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Gene-Environment Interactions in Renal Cell Carcinoma

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The majority of renal cell carcinomas (RCCs) are sporadic, and increasing incidence rates suggest that such environmental risk factors as smoking play a role in the etiology of the disease. Cases with RCC were selected from the population-based cancer registry of Orange County, California, between 1994 and 1997; controls were recruited by telephone using random digit dialing. A total of 115 case and 259 control subjects were genotyped for *N*-acetyltransferase 2 (*NAT2*), which codes for a polymorphic enzyme involved in tobacco-carcinogen metabolism. Subjects with slow acetylator genotypes were found to be at twofold increased risk (odds ratio (OR) = 1.8; 95 percent confidence interval (CI): 1.1, 2.9) of RCC. Although cancer risk doubled among smokers (OR = 2.2; 95 percent CI: 1.3, 3.7), stratified analysis revealed gene-environment interaction among slow acetylators that smoked (OR = 3.2; 95 percent CI: 1.7, 6.1) compared with rapid acetylators that smoked (OR = 1.4; 95 percent CI: 0.7, 2.9). A dose response was found for pack-years among slow acetylators ($p < 0.01$) but not among rapid acetylators ($p = 0.06$). Although smoking is a well-established risk factor of RCC, our data suggest that the risk is pronounced among slow rather than rapid acetylators. *Am J Epidemiol* 2001;153:851–9.

acetyltransferases; hypertension; kidney neoplasms; smoking

Renal cell carcinoma (RCC) is relatively rare, accounting for approximately 3 percent of adult malignancies, but incidence and mortality have increased internationally over the last 20 years and the reasons for this increase are unclear (1). RCC occurs at a 2:1 male:female ratio, and several environmental, medical, and genetic risk factors have been implicated in the etiology of this disease (2). Tobacco smoke is a major risk factor, doubling the risk of RCC in both men and women (3–8). There is a direct dose-response relation between pack-years of cigarette use and RCC risk, while smoking cessation results in a decline of RCC risk (9). It is possible that renal concentration and biotransformation of tobacco carcinogens are responsible for the increased risk of RCC among smokers; however, a toxicologic mechanism has not been elucidated. Obesity, hypertension, and analgesic or diuretic abuse are also associated with the likelihood of RCC, a finding particularly pronounced in women. The reasons for RCC susceptibility among diuretic and analgesic users are unclear, but they could be due to drug metabolism in the kidney, analogous to tobacco carcinogen metabolism. Family history of RCC is an established risk factor, and the von

Hippel-Lindau tumor suppressor gene associated with this familial cancer syndrome has been isolated (10). However, most RCCs are sporadic, and environmental agents are suspected to play a role in the etiology of the disease.

The objective of our study was to determine the extent of genetic susceptibility in RCC and to account for gene-environment interactions. We conducted a case-control study and analyzed the role of *N*-acetyltransferase 2 (*NAT2*) in RCC. *NAT2* was selected as a genetic susceptibility marker because this enzyme is polymorphic in a large proportion of the population and because it is involved in the metabolism of drugs and carcinogens, such as arylamines, from tobacco smoke (11). The polymorphic variant of *NAT2*, classified as slow acetylator genotype, affects *NAT2* enzyme activity, which in turn reduces the detoxification capacity of *NAT2* substrates (12). Thus, we hypothesized that slow acetylators are at increased risk of RCC if they smoke.

MATERIALS AND METHODS

Study population

We conducted a case-control study of histologically confirmed, newly diagnosed cases of RCC (*International Classification of Diseases for Oncology* code C64.9) identified through the population-based cancer registry of Orange County, California. The inclusion criteria required that cases were alive and resident in Orange County at the time of contact, had been diagnosed between 1994 and 1997, and were in the age group 20–74 years. Childhood cancers, such as Wilms' tumor, were therefore excluded. The Institutional Review Board (IRB) at the University of California at Irvine (UCI) approved the study proposal as well as the manner in

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Abbreviations: CI, confidence interval; *NAT2*, *N*-acetyltransferase 2 (enzyme); *NAT2*, *N*-acetyltransferase 2 (gene); OR, odds ratio; RCC, renal cell carcinoma.

