Short Communication

Glutathione *S*-Transferase M1, T1, and P1 Polymorphisms as Risk Factors for Renal Cell Carcinoma: A Case-Control Study[1]

Carol Sweeney,[2] Diana C. Farrow, Stephen M. Schwartz, David L. Eaton, Harvey Checkoway, and Thomas L. Vaughan

Departments of Epidemiology [C. S., D. C. F., S. M. S., H. C., T. L. V.] and Environmental Health [D. L. E., H. C.], School of Public Health and Community Medicine, University of Washington, Seattle, Washington 98195, and Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, Washington 98109 [D. C. F., S. M. S., T. L. V.]

Abstract

Renal cell carcinoma (RCC) has known environmental risk factors, notably smoking, and enzymes that biotransform carcinogens have high levels of activity in the kidney. However, a possible role of polymorphisms in these enzymes in RCC etiology has received little study. We investigated glutathione *S*-transferase (GST) polymorphisms in a population-based case-control study of RCC. Subjects completed a structured interview, and DNA was isolated from pathological material or buccal cells for 130 cases, and from blood for 505 controls. Genotypes for *GSTM1* and *GSTT1* were determined by multiplex PCR, and for *GSTP1* by oligonucleotide ligation assay. The frequency of *GSTM1* null genotype was 50.0% in cases and 50.5% in controls, with an adjusted odds ratio (OR) of 1.0 [95% confidence interval (CI), 0.6–1.6]. For *GSTP1*, the frequencies of genotypes AA, AG, and GG representing the Ile^{104}Val variant were: cases, 44.6%, 43.1%, and 12.3%; controls, 43.4%, 44.0%, and 12.6%; OR for AG and GG, 1.0 (95% CI, 0.6–1.6). An excess of the *GSTT1* null genotype was observed in cases compared with controls, 28.6% *versus* 18.5% (OR, 1.9; 95% CI, 1.1–3.4). The association with *GSTT1* was present among both smokers and nonsmokers, but was modified by body mass index, a recognized risk factor for RCC; among subjects in the lowest tertile of body mass index, the OR for *GSTT1* null was 4.8 (95% CI, 1.8–13.0). The association between *GSTT1* null and increased RCC risk in this population-based study suggests that activity of the GSTT1 enzyme protects against RCC. This contrasts with a recent report of reduced risk of RCC associated with *GSTT1* null in a cohort of trichloroethene-exposed workers and suggests that specific chemical exposures alter the effect of *GSTT1* on cancer risk.

Introduction

Recognized risk factors for RCC[3] include cigarette smoking, high relative body weight, and high blood pressure (1). A recent study in the United States evaluating population-attributable risks for RCC reported that after accounting for these three exposures, about 51% of RCC cases remained unexplained (2). Research on genetic factors that affect cancer susceptibility has the potential to improve our understanding of RCC etiology.

GST enzymes catalyze conjugation of electrophilic substrates with glutathione, usually resulting in detoxification of reactive intermediates (3). Common polymorphisms occur in three human GSTs and seem to influence cancer risk (4–6). Polymorphisms in which the gene is deleted and no active enzyme is expressed occur for *GSTM1* and *GSTT1* (4). A single-nucleotide variant in exon 5 of *GSTP1* results in an amino acid substitution, Ile^{104}Val, and altered enzyme activity (7, 8). GSTM1 and GSTP1 are active in the detoxification of activated forms of polycyclic aromatic hydrocarbon compounds. Substrates for GSTT1 include halogenated solvents, such as dichloromethane, and ethylene oxide, formed endogenously from ethene, which is present at high levels in cigarette smoke (3). GSTT1 and GSTM1 enzymes also have catalytic activity toward phospholipid hydroperoxide (9), evidence that GSTs may prevent DNA damage from lipid peroxides formed endogenously as a result of oxidative stress. Not all reactions catalyzed by GST enzymes result in detoxification; reactions of certain halogenated compounds catalyzed by θ-class GSTs produce mutagenic species (3).

