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World J Urol (2008) 26:103–110
DOI 10.1007/s00345-007-0220-5

ORIGINAL ARTICLE

Polymorphisms in genes related to activation or detoxification of carcinogens might interact with smoking to increase renal cancer risk: results from The Netherlands Cohort Study on diet and cancer

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Received: 27 July 2007 / Accepted: 9 October 2007 / Published online: 3 November 2007

Abstract Metabolic gene polymorphisms have previously been suggested as risk factors for renal cell carcinoma (RCC). These polymorphisms are involved in activation or detoxification of carcinogens in cigarette smoke which is another RCC risk factor. We evaluated gene–environment interactions between *CYP1A1*, *GSTμ1* and smoking in a large population-based RCC case group. The Netherlands Cohort Study on diet and cancer (NLCS) comprises 120,852 persons who completed a questionnaire on smoking and other risk factors at baseline. After 11.3 years of follow-up, 337 incident RCC cases were identified. DNA was collected for 245 cases. In a case-only analysis, interaction-odds ratios (OR) and 95% confidence intervals (95% CI) were calculated using logistic regression. We observed a moderate, not statistically significant, interaction between current smoking and *CYP1A1*2C* (OR 1.42; 95% CI 0.70–2.89) and *GSTμ1* null (OR 1.35; 95% CI 0.65–2.79). For current smokers with both a variant (heterozygous or homozygous) in *CYP1A1* and *GSTμ1* null, risk was also increased (OR 1.63; 95% CI 0.63–4.24). No interaction was observed between ever smokers, smoking duration (increments of 10 smoking years) or amount (increments of 5 cigarettes/day) and *CYP1A* or *GSTμ1*. Our results show a modest trend towards a statistically significant gene–environment interaction between *CYP1A1*, *GSTμ1* and smoking in RCC. This could indicate that RCC risk among smokers might be more increased with the *CYP1A1*2C* genotype, *GSTμ1* null, or both a *CYP1A1* variant and *GSTμ1* null.

Keywords *CYP1A1* genotype · Gene–environment interaction · *GSTμ1* genotype · Smoking · Renal cell cancer

The Dutch Kidney Foundation (grant number C99.1863) and the Netherlands Cancer Society financially supported this study.

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Introduction

Renal cell carcinoma (RCC) is the ninth most common tumour in the European Union [1, 2] with a worldwide incidence of 4.7 per 100,000 person years for men and 2.2 per 100,000 person years for women (http://www-dep.iarc.fr). Incidence rates rise steadily in industrialized countries [3].

Previous studies have identified smoking as a risk factor for the development of RCC [2, 4–8] with a relative risk of 1.45 for current smokers with a strong dose-dependent increase in risk [9]. In addition to environmental risk factors, several researchers have focused on molecular markers and have described several genetic polymorphisms that are potential risk factors for RCC e.g. [3, 10–15]. Among others, polymorphisms in genes that code for xenobiotic-metabolizing enzymes have been proposed as possible risk factors for RCC since these enzymes are involved in the activation of pro-carcinogenic compounds or detoxification of carcinogens [3].

CYP1A1 is a phase I enzyme that is involved in the metabolic activation of polycyclic aromatic hydrocarbons (PAHs), such as bezo[a]pyrenediolepoxide, which are found in cigarette smoke. Previous studies have described several polymorphisms in the *CYP1A1* gene, of which two (*CYP1A1*2A* and *CYP1A1*2C*) have been described extensively [16]. *CYP1A1*2A* is a T to C transition in the 3′ non-coding region of the *CYP1A1* gene. *CYP1A1*2A* causes an higher conversion of PAHs to electrophilic molecules which can react with DNA. *CYP1A1*2C* is an A to G transition in exon 7, this transition is associated with a twofold increase in microsomal activity of the CYP1A1 enzyme [16] as compared to the wildtype although not consistently [17]. *CYP1A1*2A* and *CYP1A1*2C* have been found to be associated with an increased risk of RCC [3].

