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Renal cell carcinoma, occupational pesticide exposure and modification by glutathione *S*-transferase polymorphisms

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This study investigated associations between occupational pesticide exposure and renal cell carcinoma (RCC) risk. To follow-up on a previous report by Buzio *et al.*, we also considered whether this association could be modified by glutathione *S*-transferase *M1* and *T1* (*GSTM1* and *GSTT1*) genotypes. About 1097 RCC cases and 1476 controls from Central and Eastern Europe were interviewed to collect data on lifetime occupational histories. Occupational information for jobs held for at least 12 months duration was coded for pesticide exposures and assessed for frequency and intensity of exposure. *GSTM1* and *GSTT1* gene deletions were analyzed using TaqMan® assays. A significant increase in RCC risk was observed among subjects ever exposed to pesticides [odds ratio (OR): 1.60; 95% confidence interval (CI): 1.00–2.55]. After stratification by genotypes, increased risk was observed among exposed subjects with at least one *GSTM1* active allele (OR: 4.00; 95% CI: 1.55–10.33) but not among exposed subjects with two *GSTM1* inactive alleles compared with unexposed subjects with two inactive alleles (*P*-interaction: 0.04). Risk was highest among exposed subjects with both *GSTM1* and *GSTT1* active genotypes (OR: 6.47; 95% CI: 1.82–23.00; *P*-interaction: 0.02) compared with unexposed subjects with at least one *GSTM1* or *T1* inactive genotype. In the largest RCC case–control study with genotype information conducted to date, we observed that risk associated with pesticide exposure was exclusive to individuals with active *GSTM1*/*T1* genotypes. These findings further support the hypothesis that glutathione *S*-transferase polymorphisms can modify RCC risk associated with occupational pesticide exposure.

Introduction

Tobacco smoking, obesity and hypertension are established risk factors for renal cell carcinoma (RCC) and account for nearly half of cases diagnosed in the USA (1,2). Given the high proportion of RCC cases unexplained by these known risk factors, research on occupational exposures and genetic risk factors that modify cancer susceptibility has the potential to improve our understanding of RCC etiology and identify susceptible subgroups.

Abbreviations: CI, confidence interval; GST, glutathione *S*-transferase; OR, odds ratio; RCC, renal cell carcinoma.

Pesticides are developed to be toxic to living organisms and represent a wide range of compounds. Those produced from halogenated alkanes, alkenes and other solvents are thought to undergo bioactivation in the kidney after conjugation to glutathione by glutathione *S*-transferases (*GST*s) (2,3). Since *GST*s are expressed and are active in the kidney (2), altered *GST* activity associated with functional polymorphisms in the glutathione *S*-transferase mu (*GSTM1*) and theta (*GSTT1*) genes may modify cancer risk because of differences in xenobiotic metabolism and bioactivation in the kidney.

Results from epidemiological investigations of occupational exposures to pesticides and RCC have been limited and inconsistent (3,4). Most gene–environment interaction studies of the *GST*s and RCC have been underpowered and have shown mixed results (2,5–7). One study (5) investigated the association between occupational pesticide exposures, RCC risk and modification by *GST* genotypes and observed higher risks among subjects with the *GSTM1*- and *GSTT1*-present genotypes compared with subjects with the null genotypes.

Therefore, the aim of the present study was to: (i) investigate whether occupational exposure to pesticides was associated with an increased risk in RCC and (ii) follow-up on a previous report by Buzio *et al.* to test the hypothesis that *GSTM1* and *GSTT1* polymorphisms would modify pesticide-related RCC risk in a larger, multicentered case–control study conducted in Central and Eastern Europe.