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which informed consent was obtained from subjects (UCI, IRB protocol 96-496). Of 313 persons that met the inclusion criteria, 189 (60 percent) agreed to participate and we interviewed 188 cases, of whom 115 (61 percent) donated a blood sample that was successfully genotyped for *NAT2*. Controls who were between 20 and 74 years of age and who had no history of cancer were randomly selected from Orange County residents using random digit dialing methodology (98 percent of households have a phone in Orange County, California). Of 427 subjects that agreed to participate in the study and to donate either a blood sample or a cheek cell sample, we analyzed and genotyped 259 (61 percent) controls with a blood sample.

Cases and controls were interviewed on the phone using a standardized instrument to determine demographic variables, tobacco use (including cigarettes, cigars, pipe, and chewing tobacco), occupational and environmental exposures, and medical history. Smokers were defined as having smoked at least 100 cigarettes in their lifetime, but we also recorded the age when smoking started/stopped, average number of cigarettes smoked per day, type of cigarettes (filtered, unfiltered), and other type of tobacco use (cigars, pipe, and chewing tobacco). The medical history interview included cancers (with age at diagnosis) and medical conditions that are known risk factors of RCC, such as hypertension, thyroid disease, diabetes, and gallstones, of proband and next of kin. Among cases, self-reported hypertension was defined as a condition diagnosed 5 years prior to the cancer diagnosis and among controls, 5 years prior to the interview. Study participants were requested to donate a blood sample for genetic analysis.

Laboratory assays

DNA was extracted from peripheral blood lymphocytes using established methods and frozen at -80°C until use. The most common functional and low activity allelic variants of *NAT2* were included in this analysis: wild type (wt), M1, M2, M3, and M4, corresponding to *NAT2*4*, *NAT2*5A*, *NAT2*6A*, *NAT2*7A*, and *NAT2*14A* (12). A specific set of primers was used to amplify a fragment that contained all four polymorphic alleles. The primers *NAT2A* (5'-GGA ACA AAT TGG ACT TGG) and *NAT2B* (5'-TCT AGC ATG AAT CAC TCT GC) were used at a concentration of 50 pmol, with 200 μmol of dinitrophenol, polymerase chain reaction buffer, and 0.5 unit of *Taq* polymerase (Gibco, Inc., Grand Island, New York). The genomic DNA concentration was adjusted to 200 ng per 50 μl of polymerase chain reaction buffer. The polymerase chain reaction was conducted in a thermocycler (MJ Research, Waltham, Massachusetts) under the following conditions: 94°C for 30 seconds, 54.3°C for 30 seconds, and 72°C for 30 seconds for 35 cycles. The restriction endonucleases *KpnI*, *TaqI*, *BamHI*, and *MspI/AluI* (New England Biolabs, Beverly, Massachusetts) were used to digest 10 μl of the amplification product in the presence of appropriate buffers to determine the presence of *NAT2*4*, *NAT2*5A*, *NAT2*6A*, *NAT2*7A*, and *NAT2*14A* polymorphic sites, respectively. Slow acetylators were defined as having at least two poly-

morphic alleles (*NAT2*5A*, *NAT2*6A*, *NAT2*7A*, and *NAT2*14A*) of *NAT2*.

The restriction digests were separated using a 3:1 NuSieve (BioWhittaker Molecular Applications, Rockland, Maine):agarose gel, stained with ethidium bromide, and photographed under ultraviolet illumination. The presence or absence of differently sized bands was scored blindly and independently by two researchers to determine the genotypes. Duplicate amplification was performed for quality control.