Most carcinogens are detoxified by phase II enzymes such as GSTμ1. GSTμ1 metabolizes, among others, reactive epoxides of PAHs. The gene that codes for GSTμ1 has been found to be homozygously deleted in 40–50% of the Caucasian population resulting in an absence of enzyme activity. This *GSTμ1* null genotype is associated with susceptibility to several forms of cancer [3].

Since cigarette smoking and metabolic gene polymorphisms may be associated with RCC development, RCC risk may be even more increased after cigarette smoke exposure in the presence of certain genotypes. In this study, we evaluated the gene–environment interaction between *CYP1A1*2A*, *CYP1A1*2C* and *GSTμ1* null and smoking in patients with RCC. Since there are no indications that smoking behaviour is associated with *CYP1A1*2A*, *CYP1A1*2C* or *GSTμ1* null, a case-only design is an efficient method to estimate a possible gene–environment interaction. However, the main effects of *CYP1A1*2A*, *CYP1A1*2C* or *GSTμ1* null or smoking cannot be assessed in a case-only design.

Materials and methods

Study population

The Netherlands Cohort Study on diet and cancer (NLCS) is a prospective cohort study, initiated in 1986 with the enrolment of 120,852 men and women. The study design has been reported in detail elsewhere [18]. Briefly, at baseline a total of 58,279 men and 62,573 women, aged 55–69 years old, were included. All cohort members completed a self-administered questionnaire on dietary habits, lifestyle, smoking, personal and family history of cancer and demographic data at baseline. Tobacco smoking was assessed as smoking status (never, ex and current), age at first and last exposure, smoking frequency, smoking duration and cigar and pipe smoking. Information on smoking status was available for all cases. Incident cancer cases are identified by computerized record linkage with the Netherlands Cancer Registry (NCR) and PALGA, a national database of pathology reports. The method of record linkage to obtain information on cancer incidence has been described in detail previously [19]. The completeness of follow-up was estimated to be over 96%. From 1986 to 1997, 355 kidney cancer cases (ICD-O-3:C64.9) were identified within the cohort. Urothelial cell carcinomas were excluded and only histologically confirmed renal cell cancers were included (ICD-O: M8010–8119, 8140–8570), leaving 337 cases.

Tissue samples

Tumour material and healthy tissue samples of kidney cancer patients were collected after approval by the Ethical Review Board of Maastricht University, the NCR and PALGA. For 273 of the 337 eligible cases, a PALGA record with information on the location of tissue blocks was available. We were able to collect DNA material for 251 cases. All HE-stained slides were reviewed by an experienced genitourinary pathologist. Tissue collection has been described in detail elsewhere [20]. RCCs were classified according to the World Health Organization classification of tumours from 2002 [21].

For 248 out of 251 cases, *CYP1A1* and *GSTμ1* genotypes were determined. Material of three cases was additionally discarded after revision because of the fact that only material from a metastasis or a biopsy was available. We used normal tissue for 191 persons and tumour material for 57 patients since normal tissue was not available for all cases. To check if *CYP1A1* and *GSTμ1* genotypes differ in normal tissue compared to tumour tissue, we performed a pilot study and selected 40 samples for each genotype (20 from normal tissue and 20 from tumour tissue) to compare genotypes in normal tissue and tumour tissue. We observed no differences in the studied genotypes between normal tissue and tumour tissue and therefore used both tissue types for the interaction analyses. Three cases have not been genotyped due to administrative problems. As a result, 245 cases were available for further analysis.

DNA extraction and genotyping

DNA was extracted as described previously [20]. In brief, paraffin was removed with xylene and DNA was extracted by salt-precipitation. *CYP1A1* and *GSTμ1* genotypes were analyzed by restriction fragment length polymorphism polymerase chain reaction (RFLP-PCR) and single specific primer polymerase chain reaction (SSP-PCR).