Materials and methods

Study population

A hospital-based case–control study of RCC was conducted between 1999 and 2003 in seven centers in four countries of Central and Eastern Europe. Cases included patients aged 20–88 years with newly diagnosed histologically confirmed RCC (IDC-O-2 codes C64) who were living in the study areas for at least 1 year and were interviewed within 3 months of diagnosis. All tumors were centrally reviewed at the National Cancer Institute to confirm diagnosis; each clear cell RCC case was histologically confirmed by Dr Maria Merino, a world expert in renal tumor pathology. The majority of renal tumors were clear cell carcinomas (83.4%), whereas other subtypes included papillary (7%), chromophobe (2.4%), oncocytoma (2.3%), oncocytic neoplasms (0.2%), transitional cell carcinomas (1.1%) and unclassified (3.6%). Controls, frequency matched to cases on age (±3 years), sex and place of residence, were selected from patients admitted to participating hospitals for diagnoses unrelated to smoking or urological disorders with the exception of benign prostatic hyperplasia. No single disease made up >20% of the control group. The final study included 1097 cases and 1476 controls. The response rates for study participation ranged from 90.0 to 98.6% for cases and from 90.3 to 96.1% for controls. All subjects provided written informed consent. This study was approved by the institutional review boards of all participating centers.

Cases and controls were interviewed by the same interviewers using identical questionnaires. During hospitalization, cases and controls were administered a standardized questionnaire by trained interviewers that included basic demographic characteristics, family history of cancer, history of tobacco consumption and diet information. Lifetime occupational information for jobs of ≥12 months duration was also collected during interviews through the use of a general occupational questionnaire and job-specific questionnaires. In addition to the information on other agents, job-specific questionnaires for farmers, gardeners and wood, chemical and tannery workers were used to collect information on (i) possible pesticide exposures; (ii) hours per week participants were exposed; (iii) the source of pesticide exposure (e.g. name of the product or how the mixture was prepared) and (iv) a description of pesticide use (e.g. the type of seeds, crops or animal feeds being treated). The occupational questionnaires were reviewed by local occupational health experts or industrial hygienists who were trained by the studies lead by industrial hygienist; the data were also reviewed by the study industrial hygienist. Every job was coded for 74 specific agents.

Occupational health experts assessed the frequency, intensity and confidence of exposure to inorganic/organic pesticides for each job held by each subject at the study center. Frequency of exposure (in hours) was coded in three

categories, representing the percentage of time in a 40 h workweek during which exposure was possible: 1–4, 5–29 and ≥30% of the time. To compute lifetime pesticide exposure across jobs that had different frequencies of exposure, frequency weights (0.03, 0.175 and 0.50) were assigned to the three frequency categories corresponding to the midpoint of the ranges. An intensity weight of '5' was assigned as the midpoint of the lowest exposure category (0 to <10 mg/h of dermal exposure), a weight of '50' was assigned as the midpoint of the middle category (10–100 mg/h of dermal exposure) and a weight of '125' was assigned to the highest exposure category (>100 mg/h of dermal exposure). Intensity of exposure to pesticides was coded as low (<10 mg/h, weight 5), medium (10–100 mg/h, weight 50) or high (>100 mg/h, weight 125). The confidence of exposure was provided by experts as 'possible' (<40%), 'probable' (40–90%) or 'certain' (>90%). Experts were blinded to the case–control status of the subjects while reviewing occupational histories and assessing exposure. Reliability of the experts' assessment for all agents across study centers was evaluated with an interteam agreement study of 19 job descriptions and 54 exposures and found that the overall quality was comparable among expert teams (8,9). Specifically, through the use of kappa scores, agreement for the presence or absence of an exposure among teams was excellent (kappa > 0.75), whereas agreement with regards to confidence, intensity and frequency of exposures was fair to good (kappa between 0.4 and 0.75) (9).