The altered GST activity associated with the polymorphisms is expected to affect cancer risk through decreased protection against DNA damage from reactive electrophiles. GSTs are expressed and have significant activity in the kidney (10–12), but few studies have considered GSTs in susceptibility to RCC. Published reports include a case-control study nested within a cohort of German workers occupationally exposed to TCE (13) and a hospital-based case-control study in France (14). Reduced risk of RCC associated with *GSTT1* null genotype was reported in the TCE-exposed cohort. The hospital-based study found no association between *GSTM1*, *GSTT1*, or *GSTP1* polymorphisms and RCC risk. Thus, previous data on associations between these candidate susceptibility genes and RCC are limited. We investigated GST polymorphisms in a population-based case-control study of RCC.

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[2] To whom requests for reprints should be addressed, at Arkansas Cancer Research Center, Slot 795, 4301 West Markham Street, Little Rock, AR 72205. Phone: (501) 296-1248, ext. 9; Fax: (501) 686-8297; E-mail: sweeneycarol@exchange.uams.edu.

[3] The abbreviations used are: RCC, renal cell carcinoma; GST, glutathione *S*-transferase; BMI, body mass index; OR, odds ratio; CI, confidence interval; TCE, trichloroethene.

Table 1 Associations between cigarette smoking, BMI, and high blood pressure and RCC

	Cases (%)	Controls (%)	OR	95% CI
Cigarette smoking (tertiles pack-years)[a]				
Never smoker	52 (30.2)	215 (35.1)	1.0	
Low (≤17)	39 (22.7)	134 (21.9)	1.2	0.7–2.1
Middle (>17–39)	37 (21.5)	131 (21.4)	1.3	0.7–2.3
High (>39)	44 (25.6)	132 (21.6)	1.8	1.0–3.2
BMI (tertiles)[b]				
Low	43 (24.9)	205 (33.4)	1.0	
Middle	62 (35.8)	206 (33.6)	1.4	0.9–2.4
High	68 (39.3)	203 (33.1)	1.7	1.0–2.9
High blood pressure[c]				
No	91 (52.9)	56 (81.2)	1.0	
Yes	81 (47.1)	13 (18.8)	4.8	2.2–10.3

[a] ORs adjusted for age, sex, county, and BMI. Test for trend across tertiles pack-years, $P = 0.03$.
[b] ORs adjusted for age, sex, county, and pack-years smoking. Cut points for tertiles of BMI were 25.0 and 27.4 for males and 22.9 and 26.4 for females. Test for trend across tertiles BMI, $P = 0.04$.
[c] Self-reported history of diagnosis of high blood pressure. Information was available for only a subset of control subjects, $n = 69$. OR adjusted for age, sex, county, BMI, and pack-years smoking.

Subjects and Methods

Incident cases of RCC (ICD-O site code 189.0) diagnosed February 1996 through January 1997 in five counties in western Washington state were identified from the Cancer Surveillance System, a population-based cancer registry. Cases, 20–74 yr of age, were eligible. Control subjects had been identified and interviewed as controls for studies of esophageal (15) and lung[4] cancer. The two control groups, one selected from counties that are largely rural and suburban, and the other from counties that contain large urban areas, were combined to provide geographic balance for the RCC cases. Controls were ascertained in 1993–1996 by random digit dialing and from Health Care Finance Administration records. Participants completed a structured telephone or in-person interview regarding exposures to risk factors for RCC. Interviews were conducted for 173 of 214 eligible case subjects (80.8%). The respondent was the index subject for 144 case interviews, with 90% taking place within 12 months of diagnosis. Interviews were conducted with 29 proxies for cases because the subject was deceased or too ill to participate. Reasons for nonresponse among cases were: subject deceased with no proxy ($n = 16$), unable to contact subject ($n = 10$), and refusal [index subject ($n = 5$), proxy ($n = 6$), and physician ($n = 4$)]. The control group included 124 subjects from the esophageal cancer study (71.8% response rate) and 490 subjects from the lung cancer study (85.2% response rate). Interviews were conducted with proxy respondents for eight controls.