CYP1A1

*CYP1A1*2A* genotype was determined using forward primer GGCCCCAACTACTCAGAGGC and reverse primer CAGTGAAGAGGTGTAGCCGCT. PCR products were digested with *Msp*I and separated by gel electrophoresis on 4% agarose gels and stained with ethidium bromide resulting in an undigested 180 bp fragment for the wildtype genotype (TT), three fragments (44, 136 and 180 bp) for the heterozygous genotype (TC) or two fragments (44 and 136 bp) for the homozygous variant (CC).

*CYP1A1*2C* genotype was determined as previously described [22]. A forward primer GGTCAACCCATCTGA GTTCC was used together with the reverse primer CCAGG AAGAGAAAGACCTCCCAGCGGGCCA. PCR products were digested with *Nco*I restriction enzymes and, separated by gel electrophoresis on 4% agarose gels and stained with ethidium bromide, resulting in an undigested 151 bp fragment for the wildtype AA genotype, three fragments (31, 120 and 151 bp) for the heterozygous genotype (AG) and two fragments (31 and 120 bp) for the GG genotype.

*GSTμ*1

GSTμ1 genotype was determined as described before by Fryer and colleagues [23] by SSP-PCR. Forward primer GCTTCACGTGTTATGGAGGTTC was used together with reverse primer: TTGGGAAGGCGTCCAAGCAC. Two additional primers for *VHL* were added as internal controls (forward: CACTGAGGATTTGGT TTT TGC and reverse TCCAGGTCTTTCTGCACATTT). PCR products were separated by gel electrophoresis on 4% agarose gel and stained with ethidium bromide. *GSTμ1* null is seen as a complete deletion of the gene and thus as a failure to amplify DNA.

*CYP1A1*2A* genotype could not be determined for four cases, *CYP1A1*2C* could be determined for all cases and *GSTμ1* could not be determined for six cases.

Statistical analyses

Data analyses were performed on 245 cases with available smoking status. Interactions between smoking, *CYP1A1*2A*, *CYP1A1*2C* or *GSTμ1* genotype and RCC risk were assessed by use of a case-only design. The association between genotype and smoking status among RCC patients was assessed with logistic regression analysis in which smoking was the dependent variable and genotype the independent variable. In this analysis, the odds ratio (OR) and corresponding 95% confidence intervals (CI) for the association between smoking status and genotype estimate the departure of the gene and environment joint effects from multiplicative interaction. In the absence of interaction, this OR is expected to be 1. Using this approach, statistical power is increased. Results were considered to be statistically significant if $P \leq 0.05$.

Smoking status contrasts in the analyses were defined as never versus ever (ex- and current-smokers) and non-current (never and ex-smokers) versus current smokers. To assess dose–response trends, analyses were performed for years of smoking (per 10 years of smoking) and the number of cigarettes smoked a day (per 5 cigarettes a day).

*CYP1A1*2A*, *CYP1A1*2C* and *GSTμ1* genotypes were combined to assess the joint effects on RCC risk. Patients with *CYP1A1*2A* wildtype (TT), *CYP1A1*2C* (AA) wildtype and presence of *GSTμ1* (heterozygous or homozygous) were considered as the reference group. Due to the low numbers of patients homozygous for *CYP1A1*2A* or *CYP1A1*2C*, these groups were combined. Patients with heterozygosity or homozygosity of *CYP1A1*2A* or *CYP1A1*2C* were considered as the variant *CYP1A1* group.

In case-only studies of interaction, analyses should be controlled for covariates that possibly influence the independence between the genetic factor and the environmental factor by including these factors in the analyses [24]. Age at baseline (years), sex, family history of RCC (yes/no), body mass index (kg/m^2), alcohol consumption (g/day), hypertension (yes/no), use of antihypertensive medication (yes/no), diabetes (yes/no), physical activity in leisure time (<30, 30–60, 60–90, >90 min/day), intake of fruit and vegetables (g/day) and pipe smoking (never, ex, current) were considered as potential confounders. The variables that were found to influence the risk estimates by more than 10% were included in the model. Confounders that were entered in the model were age at baseline, gender, BMI, alcohol consumption, physical activity, hypertension and pipe smoking. Since information of BMI was missing in several cases, we substituted the missing value by the median BMI value of the complete case group and added an indicator variable for missing values of BMI.