Laboratory analysis

Suitable genomic DNA was obtained from a subset of 925 (84.3%) newly diagnosed and histologically confirmed RCC cases and 1192 (80.8%) controls. DNAs for genotyping assays were extracted from whole blood and buffy coat samples, which were stored at −80°C and shipped to the National Cancer Institute biorepository on dry ice. DNA was extracted using phenol–chloroform extraction. Genotyping was conducted at the International Agency for Research on Cancer and at National Cancer Institute's Core Genotyping Facility. DNAs from cases and controls were blinded and randomized on polymerase chain reaction plates to avoid potential bias; duplicate genotyping was performed for a randomly selected 5% of the total series for quality control. In total, 925 (84.3%) cases and 1192 (80.8%) controls were genotyped for *GSTM1* and *GSTT1* genes. Call rates were >99% for the *GSTM1* and *GSTT1* deletion assays. Concordance rates were >99% for *GSTT1* and 97.4% for *GSTM1*. Methods for genotype assays can be found at: http://snp500cancer.nci.nih.gov/home_1.cfm.

Statistical analysis

Few subjects were exposed to specific pesticide subtypes, thus limiting our precision and power to assess RCC risk for specific types of pesticides. Therefore, pesticides were subgrouped as organic or inorganic; because organic and inorganic pesticide exposures were highly correlated ($R^2 = 0.95$), the assessments were combined to form a single pesticide exposure variable. This variable was created by selecting the type of pesticide (either organic or inorganic) with the longest duration of exposure for each job.

Pesticide exposures were calculated for ever exposure, years of exposure, hours of exposure, cumulative exposure (milligrams per years) and average exposure (milligrams). Duration of exposure in hours was calculated for each job using the following formula and summing over jobs: duration (years) × 50 (weeks/year) × 40 (hours/week) × frequency weights. Cumulative exposure was calculated for each job using the following formula and summing over all jobs: intensity weight × frequency weight × duration (years). Average exposure was calculated for each job by dividing cumulative exposure by the number of years exposed.

Subgroup analysis among those with the highest confidence of pesticide exposure was conducted by using only jobs with a confidence of exposure rating of certain. Subgroup analysis with 20 years lag period between exposure and diagnosis was also conducted to restrict analyses to subjects with a sufficient latency period from exposure to disease.

Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to estimate RCC risk by genotype and occupational exposure, using unconditional logistic regression models adjusted for sex, age, center and smoking status. Different models adjusted for body mass index, hypertension and/or family history of cancer were also used to examine the risk of kidney cancer; however, the inclusion of these adjustment variables did not affect OR values by 10% or more. Interactions were tested comparing regression models with and without interaction terms using a likelihood ratio test. Categorical exposure metrics were used to evaluate exposure–response relationships with occupational exposure. These included never- and ever-exposed groups. The exposed group for each exposure metric (as years, hours, etc.) was further divided into levels of exposure based on the 50th percentile cut-point among controls. Logistic regression and linear trend were calculated using continuous variables.

Variations in the *GSTM1* and *GSTT1* genes were evaluated as 'active' if subjects had at least one non-deleted *GSTM1* or *GSTT1* allele and 'inactive' if both copies were deleted. When combining genotypes, we used the same grouping as described previously (5). Subjects were considered active if both *GSTM1* and *GSTT1* were present (i.e. more than one allele was present for both); subjects were considered inactive if one or both genes were null since there were very few cases that had both alleles of both genes deleted.

To identify other occupational exposures that may be potential confounders, correlation analyses (Pearson and Spearman) were conducted to identify other agents associated with pesticide exposure. Of the 72 other exposures assessed, no significant coexposures were associated with pesticide exposure ($R^2 \leq 0.30$) and risk of RCC and no coexposures modified ORs by >10%.

All analyses were conducted in STATA 8.0 unless otherwise specified (STATA Corporation, College Station, TX).

Results

A description of the study subjects is presented in Table I. Cases and controls were comparable in age, but cases were more often female and more likely to have excess body mass index (>30 kg/m^2), hypertension and a first-degree relative with cancer, but a lower prevalence of smoking, although the association with smoking was not significant after adjustment for age, body mass index, hypertension, center and sex (10). Cases and controls were also comparable among occupations suspect of pesticide exposures. The prevalence of ever being occupationally exposed to pesticides was 5.3% among cases and 2.9% among controls and was not substantially altered after restricting the analysis to exposures with the highest (certain) confidence rating (5.0% among cases and 2.4% among controls).