Paraffin blocks containing fixed tissue from surgery or biopsy were the source of DNA for cases. Blocks containing only normal kidney were used if available. Multiple sections, 10 microns in thickness, were cut from each block and deparaffinized using 1200 ml of xylene, followed by ethanol wash. DNA was extracted using a commercial kit (QIAGEN, Inc.), a method similar to published procedures (16, 17). For nine case subjects with no suitable pathology specimen available, a buccal cell sample was collected for genotyping. Buccal cells were obtained using a cytology brush, which was rubbed on the inside of the subject's cheek. Samples were kept on ice during transportation, then frozen at −70°C. DNA was extracted by incubation for 5 min at 95°C with 60 ml of 500 mM NaOH, followed by neutralization with 60 ml of 1 M Tris (pH 8). For controls, DNA was obtained from blood samples, which were kept on ice during transportation, then the buffy coats were isolated and frozen at −70°C until processed for DNA extraction.

GSTM1 and *GSTT1* genotypes were determined by multiplexed PCR using three sets of primers to amplify a 215-bp sequence of the *GSTM1* gene (18), a 268-bp sequence of the β-globin gene (18), and a 480-bp segment of the *GSTT1* gene (19). Presence or absence of the β-globin band was used to determine failed PCR. The A/G polymorphism of the *GSTP1* gene (20) responsible for the $Ile^{104}Val$ substitution was detected using an oligonucleotide ligation assay, a modification of previously described methods (21). *GSTP1* DNA sequences were amplified by PCR, followed by allele-specific ligation reaction and colorimetric detection. The PCR primers were: forward primer, 5′-GACTGTGTGTTGATCAGGCG-3′, and reverse primer 5′-TGCACCCTGACCCAAGAAGG-3′. The oligomer probes for the A/G polymorphism were: wild-type probe, 5′-biotin-AGGACCTCCGCTGCAAATACA-3′; mutant probe, 5′-biotin-AGGACCTCCGCTGCAAATACG-3′; and common reporter probe, 5′-phosphorylated- TCTCCCT-CATCTACACCAACT-digoxigenin-3′. The *GSTP1* oligonucleotide ligation assay had been verified in the same laboratory by DNA sequencing of PCR products from samples from 60 subjects, with 100% concordance of results. For all genotyping assays, several quality control samples (DNA from volunteers with known genotype) were included with each batch of study samples.

Of 173 case subjects interviewed, tissue was obtained for 132 (85 from tissue blocks containing normal kidney preserved from surgery, 23 from tissue blocks from surgery containing all or part tumor tissue, 15 from tissue blocks from biopsy, and 9 from buccal cell samples). No DNA samples were obtained for 18 interviewed cases who did not return consent forms for access to tissue and for 23 cases for whom tissue was unavailable from the hospitals. The number of cases with interpretable genotype results was 126 for *GSTM1*, 126 for *GSTT1*, and 130 for *GSTP1*. DNA from blood samples was obtained for 505 control subjects, with genotype results available for 505 for *GSTM1*, 504 for *GSTT1*, and 491 for *GSTP1*.

ORs were calculated using unconditional logistic regression. Effect modification among *GST* genotypes and between

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Table 2 GST *M1, T1,* and *P1* genotypes in RCC cases and controls[a] by selected characteristics

	n		*GSTM1* (% null)[a,b]		*GSTT1* (% null)[a,b]		*GSTP1* (% AG or GG)[a,c]	
	Cases	Controls	Case	Control	Case	Control	Case	Control
Sex								
Male	94	401	53.9	50.4	26.4	18.3	53.2	57.3
Female	36	104	40.0	51.0	34.3	19.2	61.1	53.9
Age								
<45 yr	14	39	57.1	51.3	14.3	18.0	64.3	75.7
45–54 yr	26	87	46.2	49.4	26.9	17.2	61.5	51.2
55–64 yr	46	188	52.3	52.7	38.6	19.7	47.8	55.7
65–74 yr	44	191	47.6	48.7	23.8	17.9	56.8	56.1
Race								
White	121	489	48.7	50.5	24.8	18.0	56.2	56.7
African American	4	2	75.0	50.0	75.0	50.0	50.0	50.0
Asian	2	7	100.0	42.9	100.0	42.9	0.0	50.0
Native American	2	3	0.0	33.3	50.0	0.0	50.0	33.3
Other	1	4	100.0	75.0	100.0	25.0	100.0	75.0
Country of birth								
United States	120	466	49.2	49.6	28.0	18.5	57.5	56.5
Other	10	39	62.5	61.5	37.5	18.0	30.0	58.3
Hispanic origin								
No	128	496	49.2	50.4	29.0	18.2	54.7	56.1
Yes	2	9	100.0	55.6	0.0	33.3	100.0	87.2
County of residence								
King	61	55	48.3	61.8	37.9	14.6	50.8	57.4
Pierce	31	20	41.9	45.0	22.6	20.0	67.7	45.0
Skagit	3	72	100.0	44.4	33.3	9.7	33.3	53.5
Snohomish	26	252	57.7	48.8	11.5	17.1	57.7	59.3
Whatcom	9	106	50.0	53.8	37.5	29.3	44.4	54.4
Stage at diagnosis								
Local	72		48.6		29.2		56.9	
Regional	33		45.5		24.2		57.6	
Distant	23		63.2		26.3		47.8	
Unstaged	2		50.0		100.0		50.0	