Results

Table 1 presents baseline characteristics for the 245 cases that were included in the analyses. The mean age of our

Table 1 Description of baseline characteristics for renal cell cancer cases, Netherlands Cohort Study on diet and cancer, 1986–1997

Total population (*N*, %)	245 (100)
Patient characteristics	
Age (mean, SD)	61.9 (3.89)
Gender (male, *N*,%)	157 (64.1)
Family history (No., *N*,%)	242 (98.8)
BMI (kg/m^2, mean, SD)	25.41 (2.89)
Alcohol (mean, SD, grams)	11.01 (14.56)
Diabetes (No., *N*, %)	236 (96.3)
Hypertension (No., *N*, %)	176 (71.8)
Antihypertensive medication (No., *N*, %)	225 (91.8)
Physical activity (<30 min/day) (*N*, %)	54 (22.4)
Physical activity (30–60 min/day) (*N*, %)	73 (30.3)
Physical activity (60–90 min/day) (N,%)	49 (20.3)
Physical activity (>90 min/day) (N, %)	65 (27.0)
Smoking information	
Never smoker (*N*, %)	64 (26.1)
Current smoker (*N*, %)	86 (35.1)
Ex-smoker (*N*, %)	95 (38.8)
Zero years of smoking (*N*, %)	64 (26.7)
One to 40 years of smoking (*N*, %)	102 (42.5)
>40 years of smoking (*N*, %)	74 (30.8)
Genotype information	
*CYP1A1*2A*	
Wildtype (TT) (*N*, %)	212 (88.0)
Heterozygote (TC) (*N*, %)	27 (11.2)
Homozygote (CC) (*N*, %)	2 (0.8)
*CYP1A1*2C*	
Wildtype (AA) (*N*, %)	172 (70.2)
Heterozygote (AG) (*N*, %)	65 (26.5)
Homozygote (GG) (*N*, %)	8 (3.3)
GSTμ1	
Present (*N*, %)	87 (36.4)
Null (*N*, %)	152 (63.6)
CYP1A1 wildtype & *GSTμ1* wildtype (*N*, %)	57 (23.8)
CYP1A1 variant[a] & *GSTμ1* wildtype (*N*, %)	30 (12.6)
CYP1A1 wildtype & *GSTμ1* null (*N*, %)	100 (41.8)
CYP1A1 variant[a] & *GSTμ1* null (*N*, %)	52 (21.8)

[a] heterozygous variant: *CYP1A1*2A* (TC), *CYP1A1*2C* (AG) & homozygous variant: *CYP1A1*2A* (CC), *CYP1A1*2C* (GG)

population was 61.9 years and the majority, 64.1%, of the patients were men. Most patients, 98.8%, did not have a family history of RCC and had not reported diabetes (96.3%) or hypertension (71.8%) at baseline. Since the homozygote variants for *CYP1A1*2A* and *CYP1A1*2C* were rare (0.8% for 2A and 3.3% for 2C), patients with homozygote and heterozygote variants for *CYP1A1* were combined in the analyses to increase power.

Table 2 presents the logistic regression results on the association between *CYP1A1* and *GSTμ1* genotype and smoking among RCC patients from the NLCS. We observed moderate departure, although not statistically significant, from multiplicative interaction between the *CYP2A1*2C* heterozygous or homozygous genotype and current versus non-current smoking; OR 1.42 (95% CI 0.70–2.89) and between *GSTμ1* null and current versus non-current smoking; OR 1.35 (95% CI 0.65–2.79). Also, for the group with both a variant in *CYP1A1* and *GSTμ1* null genotype, we observed a moderate interaction, although not statistically significant, between genotype and current versus non-current smoking; OR 1.63 (95% CI 0.63–4.24). No interaction was observed between any of the genotypes and ever versus never smoking or between the genotypes and an increment of 10 smoking years or 5 cigarettes/day.