RCC risk was elevated among subjects ever occupationally exposed to pesticides compared with those never exposed (OR: 1.60; 95% CI: 1.00–2.55) (Table II); elevated RCC risk was also observed after restriction to only high confidence exposures (OR: 1.82; 95% CI: 1.10–3.00). Among all subjects, increased renal cancer risk and significant exposure–response relationships were observed for years (*P*-trend: 0.01), hours (*P*-trend: 0.03) and cumulative exposure indices (*P*-trend: 0.04) but not for average exposure. All relationships were strengthened after restriction to only high confidence exposures. Results did not differ when analyses were restricted to clear cell carcinomas (data not shown). Additionally, similar associations were observed in both men and women separately (data not shown). Among pesticide-exposed subjects, no particular job/industries appeared to drive results. Furthermore, excess risks (OR: 1.67; 95% CI: 1.02–2.76 for ever exposed) were observed for all exposure metrics (*P*-trend: 0.01 for years; *P*-trend: 0.02 for hours; *P*-trend: 0.03 for cumulative exposure) except average exposure (*P*-trend: 0.08) when the risk of RCC and pesticide exposure was assessed after consideration of 20 years lag period (data not shown).

Table III shows the joint effects of both *GST* genotypes and pesticide exposure on RCC risk. The main effects for RCC risk associated with the active compared with the null *GST* genotypes were not significant [*GSTM1* (OR: 0.93; 95% CI: 0.78–1.12), *GSTT1* (OR: 0.94; 95% CI: 0.75–1.19) and *GSTT1/GSTM1* combined (OR: 0.93; 95% CI: 0.78–1.12)]. After consideration of pesticide exposure, a significant increased risk was observed only among pesticide-exposed subjects with the *GSTM1* active genotype (OR: 4.00; 95% CI: 1.55–10.33), whereas no excess risk was observed for unexposed subjects with an active genotype (OR: 0.99; 95% CI: 0.80–1.23) or exposed subjects with an inactive genotype (OR: 1.03; 95% CI: 0.50–2.14) when compared with unexposed subjects with the inactive genotype (*P*-interaction: 0.04). Similarly, pesticide-exposed subjects with an active *GSTT1* genotype showed a significantly elevated risk for RCC (OR: 2.28; 95% CI: 1.11–4.67) when compared with unexposed subjects with the *GSTT1* null genotype (*P*-interaction: 0.15). Combined effects of *GSTM1* and *GSTT1* active genotypes showed a greater than 6-fold risk (OR: 6.47; 95% CI: 1.82–23.00) among pesticide-exposed subjects with both *GSTM1* and *GSTT1* active genotypes compared with unexposed subjects with at least one inactive genotype (*P*-interaction: 0.02). No elevated risk was observed among pesticide individuals with null *GST* genotypes.

Discussion

The results of this study showed an increased risk of RCC with pesticide exposure. The excess risk was linearly associated with indices