Statistical methods

Univariate unconditional logistic regression models were generated using standard SAS programs to estimate odds ratios and 95 percent confidence intervals (13). Statistical significance was attributed to p values of <0.05 for two-sided t statistics, and significant predictors were included in a multivariate model. The effects of *NAT2* genotype and exposure to smoking on the risk of RCC were examined in multivariate unconditional logistic regression models that controlled for suspected risk factors that may confound and/or modify the effects of interest (14). These risk factors include sex, age at diagnosis, race/ethnicity, educational level, occupation, and medical conditions. The exposure variables, such as smoking, were analyzed as continuous variables and as categorical variables by quartiles and by tertiles based upon the distribution of the exposure variables in controls. The effect of the exposure variables and *NAT2* genotype on the risk of RCC was first assessed separately in bivariate models and then controlling for confounding variables. Confounding was assumed if the parameter estimate for *NAT2* or the exposure variable changed by at least 15 percent. To assess the possibility of effect modification, the effect of exposure variables was examined within the two strata of the *NAT2* metabolic genotype (rapid and slow acetylators) and by sex. Interactions between the *NAT2* genotype and exposure variables were evaluated on a multiplicative scale with a product-term coefficient in logistic regression models. We also used dummy variables to compare nonsmoking rapid acetylators with nonsmoking slow acetylators, smoking rapid acetylators, and smoking slow acetylators in a multivariate model. Rothman's synergy index (S) was calculated to determine a relative scale of measurement of the interaction of *NAT2* and smoking as a risk factor of RCC (15, 16). We calculated S as follows:

$$S = \frac{RR(AB) - 1}{RR(\overline{A}\overline{B}) + RR(\overline{A}B) - 2}$$

where the rate ratio (RR) of the interaction [RR (AB)] between slow acetylators (A) and smoking (B) minus one is divided by the sum of the main effects in the absence of the other minus two.

RESULTS

Of 445 blood samples that were available for genetic analysis, we successfully genotyped 374 (84 percent). There were no differences ($p > 0.05$) between the genotyped and

nongenotyped subjects with respect to age, race, ethnicity, education, or smoking status. However, there was a difference with respect to sex ($p = 0.03$), but among those tested the distribution of rapid and slow acetylator genotype did not differ by sex ($p = 0.6$). The demographic composition of the study population is presented in table 1. The mean age of cases was 54.7 years compared with 53.7 years among controls. Our study population was predominantly Caucasian, and 77 percent of cases and 82 percent of controls worked in “white collar” occupations, reflecting the population of Orange County, California. The majority (82 percent) of slow acetylators among cases and controls carried two combinations of low activity alleles of *NAT2*:

(*NAT2*5A/NAT2*5A* and *NAT2*5A/NAT2*6A*) as presented in table 2. Sixty-three percent of cases were classified as slow acetylators compared with 48 percent of controls.

NAT2 and smoking

Multivariate unconditional logistic regression analysis revealed a twofold increased risk (odds ratio (OR) = 1.8; 95 percent confidence interval (CI): 1.1, 2.9) of RCC for slow acetylators, controlling for sex, age, race/ethnicity, and other risk factors (table 3).

Because there were more female controls and more male cases, we stratified by sex and found that the risk of RCC was evident in women with a slow acetylator genotype (OR = 2.0; 95 percent CI: 1.1, 4.0), whereas in men with a slow acetylator genotype the risk of RCC was not significantly elevated (table 4). In controls, the distribution of the genetic variants was identical in both sexes (48 percent of slow acetylators), whereas it differed among cases for women and men (69 percent and 55 percent of slow acetylators, respectively).

Our observation that metabolically slow acetylators are at increased risk of RCC suggests that susceptible individuals could experience an altered risk from environmental exposures. Tobacco use has been established as a risk factor of RCC, and since tobacco carcinogens are metabolized by *NAT2*, we assessed the importance of smoking in the context of acetylator genotype. In our population, cases were more likely than were controls to smoke (68 percent of cases and 46 percent of controls smoked at least 100 cigarettes in their lifetime), reflected in a twofold increased risk (OR = 2.2; 95 percent CI: 1.3, 3.7) for RCC among smokers compared with nonsmokers (table 3).

To assess the association between smoking and *NAT2* genotype, we stratified by slow and rapid acetylators. Smoking-related variables are listed in table 5. We found subjects classified as smokers with a slow acetylator genotype to be at threefold (OR = 3.2; 95 percent CI: 1.7, 6.1) increased risk of RCC, whereas the risk for rapid acetylators who smoked was not significant (OR = 1.4; 95 percent CI: 0.7, 2.9) (table 5). We also evaluated whether the risk of intensity and duration of tobacco use was modified by *NAT2* genotype. A statistically significant dose response for the average number of cigarettes smoked per day (intensity) was found for the slow acetylators (p for trend < 0.01), although not for the rapid acetylators (p for trend < 0.16). Similarly, duration of smoking increased the risk in a dose-response manner for slow acetylators. A duration of less than 20 years of smoking was associated with a threefold increased risk, whereas a duration of greater than 20 years was associated with a fourfold increased risk. Years of smoking increased the risk of RCC for slow acetylators among both women and men ($p = 0.006$). The risk of smoking duration was modified by *NAT2* genotype, reflected by the fact that the dose response was significant among slow acetylators ($p < 0.01$) but not among rapid acetylators ($p = 0.11$).