[a] Results from 132 cases and 505 controls with DNA available, of 173 cases and 614 controls interviewed. The number of subjects with genotype results was 126 cases and 505 controls for *GSTM1*; 126 cases and 504 controls for *GSTT1*; and 130 cases and 491 controls for *GSTP1*.
[b] Percentage of genotyped subjects in each category homozygous null for *GSTM1* or *GSTT1*.
[c] Percentage of genotyped subjects in each category heterozygous or homozygous for *GSTP1* exon 5 A/G.

genotypes and age, sex, cigarette smoking, and BMI was evaluated using logistic models including interaction terms between categories of the variables of interest, with statistical significance determined by likelihood ratio tests comparing models with and without the interaction term(s). Stata software (Stata Corporation, College Station, TX) was used for statistical analysis.

Results

Exposures associated with increased RCC risk included cigarette smoking, high BMI, and high blood pressure (Table 1). There was no increased risk associated with cigar or pipe smoking or residential second-hand smoke exposure (data not shown). Among cases, current smoking at reference date was reported more frequently by proxy respondents (34.5%) than by index subjects (22.4%). However, ORs for pack-years of smoking, BMI, high blood pressure, and other exposures were essentially unchanged when information obtained from proxy respondents was excluded. Control subjects from the study of esophageal cancer were older than those from the study of lung cancer (48.9% older than 64 *versus* 35.5% older than 64), but the two groups had similar distributions of other characteristics such as sex, race, smoking history, and BMI.

Demographic characteristics and exposure histories of cases and controls with DNA available were similar to all interviewed subjects. However, cases with DNA were more likely to have been diagnosed with local or regional stage disease than all interviewed cases. Among cases with DNA available, the percentages with local, regional, distant, and unstaged disease were 55.4%, 25.4%, 17.7%, and 1.5%, respectively, whereas the corresponding percentages among all interviewed cases were 49.1%, 23.1%, 23.7%, and 4.1%. (The percentages among all 214 eligible cases were 48.6%, 19.2%, 26.6%, and 5.6%.). Most cases with distant disease at diagnosis were not treated by surgery, and, therefore, pathological material was unavailable for these cases.

Frequencies of *GSTM1*, *GSTT1*, and *GSTP1* genotypes were similar between the two different study groups that formed the control group (data not shown). Among controls, genotype frequencies were similar by gender and across age categories (Table 2). Hispanic controls had a higher frequency of *GSTP1* AG or GG genotypes than non-Hispanic controls ($P = 0.08$). No other important differences were detected by race, ethnicity, or country of birth. The numbers of non-white and Hispanic subjects were small. The distribution of *GSTT1* genotypes varied by county of residence ($P = 0.01$) among controls. When control subjects who were non-white, Hispanic, or born outside the United States were excluded from the comparison, the differences in genotypes across counties persisted. Among cases, the frequency of *GSTM1*, *GSTT1*, and *GSTP1* genotypes did not differ significantly by source of DNA

Table 3 GST *M1*, *T1*, and *P1* polymorphisms and RCC risk

	Cases (%)	Controls (%)	OR[a]	95% CI	OR[b]	95% CI
GSTM1						
Present	63 (50.0)	250 (49.5)	1.0		1.0	
Null	63 (50.0)	255 (50.5)	1.0	0.7–1.4	1.0	0.6–1.6
GSTT1						
Present	90 (71.4)	411 (81.6)	1.0		1.0	
Null	36 (28.6)	93 (18.5)	1.8	1.1–2.8	1.9	1.1–3.4
GSTP1[c]						
AA	58 (44.6)	213 (43.4)	1.0		1.0	
AG	56 (43.1)	216 (44.0)	1.0	0.6–1.4	1.0	0.6–1.7
GG	16 (12.3)	62 (12.6)	0.9	0.5–1.8	1.0	0.4–2.0