Table I. General characteristics of participants

Variables	Cases		Controls		OR	95% CI	*P*-value
	n	%	*n*	%			
Participants	1097	42.6	1476	57.4			
Sex							
Males	648	59.1	952	64.5	1.00		0.01
Females	449	40.9	524	35.5	1.26	1.07–149	
Age at interview							
<45	86	7.8	122	11.1	1.00		0.61
45–54	278	25.3	379	34.5	1.05	0.76–1.43	
55–64	335	30.5	460	41.9	1.03	0.76–1.41	
65–74	353	32.2	452	41.2	1.11	0.81–1.51	
75+	45	4.1	63	5.7	1.01	0.63–1.62	
Mean age (standard)	59.6 years (10.3)		59.3 years (10.3)				
Center							
Romania—Bucharest	95	8.7	160	10.8	1.00		<0.001
Poland—Lodz	99	8.7	198	13.4	0.84	0.59–1.20	
Russia—Moscow	317	28.9	463	31.4	1.15	0.86–1.54	
Czech Republic[a]	586	53.4	655	44.4	1.51	1.14–1.99	
Body mass index at interview							
<25	329	30.0	535	36.4	1.00		0.01
25–29.9	474	43.2	617	41.9	1.25	1.04–1.50	
30+	293	26.7	319	21.7	1.49	1.21–1.84	
Tobacco status							
Never	510	46.6	599	40.6	1.00		0.003
Ever	584	53.4	876	59.4	0.78	0.67–0.92	
Hypertension							
No	600	54.7	906	61.4	1.00		0.001
Yes	496	45.3	569	38.6	1.32	1.12–1.54	
Familial history of cancer							
No first-degree relative with cancer	733	66.8	1074	72.8	1.00		0.002
First-degree relative with cancer	364	33.2	402	27.2	3.26	1.12–1.57	
Farmers							
No	1077	98.2	1458	98.8	1.00		0.16
Yes	20	1.8	17	1.2	1.59	0.83–3.10	
Gardeners							
No	1094	99.7	1467	99.5	1.00		0.31
Yes	3	0.3	8	0.5	0.50	0.13–1.90	
Wood workers							
No	1064	97.0	1442	97.8	1.00		0.22
Yes	33	3.0	33	2.2	1.36	0.83–2.21	
Chemical workers							
No	1089	99.3	1469	99.6	1.00		0.28
Yes	8	0.7	6	0.4	1.80	0.62–5.20	
Tannery workers							
No	1096	99.9	1473	99.9	1.00		0.75
Yes	1	0.1	2	0.1	0.67	0.06–7.42	
Pesticide exposure							
No	782	94.7	1152	97.1	1.00		0.01
Yes	44	5.3	34	2.9	1.91	1.21–3.01	
Pesticide exposure high confidence[b]							
No	782	95.0	1152	97.6	1.00		0.002
Yes	41	5.0	28	2.4	2.16	1.32–3.52	

[a]Brno, Olomouc, Prague and Ceske Budejovice.
[b]Representative of participants with a high confidence (probable or certain) of pesticide exposure.

of duration and cumulative exposure. All associations were stronger after restriction to exposures assessed with high confidence and after inclusion of 20 years lag period between initial exposure and disease. We also replicated in a much larger case–control study the results reported by Buzio *et al.* (5) who reported that kidney cancer risk associated with pesticide exposure was modified by *GSTM1* and *GSTT1* genotypes. Specifically, that excess risk was only associated with exposure among individuals with active genotypes but not among those with null genotypes when unexposed *GST* null subjects were used as a comparison group.

The relationship between RCC and occupational pesticide exposure has been examined previously in five epidemiological studies and results have been mostly negative. No association was shown between RCC risk and occupational pesticide exposure in a large international multicenter population-based study of 1723 cases and 2309 controls (11) or in a smaller study conducted in Germany (12). Non-significant increased risks were observed in two European case–control studies (3,13); however, when analyses were restricted to subjects occupationally exposed for 20 years or more, a significant 4-fold RCC risk was shown in men (OR: 3.9; 95% CI: 1.0–15.0) (13). Likewise, a significantly elevated RCC risk was reported among males exposed to herbicides (OR: 1.6; 95% CI: 1.3–2.0) and pesticides (OR: 1.8; 95% CI: 1.4–2.2) in a large Canadian case–control study of 1279 cases and 5370 controls (4).