The mean pack-years of cigarettes smoked were 15.0 (standard deviation, 22.7; range, 0–102) among slow acetylators and 13.4 (standard deviation, 22.9; range, 0–108) among rapid acetylators (not statistically different, $p =$

TABLE 1. Demographic characteristics of cases and controls, Orange County RCC* Study, 1994–1997†

	Cases (n = 115)		Controls (n = 259)	
	No.	%	No.	%
Sex				
Female	49	43	145	56
Male	66	57	114	44
Race/ethnicity				
White	98	85	205	79
African American	3	3	4	2
Hispanic	8	7	33	13
Asian	4	3	7	3
Other	2	2	9	3
Mean age				
Years (SD*)	54.7 (12.6)		53.7 (13)	
Education				
≤10th grade	4	4	11	4
11th–12th grade	40	35	53	21
College	51	45	147	57
Postgraduate and professional school	8	14	45	18
Vegetarian				
No	118	98	241	96
Yes	2	2	11	4
Self-reported conditions				
Hypertension‡	32	28	32	12
Gallstones	17	15	16	6
Thyroid disease	15	13	16	6
Diabetes§	5	4	6	2
Family history of kidney cancer¶	2	2	2	1

* RCC, renal cell carcinoma; SD, standard deviation.

† Missing data are due to incomplete questionnaire information.

‡ Hypertension, “high” defined as self-reported onset of high blood pressure 5 years prior to cancer diagnosis (cases) or interview (controls).

§ Diabetes, self-reported insulin and non-insulin-dependent diabetes.

¶ Family history of kidney cancer, self-reported maternal and/or paternal kidney cancer.

TABLE 2. Allele frequency of NAT2* for cases and controls, Orange County RCC* Study, 1994–1997

Genotype†	Cases (n = 115)		Controls (n = 259)		Total (no.)
	No.	%	No.	%	
Slow acetylators‡					
NAT2*5A/NAT2*5A	21		45		66
NAT2*6A/NAT2*6A	7		8		15
NAT2*7A/NAT2*7A	0		0		0
NAT2*14A/NAT2*14A	2		0		2
NAT2*5A/NAT2*6A	35		61		96
NAT2*5A/NAT2*7A	1		7		8
NAT2*5A/NAT2*14A	1		0		1
NAT2*6A/NAT2*7A	5		4		9
NAT2*6A/NAT2*14A	0		0		0
NAT2*7A/NAT2*14A	0		0		0
Subtotal	72	62	125	48	197
Rapid acetylators					
NAT2*4 (wt)/NAT2*4 (wt)*	6		24		30
NAT2*4 (wt)/NAT2*5A	26		57		83
NAT2*4 (wt)/NAT2*6A	11		46		57
NAT2*4 (wt)/NAT2*7A	2		7		9
NAT2*4 (wt)/NAT2*14A	0		0		0
Subtotal	45	39	134	52	179

* NAT2, N-acetyltransferase 2 (gene); RCC, renal cell carcinoma; NAT2*4 (wt), wild-type alleles where polymorphic variants are absent.

† Functional and low activity alleles of NAT2 include wt, M1, M2, M3, and M4, corresponding to NAT2*4, NAT2*5A, NAT2*6A, NAT2*7A, and NAT2*14A. Rapid acetylators were defined as having ≤ 1 low activity allele, and slow acetylators were defined as having ≥ 2 low activity alleles of NAT2.

‡ Two cases with slow acetylator genotype carried the NAT2*14A/NAT2*14A variant, as well as the NAT2*5A/NAT2*6A variant.

0.50). Although the twofold increased risk of RCC from smoking in our population (table 3) is consistent with results from other epidemiologic studies, our molecular data suggest that this risk is mainly confined to slow acetylators.