Discussion

Polymorphisms in metabolic genes may alter the risk of cancer by activation of pro-carcinogens or detoxification of carcinogens [3]. *CYP1A1* polymorphisms and *GSTμ1* null genotype have been associated with an increased risk of several types of cancer, among which lung cancer, pancreatic cancer and colorectal cancer, although not consistently [25, 26]. Up till now, few studies have considered the influence of *CYP1A1* and *GSTμ1* genotype on RCC risk. *CYP1A1* genotype has previously been found to be associated with an increased risk of RCC [3]. *GSTμ1* genotype has not been associated with an alteration in RCC risk. However, it was suggested that *GSTμ1* genotype modified RCC risk in combination with other genotypes [3]. Since *CYP1A1* and *GSTμ1* genotype are involved in the metabolism of carcinogens in cigarette smoke, a known risk factor for RCC [9], genotype and smoking may have a synergistic effect on RCC risk.

In the present study, we evaluated a possible gene–environment interaction between *CYP1A1* and *GSTμ1* genotype and smoking in patients with RCC. We observed moderate departure from multiplicative interaction between *CYP1A1*2C* heterozygosity or homozygosity and current versus non-current smoking and between *GSTμ1* null and current versus non-current smoking. Moreover, our observations suggest an interaction between patients with both a variant in *CYP1A1* and *GSTμ1* null genotype and current versus non-current smoking. Our study implies that polymorphisms in metabolic genes might increase susceptibility to RCC, possibly by interfering with the detoxification of carcinogens present in cigarette smoke. However, none of the observed associations reached statistical significance although we observed a modest trend towards statistical significance.

Table 2 Adjusted odds ratios (OR) and corresponding 95% confidence intervals (CI) for the interaction between *CYP1A1* and *GSTμ1* genotype and smoking among renal cell cancer patients

		Never smoker (%, N)	Ever smoker (%, N)	OR[a]	*P* value	No current smoker (%, *N*)	Current smoker (%, *N*)	OR[a]	*P*value
CYP1A1*2a	Wildtype	25.5 (54)	74.5 (158)	1 (ref)		65.1 (138)	34.9 (74)	1 (ref)	
	Heterozygous or homozygous genotype	27.6 (8)	72.4 (21)	0.77 (0.22–2.71)	0.69	62.1 (18)	37.9 (11)	1.02 (0.34–3.09)	0.97
CYP1A1*2c	Wildtype	22.7 (39)	77.3 (133)	1 (ref)		62.1 (112)	34.9 (60)	1 (ref)	
	Heterozygous or homozygous genotype	34.3 (25)	65.7 (48)	0.72 (0.34–1.52)	0.39	64.4 (47)	35.6 (26)	1.42 (0.70–2.89)	0.33
GSTμ1	Present	27.6 (24)	72.4 (63)	1 (ref)		67.8 (59)	32.2 (28)	1 (ref)	
	Null	25.7 (39)	74.3 (113)	1.10 (0.49–2.44)	0.82	63.2 (96)	36.8 (56)	1.35 (0.65–2.79)	0.42
CYP1A1 GSTμ1	Wildtype Wildtype	22.8 (13)	77.2 (44)	1 (ref)[b]		66.7 (38)	33.3 (19)	1 (ref)[c]	
CYP1A1 GSTμ1	Variant Wildtype	36.7 (11)	63.3 (19)	0.53 (0.16–1.74)[b]	0.29	70.0 (21)	30.0 (9)	0.69 (0.16–2.92)[c]	0.61
CYP1A1 GSTμ1	Wildtype Null	22.0 (22)	78.0 (78)	1.00 (0.36–2.77)[b]	0.99	65.0 (65)	35.0 (35)	1.05 (0.46–2.44)[c]	0.90
CYP1A1 GSTμ1	Variant Null	32.7 (17)	67.3 (35)	0.69 (0.23–2.02)[b]	0.50	59.6 (31)	40.4 (21)	1.63 (0.63–4.23)[c]	0.31
		Increment, 10 smoking years				Increment, 5 cigarettes/day			
		OR[a]		*P* value		OR[a]			*P* value
CYP1A1*2a	Wildtype	1 (ref)				1 (ref)			
	Heterozygous or homozygous genotype	0.81 (0.23–2.85)		0.74		0.78 (0.20–3.01)			0.71
CYP1A1*2c	Wildtype	1 (ref)				1 (ref)			
	Heterozygous or homozygous genotype	0.66 (0.31–1.41)		0.29		0.76 (0.35–1.63)			0.48
GSTM1	Present	1 (ref)				1 (ref)			
	Null	1.14 (0.51–2.54)		0.75		1.00 (0.44–2.29)			0.99
CYP1A1 GSTμ1	Wildtype Wildtype	1 (ref)[d]				1 (ref)[e]			
CYP1A1 GSTμ1	Variant Wildtype	0.43 (0.13–1.45)[d]		0.17		0.51 (0.15–1.78)[e]			0.29
CYP1A1 GSTμ1	Wildtype Null	0.99 (0.36–2.73)[d]		0.98		0.88 (0.31–2.48)[e]			0.811
CYP1A1 GSTμ1	Variant Null	0.68 (0.23–2.02)[d]		0.49		0.69 (0.22–2.10)[e]			0.51