[a] Crude ORs.
[b] ORs adjusted for age, sex, race, county, pack-years of smoking, and BMI.
[c] For combined categories GSTP1 AG and GG *versus* AA (OR, 1.0; 95% CI, 0.6–1.6).

Table 4 Combined *GSTT1*, *GSTM1*, and *GSTP1* genotypes and RCC risk

	GSTT1 present				*GSTT1* null			
	Cases	Controls	OR[a]	95% CI	Cases	Controls	OR[a]	95% CI
GSTM1 present, *GSTP1* AA	17	91	1.0		5	20	2.9	0.8–10.6
GSTM1 present, *GSTP1* AG or GG	27	110	1.8	0.8–4.0	14	19	4.1	1.4–12.4
GSTM1 null, *GSTP1* AA	22	80	1.8	0.8–4.3	10	21	3.0	0.9–9.5
GSTM1 null, *GSTP1* AG or GG	24	120	1.3	0.6–2.9	7	28	1.8	0.5–6.0

[a] ORs adjusted for age, sex, race, county, pack-years of smoking, and BMI.

(normal tissue block, tumor tissue block, biopsy, or buccal cells) or by stage at diagnosis.

An excess of the *GSTT1* null genotype was observed in cases (Table 3). The frequencies of *GSTM1* and *GSTP1* genotypes in cases were similar to controls. Among non-Hispanic white subjects, results for *GSTT1* (OR, 1.8; 95% CI, 1.0–3.2), *GSTM1* (OR, 0.9; 95% CI, 0.6–1.6), and *GSTP1* (for AG and GG *versus* AA genotypes, OR, 1.0; 95% CI, 0.6–1.7) were similar to the race-adjusted results for all subjects.

Table 4 shows numbers of cases and controls according to combined genotypes for *GSTT1*, *GSTM1*, and *GSTP1*, and ORs using subjects with *GSTT1* present, *GSTM1* present, and *GSTP1* AA as the reference category. Although CIs were large due to the small number of subjects in some categories, there was increased risk associated with *GSTT1* null genotype in all categories of *GSTM1* and *GSTP1* genotypes. There seems to be a slightly elevated risk associated with *GSTM1* null or *GSTP1* AG or GG among *GSTT1* present subjects, but when interaction of each genotype with *GSTT1* was tested in the logistic model, there was no statistical evidence of interaction between *GSTT1* and *GSTM1* ($P = 0.41$), nor of interaction between *GSTT1* and *GSTP1* ($P = 0.78$), nor between *GSTT1* and combined categories *GSTM1* null and *GSTP1* AA or AG ($P = 0.56$).

An excess of the *GSTT1* null genotype in cases was observed for never smokers as well as for smokers (Table 5). There was no indication of a trend in OR for *GSTT1* null with pack-years of smoking. ORs for the association between heavy smoking (highest tertile of pack-years compared with never smokers) and RCC were 1.6 (95% CI, 0.7–3.4) and 2.0 (95% CI, 0.5–7.6) among *GSTT1* present and *GSTT1* null subjects, respectively. ORs for *GSTM1* null and for GSTP1 AG and GG genotypes were near 1.0 for never smokers and for smokers, and across categories of pack-years of smoking.

The association between *GSTT1* genotype and RCC varied across categories of BMI (Table 5). The elevated risk associated with *GSTT1* null genotype occurred primarily among subjects in the lowest BMI category. A similar pattern was observed for *GSTM1*, with increased risk associated with *GSTM1* null genotype only within the lowest tertile of BMI. *GSTP1* genotype frequencies did not vary by BMI. ORs for the association between high BMI (highest tertile *versus* lowest tertile) and RCC by genotype were 2.3 (95% CI, 1.1–4.6) and 0.5 (95% CI, 0.2–1.7) among *GSTT1* present and *GSTT1* null subjects, respectively, and 2.5 (95% CI, 1.0–6.2) and 0.8 (95% CI, 0.4–1.9) among *GSTM1* present and *GSTM1* null subjects. The excess of *GSTT1* genotypes was present both among cases with (17 of 57) and without (19 of 68) a history of high blood pressure.