With the goal of exploring the nature of this gene-environment interaction, we compared nonsmoking rapid acetylators with three other combinations of genotype and tobacco exposure (table 6). The risk for nonsmoking slow

TABLE 3. Multivariate unconditional logistic regression model for NAT2* genotype and other risk factors, Orange County RCC* Study, 1994–1997†

Variable	Cases (n = 115)		Controls (n = 259)		Overall	
	No.	%	No.	%	OR*	95% CI*
Acetylators, rapid	45	39	132	52		
Acetylators, slow	70	61	123	48	1.8	1.1, 2.9
Nonsmokers	37	32	139	54		
Smokers‡	78	68	116	46	2.2	1.3, 3.7
Normotension	83	74	223	87		
Hypertension	32	26	32	13	2.4	1.3, 4.3
Gallstones, no	96	85	239	94		
Gallstones, yes	17	15	16	6	2.6	1.2, 5.7
Age (mean years (SD*))	54.7	(12.6)	54	(13.3)	1.0	0.9, 1.0
Sex, male	66	57	114	44		
Sex, female	49	43	145	56	0.6	0.4, 1.0
Race, non-White§	17	15	52	20		
Race, White	98	85	203	80	0.9	0.5, 1.7

* NAT2, N-acetyltransferase 2; RCC, renal cell carcinoma; OR, odds ratio; CI, confidence interval; SD, standard deviation.

† Missing data are due to incomplete questionnaire information.

‡ Smokers, defined as having smoked at least 100 cigarettes in lifetime.

§ Race, reference group non-White: African American, Hispanic, Asian American, and "other."

TABLE 4. Risk of renal cell carcinoma by NAT2* polymorphisms and sex, Orange County RCC* Study, 1994–1997

NAT2	Cases (n = 115)		Controls (n = 259)		Total (n = 374)		OR*,†	95% CI*
	No.	%	No.	%	No.	%		
<i>Women</i>								
Rapid acetylators‡	15	31	75	52	90	46	1.0	
Slow acetylators	34	69	70	48	104	54	2.0	1.1, 4.0
<i>Men</i>								
Rapid acetylators‡	30	45	59	52	89	49	1.0	
Slow acetylators	36	55	55	48	91	51	1.3	0.7, 2.4

* NAT2, N-acetyltransferase 2 (gene); RCC, renal cell carcinoma; OR, odds ratio; CI, confidence interval.

† The odds ratio and the 95% confidence interval were determined through unconditional logistic regression, controlling for age and race/ethnicity.

‡ The rapid acetylators were used as a reference category.

acetylators was not statistically significant in either men or women and was only marginally elevated in women when compared with nonsmoking rapid acetylators. Similarly, the risk of RCC among female and male smokers with a rapid acetylator genotype was not statistically significant in our model (although males experienced a nonsignificant twofold increased risk) when compared with nonsmoking rapid acetylators. In contrast, when smokers with a slow acetylator genotype were compared with this referent group, we found an odds ratio of 3.3 (95 percent CI: 1.2, 9.2) for women and an odds ratio of 3.7 (95 percent CI: 1.4, 9.5) for men, controlling for hypertension, age, and race/ethnicity. This statistically significant gene-environment interaction can also be calculated using the synergy index as a relative scale of measurement for an interaction. Because of the established difference in risk by sex, we performed a stratified analysis and calculated the extent of interaction for women and men independently. The extent of effect modification by NAT2 was particularly pronounced among women who smoked. The synergy index to measure the interaction between smoking and NAT2 in RCC was calculated to be 15.7 in women, indicating a strong positive interaction. In men the effect modification was not as pronounced as in women, with a positive synergy index of 2.1.

NAT2 and medical conditions

Because NAT2 is also involved in the metabolism of drugs, including diuretics, we investigated the role of polymorphic variants of NAT2 on self-reported conditions, such as high blood pressure and diabetes. Twenty-eight percent of the RCC cases suffered from hypertension (diagnosed 5 years prior to the cancer diagnosis) compared with 12 percent of the controls (diagnosed 5 years prior to the interview) (table 1). In the multivariate model presented in table 3, hypertension was associated with a twofold increased risk (OR = 2.4; 95 percent CI: 1.3, 4.3) of RCC controlling for age, sex, and race/ethnicity. Because women are more likely to develop cholecystitis and gallstones and to smoke less than men, we conducted a stratified analysis by sex and

found that women are at a fivefold increased risk of RCC if they suffer from high blood pressure. We also calculated the synergy index as a relative scale of measurement for the interaction of hypertension and slow acetylator genotype. In women the synergy index was 27.4, indicating a strong interaction, whereas in men the modifying effect of NAT2 on RCC risk from hypertension was not as pronounced (synergy index = 3.3).