[a] Adjusted for age, gender, physical activity, alcohol, BMI, hypertension and pipe smoking

[b] *P* for trend = 0.77

[c] *P* for trend = 0.34

[d] *P* for trend = 0.79

[e] *P* for trend = 0.69

We did not observe a departure from multiplicative interaction between genotype and ever versus never smokers or between genotype and an increment of 10 years of smoking or 5 cigarettes/day. Unexpectedly, for ever versus never smoking, ORs dropped below 1. This could indicate that ever smoking is not the optimal variable to use in analyses on the association between *CYP1A1* and *GSTμ1* genotype and smoking in patients with RCC.

As a moderate, but not statistically significant, departure from multiplicative interaction was only observed in current versus non-current smokers, this could imply that in patients with a high-risk genotype, smoking is involved in tumour promotion rather than tumour initiation. Tumour promotion requires multiple exposures to the carcinogens in cigarette smoke before the development of a tumour. Hypothetically, it is possible that RCC risk in smokers is only increased among patients with both a variant in *CYP1A1* and *GSTμ1* null genotype after several recent exposures to the carcinogens from tobacco smoke. Previously, an association between RCC and the number of cigarettes smoked per day was suggested in our population [2], however, we did not observe an interaction between *CYP1A1*, *GSTμ1* genotype and an increment of 10 years of smoking or 5 cigarettes/day. It would have been interesting to evaluate the influence of *CYP1A1*2A, CYP1A1*2C* and *GSTμ1* genotype in more subgroups of smoking, such as ex-smokers. However, this was not possible in our study due to the population size.

For several types of cancer, such as lung cancer, *CYP1A1*2A*, *CYP1A1*2C* and *GSTμ1* genotype have previously been associated with an increase in the smoking-related cancer risk. Based on a review of the literature, Vineis and colleagues reported an overall RR of lung cancer in Caucasian patients with the *CYP1A1*2A* variant of 1.04 (95% CI 0.85–1.27), an RR of 1.30 (95% CI 0.89–1.90) for the *CYP1A1*2C* variant and an RR of 1.21 (95% CI 1.06–1.39) for patients with *GSTμ1* null [27].

Many genes are thought to be involved in the development of RCC or in the metabolism of carcinogens. In our study we evaluated only two genes, *CYP1A1* and *GSTμ1*. However, the choice to assess the influence of these two genes was hypothesis-driven, based on previous information that suggests an association with RCC. In addition, these genes are known to be involved in the metabolism of carcinogenic compounds such as cigarette smoke, either through activation of the carcinogen (CYP1A1) or through detoxification (GSTμ1). Previous studies have shown that polymorphisms in *CYP1A1* are functional, leading to increased *CYP1A1* inducibility and increased enzymatic activity [16]. However, Zhang et al. [17] suggested that associations between lung cancer and *CYP1A1*2C* are possibly not the result of an increased carcinogen bioactivation as they found only minor differences in kinetic behaviour between the variant CYP1A1 proteins.

