-52A genotype, which confirms a previous study that found that the sum of GSTA1 and GSTA2 concentration measured in human hepatic samples did not differ according to genotype (8). However, in the same study, AA carriers had lower GSTA1 concentration, whereas GSTA2 concentration was higher than for GG carriers. Thus, it seems that the lower transcriptional activity of GSTA1 is compensated for by a higher expression of GSTA2. This might explain that we did not found effect modification of glucosinolate intake and prostate cancer risk by *GSTA1* G-52A genotype.

Interestingly, the association between glucosinolate intake and GST-α concentrations was inverse. This is in

Table 5. OR (95% CI) for association of serum GST-α concentrations and prostate cancer in the EPIC-Heidelberg nested case-control study (*n* = 715)

GST-α	Cases/controls	Crude* OR (95% CI)	Adjusted† OR (95% CI)	Adjusted‡ OR (95% CI)
1st tertile (<2.17 μg/L)	106/157	1.00	1.00	1.00
2nd tertile (2.18-3.72 μg/L)	81/157	0.79 (0.55-1.14)	0.78 (0.54-1.12)	0.77 (0.53-1.11)
3rd tertile (≥3.73 μg/L)	56/158	0.54 (0.37-0.80)	0.53 (0.36-0.80)	0.53 (0.35-0.80)
Continuous§		0.77 (0.66-0.91)	0.77 (0.65-0.91)	0.75 (0.63-0.90)

*Conditional logistic regression stratified by case set.
†Additionally adjusted for BMI and alcohol intake.
‡Additionally adjusted for glucosinolate intake.
§Per unit increment of log-transformed GST-α.

contrast with our assumption based on two short-term human feeding trials (4, 28) that higher glucosinolate intake would lead to an induction of the *GSTA1* gene and therefore higher serum concentrations. Given a rapid clearance from plasma (half-life of 1 h; ref. 30), the GST-α concentration might reflect short-term dietary intervention but not habitual diet over longer periods, as assessed in our study. Additionally, higher doses used in experimental studies or better standardization of food preparation minimizing variation in isothiocyanate exposure or other dietary factors inducing GST expression might explain this result. We tried to control for these dietary factors by adjusting our analyses for vegetable and fruit intake but still got the same results. Lastly, serum GST-α most probably reflects enzyme release from hepatic cells during normal cell turnover (31). Thus, conditions impacting on liver cell turnover (viral infections and certain drugs) will influence serum GST-α concentration.

GST-α concentrations were inversely associated with prostate cancer risk in our study. Because GST-α plays an important role in eliminating potential carcinogens from the body, this finding underlines that those with higher concentrations seem less likely to develop cancer. However, we measured the concentration in serum and not in prostate tissue, assuming the serum concentration to be a good indicator of prostate tissue concentrations, which may not hold true. Furthermore, GST-α expression is mediated not only by chemopreventive agents but also by oxidative stress derived from a variety of chemicals (32); therefore, high concentrations of GST-α might reflect induction of expression caused by high oxidative stress. Therefore, we repeated our analysis after excluding cases diagnosed within the first 2 years of follow-up to minimize the potential effect of oxidative stress on GST-α expression due to underlying prostate cancer. This strengthened slightly the inverse association. Given the high variability of the measured GST-α concentrations and the effect of other inducing factors, interpretation of these results should be cautious.

A major strength of the present nested case-control study is its prospective design and the high follow-up rate (>90%), which minimizes the risk of selection bias and allowed for collecting subjects' characteristics and biomaterial before disease diagnosis. Furthermore, we were able to adjust our analyses with respect to known or suspected confounders. However, our sample size of 248 cases was quite limited. We improved study power by matching two controls per case and analyzing stratified by case set. Although we were able to determine some diet-gene interaction effects, we might not have had enough power to detect some associations with smaller effects. Moreover, we did not adjust for multiple testing. Two gene polymorphisms were not in Hardy-Weinberg equilibrium. These deviations might arise due to genotyping errors, chance, or failure of assumptions underlying Hardy-Weinberg expectations (33). However, the genotype frequencies were in accordance with those reported in other studies in Caucasians.

In conclusion, this study indicated that the inverse association between glucosinolate intake and prostate cancer risk is modified by polymorphisms in biotransformation enzymes such as *GSTT1, GSTM1*, or *NQO1*. Considering genetic variation is an important step in elucidating the mechanism of action of potentially protective substances *in vivo*. Based on our results, men with wild-type *NQO1* or deletions of the *GSTT1* and/or *GSTM1* gene might benefit from increasing glucosinolate intake. In contrast, the only other study published on this topic found protective effects in subject with the *GSTM1* present genotype. This discrepancy is known from studies evaluating interaction of glucosinolate intake and genotype with respect to other cancer sites. Studies supporting one or the other side seem to cluster in geographical regions, an observation that needs to be addressed in future research. It might be helpful to quantify individual glucosinolates consumed and to develop validated biomarkers for long-term glucosinolate intake.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Astrid Steinbrecher, Sabine Rohrmann, Maria Timofeeva, et al.

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