A separate analysis of controls only, to reflect a random sample of Orange County, confirmed the role of NAT2 as an independent risk factor for high blood pressure. Among controls we found that the slow acetylator genotype increased the risk of high blood pressure threefold (OR = 2.7; 95 percent CI: 1.2, 6.0), controlling for age, sex, and race/ethnicity. Again, this risk was particularly pronounced in women (OR = 24.1; 95 percent CI: 2.8, 206.4).

DISCUSSION

These results indicate that subjects with slow NAT2 genotypes, in particular women, are at increased risk of RCC. Moreover, the NAT2 genotype modifies the risk from tobacco use, which is reflected in a threefold increased risk from smoking among slow acetylators but a nonsignificant 40 percent increased risk among rapid acetylators. Similarly, hypertension was found to be a risk factor of RCC, particularly among women with a slow acetylator genotype.

Smoking and NAT2

Several case-control and cohort studies have examined tobacco smoke exposure as a risk factor of renal cancer, and the twofold increased risk among smokers relative to nonsmokers in our case-control study is consistent with results from other epidemiologic studies (3–8, 17–21). The intensity and duration of tobacco use affected the RCC risk, and the observed dose-response relation for both intensity and duration is also consistent with the relation from other studies (3, 7, 17, 20, 21).

TABLE 5. Risk of renal cell carcinoma by smoking characteristics and NAT2* genotype, Orange County RCC* Study, 1994–1997†

Characteristic	Slow acetylators						Rapid acetylators					
	Cases (n = 70)		Controls (n = 125)		OR*,‡	95% CI*	Cases (n = 45)		Controls (n = 134)		OR‡	95% CI
	No.	%	No.	%			No.	%	No.	%		
Nonsmokers	19	27	69	55	1.0		18	40	70	52	1.0	
Smokers§	51	73	56	45	3.2	1.7, 6.1	27	60	64	48	1.4	0.7, 2.9
No. of cigarettes smoked per day												
0	19	27	69	55	1.0		18	40	70	52	1.0	
1–20	19	27	27	22	2.5	1.1, 5.4	11	24	37	28	1.1	0.5, 2.6
>20	31	44	27	22	3.7	1.8, 7.8	15	33	26	20	1.7	0.7, 4.0
<i>p</i> for trend					<0.01						0.16	
Mean (SD*)	19.1 (14.4)		16.2 (11.3)				18.3 (15.0)		17.3 (15.4)			
Total duration of smoking (years)												
0	19	27	72	58	1.0		18	40	70	53	1.0	
1–20	12	17	15	12	3.0	1.2, 7.6	5	11	24	18	0.8	0.3, 2.3
>20	38	54	33	26	4.0	2.0, 7.9	21	47	37	28	1.8	0.8, 3.9
<i>p</i> for trend					<0.01						0.11	
Mean (SD)	21.2 (17.0)		10.6 (15.9)				18.6 (17.8)		11.6 (15.9)			
Age smoking began (years)												
Mean (SD)	17.2 (3.8)		18.1 (4.6)				16.4 (3.1)		17.8 (3.6)			
Pack-years smoked												
0	19	27	72	58	1.0		18	40	70	52	1.0	
1–20	19	27	27	22	2.6	1.2, 5.7	8	8	36	27	0.8	0.3, 2.1
>20	31	44	23	18	4.6	2.2, 9.8	18	40	24	18	2.4	1.1, 5.4
<i>p</i> for trend					<0.01						0.06	
Mean (SD)	14.6 (22.5)		9.5 (17.7)				213.2 (22.8)		10.7 (21.0)			

* NAT2, N-acetyltransferase 2; RCC, renal cell carcinoma; OR, odds ratio; CI, confidence interval; SD, standard deviation.

† Missing data are due to incomplete questionnaire information.

‡ All categories adjusted for age, race/ethnicity, and sex.