The frequency of *GSTT1* null (18.1%) and *GSTM1* null (47.5%) polymorphisms in our control population in the present study was

Table II. Risk of RCC and exposure to pesticides

Pesticide	All subjects							High confidence[a]						
	Cases		Controls		OR	95% CI	*P*-trend	Cases		Controls		OR	95% CI	*P*-trend
	n	%	*n*	%				*n*	%	*n*	%			
Pesticide exposure														
Never	782	94.7	1152	97.1	1.00		0.05	782	95.0	1152	97.6	1.00		0.02
Ever	44	5.3	34	2.9	1.60	1.00–2.55		41	5.0	28	2.4	1.82	1.10–3.00	
Years exposed														
Never	782	94.7	1152	97.1	1.00		0.01	782	95.0	1152	97.6	1.00		0.01
≤8.00	13	1.6	18	1.5	0.84	0.41–1.76		12	1.5	14	1.2	1.00	0.45–2.21	
>8.00	31	3.8	16	1.3	2.46	1.33–4.58		29	3.5	14	1.2	2.66	1.38–5.12	
Hours exposed														
Never	782	94.7	1152	97.1	1.00		0.03	782	95.0	1152	97.6	1.00		0.01
≤1230	18	2.2	17	1.4	1.16	0.59–2.29		18	2.2	14	1.2	1.43	0.70–2.93	
>1230	26	3.1	17	1.4	2.07	1.11–3.88		23	2.8	14	1.2	2.24	1.13–4.43	
Cumulative exposure														
Never	782	94.7	1152	97.1	1.00		0.04	782	95.0	1152	97.6	1.00		0.02
≤0.86	20	2.4	17	1.4	1.32	0.68–2.57		18	2.2	13	1.1	1.60	0.77–3.32	
>0.86	24	2.9	17	1.4	1.89	1.00–3.57		23	2.8	15	1.3	2.02	1.04–3.94	
Average exposure														
Never	782	94.7	1152	97.1	1.00		0.09	782	95.0	1152	97.6	1.00		0.06
≤0.03	29	3.5	20	1.7	1.73	0.96–3.13		27	3.3	15	1.3	2.21	1.15–4.25	
>0.03	15	1.8	14	1.2	1.39	0.66–2.94		14	1.7	13	1.1	1.37	0.63–2.96	

All values adjusted for age (continuous), sex, center and smoking status (ever, never). Tertile values based on median exposure levels among controls.
[a]Representative of participants with a high confidence (probable or certain) of pesticide exposure.

Table III. RCC risk, occupational pesticide exposure and *GSTT1* and *GSTM1* genotypes

Variable	*GSTM1*						*GSTT1*						*GSTM1/GSTT1*[a]					
	Cases		Controls		OR	95% CI	Cases		Controls		OR	95% CI	Cases		Controls		OR	95% CI
	n	%	*n*	%			*n*	%	*n*	%			*n*	%	*n*	%		
No pesticide exposure																		
Inactive (null)[b]	286	48.6	416	48.1	1.00		121	20.4	153	17.2	1.00		341	58.1	489	57.0	1.00	
Active (present)[c]	302	51.4	448	51.9	0.99	0.80–1.23	471	79.6	737	82.8	0.84	0.64–1.10	246	41.9	369	43.0	0.98	0.79–1.22
Pesticide exposure																		
Inactive (null)[b]	17	47.2	17	73.9	1.03	0.50–2.14	8	22.2	8	34.8	0.96	0.33–2.73	22	61.1	19	86.4	120	0.62–2.33
Active (present)[c]	19	52.8	6	26.1	4.00	1.55–10.33	28	77.8	15	65.2	2.28	1.11–4.67	14	38.9	3	13.6	6.47	1.82–23.00
P-interaction[d]						0.04						0.15						0.02

Main effect null versus present: *GSTM1* OR = 0.93 (0.78–1.12), *GSTT1* OR = 0.94 (0.75–1.19) and *GSTT1/GSTM1* OR = 0.93 (0.78–1.12). Adjusted for age (continuous), sex, center and smoking habit (ever, never).
[a]Inactive (null) if one or both genes inactive; active (present) if both genes more than one active allele.
[b]No active alleles.
[c]More than one active allele.
[d]*P*-value for interaction using likelihood ratio test.