Discussion

We observed an excess of the *GSTT1* null genotype in RCC cases compared with controls. There was no overall association between *GSTM1* or *GSTP1* genotypes and RCC and no effect modification of one *GST* genotype by the other genotypes.

Associations between recognized risk factors for RCC, smoking, high blood pressure, and BMI in this population-based case-control study were consistent with what has been reported in previous literature (1, 22, 23). Adjustment for these exposures did not affect the relationship between *GSTT1* null genotype and RCC. The major selection factor that differed between participating and nonparticipating cases in our study was stage at diagnosis, which was unrelated to *GSTT1* genotype among participating cases and, thus, seems unlikely to bias the observed association between RCC and *GSTT1* null. For *GSTM1* and *GSTP1*, the distribution of genotypes among the distant stage cases who were genotyped were similar enough to local and regional stage cases that bias, if any, related to lower participation by distant stage cases would be small. Although association between BMI and prognosis has been reported (24) in RCC cases, BMI was not associated with stage at diagnosis among cases in the present study, so stage bias is unlikely to

Table 5 *GSTT1*, *GSTM1*, and *GSTP1* polymorphisms and RCC risk, by smoking history and BMI

	GSTT1				*GSTM1*				*GSTP1*			
	Cases (%)	Controls (%)	OR	95% CI	Cases (%)	Controls (%)	OR	95% CI	Cases (%)	Controls (%)	OR	95% CI
Smoking[a]												
Never												
Present	27 (69.2)	138 (79.3)	1.0		19 (48.7)	86 (49.1)	1.0		17 (42.5)	73 (45.0)	1.0	
Null	12 (30.8)	36 (20.7)	2.3	0.9–5.7	20 (51.3)	89 (50.9)	1.0	0.4–2.3	23 (57.5)	93 (55.0)	1.1	0.5–2.5
Ever												
Present	63 (72.4)	273 (82.7)	1.0		44 (50.6)	164 (49.7)	1.0		41 (45.6)	137 (42.6)	1.0	
Null	24 (27.6)	57 (17.3)	1.7	0.8–3.4	43 (49.4)	166 (50.3)	1.0	0.6–1.8	49 (54.4)	185 (57.5)	0.9	0.5–1.6
BMI[b] (tertiles)												
Low												
Present	17 (51.5)	132 (81.0)	1.0		11 (33.3)	78 (47.9)	1.0		12 (36.4)	64 (40.8)	1.0	
Null	16 (48.5)	31 (19.0)	4.8	1.8–13.0	22 (66.7)	85 (52.2)	2.2	0.9–5.6	21 (63.6)	93 (59.2)	1.2	0.5–3.1
Middle												
Present	33 (73.3)	141 (82.9)	1.0		23 (51.1)	77 (45.2)	1.0		22 (47.8)	75 (44.6)	1.0	
Null	12 (26.7)	29 (17.1)	1.5	0.6–3.9	22 (48.9)	93 (54.7)	0.7	0.3–1.6	24 (52.2)	93 (55.4)	1.1	0.5–2.3
High												
Present	40 (83.3)	138 (80.7)	1.0		29 (60.4)	95 (55.2)	1.0		24 (47.1)	74 (44.6)	1.0	
Null	8 (16.7)	33 (19.3)	1.0	0.3–2.7	19 (39.6)	77 (44.8)	0.7	0.3–1.6	27 (52.9)	92 (55.4)	0.8	0.4–1.7

[a] ORs adjusted for age, sex, race, county, and BMI. Tests for interaction: *GSTT1* genotype and never/ever smoking, $P = 0.60$; *GSTM1* genotype and never/ever smoking, $P = 0.97$; *GSTP1* genotype and never/ever smoking, $P = 0.70$.
[b] ORs adjusted for age, sex, race, pack-years smoking, and county. Tests for interaction: *GSTT1* genotype and tertile BMI, $P = 0.07$; *GSTM1* genotype and tertile BMI, $P = 0.13$; *GSTP1* genotype and tertile BMI, $P = 0.76$.

have influenced the relationships among GSTM1, BMI, and case-control status.