GSTμ1 null genotype causes a deficient detoxification through the loss of protein expression [23, 28]. Other genes, such as *GSTP1*, are also known to detoxify reactive epoxides of PAHs [10]. We did not include these genes in our study. Possibly, additional studies including *GSTP1* could elucidate gene–environment interactions in RCC. In addition, we are aware that other *CYP1A1* variants such as *CYP1A1*4*, have previously been described in RCC [3], but since this variant has a population frequency of only 3% [11], we did not include this variant in our analyses.

In a case-only design, the assumption of independence of genotype and exposure is required for a valid interpretation of the interaction odds ratio. Although it could be hypothesized that polymorphisms in metabolic genes could influence smoking behaviour, a large study on healthy controls from the database of the International Collaborative Study on Genetic Susceptibility to Environmental Carcinogens showed no association between *CYP1A1* and *GSTμ1* genotype and smoking [29].

The important strengths of our study include the design of the study, a case-only design, which needs smaller sample sizes as compared to a case–control design. However, even using a case-only design, the population was too small to conduct subgroup analyses such as the comparison of ex-smokers and current smokers. Selection and recall bias are unlikely in our study since exposure was assessed prior to cancer diagnosis and only incident cancer cases were included. Moreover, it is unlikely that selection bias has occurred in the collection of tissue material. Since we used a case-only design to assess the magnitude of the association between smoking and *CYP1A1* and *GSTμ1* genotype in RCC, we were only able to detect departure from multiplicative interaction [30, 31]. In the epidemiologic literature, there continues to be discussion on the appropriate definition and interpretation of interaction, suggesting that especially departure from an additive model represents the true underlying model of joint effects [32, 33]. As a case-only design is only able to detect departure from multiplicative interaction, we could have missed a departure from additive interaction. Moreover, it was not possible to estimate the main effects of smoking and *CYP1A1* and *GSTμ1* genotype on RCC risk due to the case-only design.

To our knowledge, this is the first study to consider a possible interaction between *CYP1A1*2A*, *CYP1A1*2C* and *GSTμ1* genotype and smoking in patients with renal cancer. Our results suggest a possible modest interaction between *CYP1A1*2C* genotype and current smoking and between *GSTμ1* null genotype and current smoking. Also, results indicate a possible interaction between cases with both a variant in *CYP1A1* and *GSTμ1* null genotype and current smoking. These results suggest that the risk of RCC in smokers may even be more increased in the presence of the *CYP1A1*2C* heterozygous or homozygous genotype or

the *GSTμ1* null genotype. However, none of the observed associations reached statistical significance although we observed a moderate trend towards statistical significance. Results should be replicated in future, larger studies before a definite conclusion on gene–environment interactions between *CYP1A1* and *GSTμ1* genotype and smoking in RCC can be drawn.

Acknowledgments We thank Pascal Smeets for his help on laboratory and data analyses. We are indebted to the participants of this study and further wish to thank the cancer registries (IKA, IKL, IKMN, IKN, IKO, IKR, IKST, IKW, IKZ and VIKC), The Netherlands nationwide registry of pathology (PALGA) and the pathology laboratories for providing the tissue samples (for a complete list see [13]). We also thank Dr. E. Dorant, C.A. de Brouwer, prof.dr. A. Geurts van Kessel and prof.dr. D.J. Ruiter for their preparatory work for this study; H. Gorissen for the laboratory analysis, Dr. A. Volovics and Dr. A. Kester for statistical advice; S. van de Crommert, H. Brants, J. Nelissen, C. de Zwart, W. van Dijk, M. Jansen, and A. Pisters for assistance; and H. van Montfort, L. van den Bosch, and J. Berben for programming assistance.

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