similar to what was published previously in meta-analyses and pooled analyses among Caucasians (14–16). Furthermore, the finding that excess RCC risk was only observed among individuals with active *GST* genotypes is biologically plausible. The *GST* enzyme is required for metabolism of some groups of pesticides through *GST* conjugation and excretion (17,18). Generally, conjugation of foreign compounds with glutathione leads to formation of less reactive products that are readily excreted. However, in specific tissues and with certain exposures, the glutathione conjugate is more reactive than the parent compound and there is evidence that this is particularly true in the kidney (2,19). For halogenated compounds, in particular, the glutathione conjugate mediated by the *GST* serves as a substrate for a subsequent enzyme, renal cysteine conjugate β-lyase (20). Metabolism occurs in the kidney and has been shown to form reactive chlorothioketenes that are directly damaging to the kidney. Therefore, an active *GST* enzyme will be required to conjugate substrates and form more reactive intermediates that directly damage kidney tissues. Conversely, the deleted variant *GST* genotype will form an inactive enzyme and therefore metabolism of halogenated compounds will occur through oxidation, without formation of reactive intermediates in the kidney (2,21,22).

Results from previous studies of *GST* polymorphisms and RCC have been inconsistent. Our results were similar to Buzio *et al.* (5) where they reported an increased risk of RCC in participants occupationally exposed to pesticides with active *GSTM1* (OR: 3.46; 95% CI: 1.12–10.74), *GSTT1* (OR: 6.54; 95% CI: 1.49–18.81) and both *GSTM1* and *GSTT1* active genotypes (OR: 6.64; 95% CI: 1.81–24.45); but USA study of 130 renal cancer cases and 505 controls found a significant increase in RCC risk among unexposed subjects with the *GSTT1* null (OR: 1.8; 95% CI: 1.1–2.8) but not the *GSTM1* null genotype (OR: 1.0; 95% CI: 0.7–1.4) (2). A French case–control study (173 RCC and 211 controls) did not observe effect modification by genotype (18). These inconsistencies may be attributable to several aspects of design such as sample size or misclassification of exposures. All studies to date have been underpowered to observe main

effects and interactions and have failed to identify the exact types of pesticide exposures. Underpowered studies of gene–environment interactions and exposure misclassification stemming from the quality of exposure assessment may dilute any real associations.

Strengths of our study include a large sample size, a high participation rate, inclusion of only histologically confirmed cancers, collection of biological materials from a high proportion of subjects, use of job-specific questionnaire modules to collect individual-specific exposure information and expert-based exposure assessment. The large sample size of this study provided sufficient statistical power to detect relatively small associations between genotypes and risk; however, only 44 cases and 34 controls were exposed, limiting the precision of our measurements that was reflected in the wide CIs for some ORs, the power to detect interactions and our ability to assess risk for specific types of pesticides. Data regarding the use of personal protective equipment and non-occupational exposure to pesticides were not ascertained, although we believe that the risk of non-occupational exposures would have been minimal. Lastly, while hospital-based case–control studies have potential limitations due to the lack of population controls, these studies can improve response rates for the intense collection of biological specimens and therefore reduces the chances of bias in the assessment of gene–environment interactions (23).

In conclusion, the results of this study suggest that occupational pesticide exposures may increase RCC risk. *GSTM1* and *GSTT1* variants significantly modified RCC risk among participants occupationally exposed to pesticides but not among unexposed subjects, thus replicating the work conducted previously by Buzio *et al.* in a larger case–control study. These findings speculate for the role of common variation within the *GSTM1* and *GSTT1* genes to modify the effect of occupational exposure and cancer risk. Additional studies with detailed information on specific types of pesticide exposures and larger numbers of exposed subjects will be important to replicate and extend these findings.

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