The frequencies of the *GSTM1*, *GSTT1*, and *GSTP1* genotypes among controls were comparable with what has previously been reported for study populations drawn from Caucasians in North America and Europe (4, 20, 25, 26). Evidence from other studies indicates that frequencies of the *GSTM1* null and possibly the *GSTT1* null genotypes vary by race (4). In our multivariate model, race-adjusted ORs for GST genotypes were similar to crude ORs, and the associations based on all subjects were qualitatively similar to results of models limited to non-Hispanic white subjects. The variation in the frequencies of *GSTT1* genotypes among controls by county of residence was not explained by race, and is most likely attributable to chance.

The use of different tissue sources to obtain DNA for cases and controls is a potential limitation of these data. Pathological material was the source of DNA for most cases, whereas blood was used for controls. However, it is unlikely that the source of DNA introduced bias in measurement of genotypes. Tissue blocks containing only normal tissue were used for the majority of case subjects. In a study comparing sources of DNA for PCR-based genotyping, genotypes for GSTM1 and several other genes determined from fixed, paraffin-embedded tissue were 97–100% concordant with genotypes determined from fresh frozen tissue from the same subject (27).

Our finding of an increased frequency of *GSTT1* null genotypes among cases suggests that activity of the GSTT1 enzyme protects against development of RCC. Because GST enzymes are active in detoxifying chemicals from cigarette smoke, researchers have hypothesized that the role of the polymorphisms in human cancer would be to modulate the effect of exposures to cigarette smoke. However, in this study the association between *GSTT1* and RCC was similar for smokers and nonsmokers. GST enzymes catalyze conjugation of glutathione with a broad range of substrates (3), including lipid peroxides formed endogenously as a result of oxidative stress (9). Kidney tissue has high metabolic activity and oxygen demand, a situation in which enhanced endogenous formation of reactive oxidants is possible; a high level of somatic mutations in kidney compared with other tissues has been reported (28). The association between *GSTT1* null genotype and increased RCC risk may be due to reduced protection against endogenous reactive oxidants.

The association between *GSTT1* null genotype and RCC risk seemed to vary by BMI, with the strongest association among subjects in the lowest tertile of BMI who are otherwise at low risk of RCC. The association between *GSTT1* and RCC among low-BMI subjects may indicate that different etiological pathways are involved in development of RCC in low-BMI *versus* high-BMI individuals. The mechanism for the association between high BMI and RCC is not well understood. If this mechanism involves reactive oxidants, a possible interpretation of our results is that protection by GSTT1 is important among individuals with low levels of exposure, but becomes overwhelmed and does not affect risk among the highly exposed. We assessed potential interaction between *GSTT1* genotype and several exposure variables, so it is possible that the *GSTT1*-BMI interaction is a chance finding. Statistical power to detect the *GSTT1*-BMI interaction in the present study was 70% at $\alpha = 0.05$.

The present study is the only report of increased risk of RCC associated with *GSTT1* null. The inconsistent results compared with other studies may reflect chance variation, but it is possible that the role of GSTT1 is different in the presence of different patterns of exposures to environmental risk factors for RCC. The case-control study in France (14), which reported no association with *GSTT1*, provided no information on the subjects' exposures to risk factors for RCC. In the study of solvent-exposed workers in Germany (13), *GSTT1* null genoype was associated with reduced risk of RCC (OR, 0.2; 95% CI, 0.1–0.9), based on a small number of cases. The authors note that their result is consistent with a model of TCE toxicity to the kidney through GST-catalyzed formation of glutathione conjugates, which are further metabolized in the kidney to toxic compounds, a mechanism that may not be relevant in individuals without occupational solvent exposure. The decreased risk of RCC associated with *GSTT1* null genotype among TCE-exposed workers contrasts markedly with the increased risk

observed in the present population-based study, in which we would expect that few subjects would have been exposed to TCE. The contrasting results may imply different effects of GSTT1 enzymes according to the presence of specific chemical exposures.

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Carol Sweeney, Diana C. Farrow, Stephen M. Schwartz, et al.

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