§ Smokers, defined as having smoked at least 100 cigarettes in lifetime.

TABLE 6. Interaction of smoking and NAT2* genotype by sex, in a multivariate unconditional logistic regression model, Orange County RCC* Study, 1994-1997†

Variable	Women				Men					
	Cases (n = 49)		Controls (n = 145)		Cases (n = 66)		Controls (n = 114)			
	No.	%	No.	%	No.	%	No.	%		
Nonsmoking, rapid acetylators	9	18	40	28	9	14	30	26	1.0	95% CI
Nonsmoking, slow acetylators	12	24	41	28	7	11	28	25	0.9	0.3, 2.8
Smoking,§ rapid acetylators	6	12	35	24	21	32	29	25	2.4	0.9, 6.2
Smoking, slow acetylators	22	45	29	20	29	44	27	24	3.7	1.4, 9.5
Hypertension	14	28	13	9	18	27	19	17	1.7	0.8, 3.7

* NAT2, N-acetyltransferase 2; RCC, renal cell carcinoma; OR, odds ratio; CI, confidence interval.

† Missing data are due to incomplete questionnaire information.

‡ Adjusted for age and race/ethnicity.

§ Smokers, defined as having smoked at least 100 cigarettes in lifetime.

To elucidate the metabolic basis of the increased risk among smokers, we analyzed polymorphic variants at the NAT2 locus that affect the metabolism of tobacco carcinogens. Arylamines from cigarette smoke are known bladder carcinogens in both animals and humans (22). Arylamines are metabolically activated to electrophilic compounds in the liver through N-hydroxylation by cytochrome P450 IA2. If these metabolites such as N-hydroxyarylamines are not detoxified by N-acetyltransferases, they can react with urothelial DNA (11).

There are two acetyltransferases that have a broad substrate specificity (23): N-acetyltransferase 1, which acetylates p-aminobenzoic acid; and NAT2, which acetylates sulfmethazine. Aromatic amines found in dyes, pesticides, and tobacco smoke are detoxified and bioactivated by NAT2; this study focuses on NAT2 because of its substrate specificity. The genetic basis for variation in phenotypic activity has been established for both genes (24, 25), and genetic variations in different ethnic groups have been documented (26).

A recent meta-analysis of 22 case-control studies found that slow acetylators are at increased risk of bladder cancer compared with rapid acetylators, particularly if they smoked (27), but no difference was found for rapid and slow acetylators with lung cancer (28). Postmenopausal women who are slow acetylators have been found to be at risk of breast cancer if they smoke (29). In contrast, rapid acetylators were found to be at increased risk of colon cancer (30, 31). Our study indicates that slow acetylators, particularly women, are at increased risk of RCC. Moreover, slow acetylators are at increased risk if they smoke, suggesting a similar mechanism of carcinogenesis as discussed above for bladder cancer. The impact of smoking on RCC risk is greater in men compared with women. Recently, a French study comparing 173 hospital patients with sporadic RCC and 211 healthy controls from a preventive medicine clinic documented a twofold increased risk of RCC among slow acetylators, a finding that is consistent with our results; however, that study did not include an analysis of gene-environment interactions (32).

Our data suggest that NAT2 has a strong etiologic role in the carcinogenic process for RCC resulting from smoking. They indicate that the effect of smoking is modified by the NAT2 genotype and that slow acetylators are at increased risk of RCC if they also smoke.

Self-reported conditions and NAT2

Several self-reported conditions were overrepresented in the RCC population. Among these medical conditions, hypertension increased the risk of RCC twofold and even more in women with a slow acylator genotype. High blood pressure has been identified as a risk factor of RCC in epidemiologic studies (20). However, it is possible that this association can be explained in part by the use of hypertension medication. Slow acetylators are at increased risk of toxic side effects from antihypertensive agents, such as hydralazine (see below), known to be metabolized by NAT2, reducing its mutagenic potential (33). These experimental findings are consistent with our epidemiologic observations.

Since NAT2 is involved in the metabolism of tobacco carcinogens as well as diuretics and analgesics, it is possible that the observed risk from hypertension and the slow acetylator genotype is a consequence of drug use. Diuretics and analgesics have been shown to increase the risk of RCC (7, 20, 21). The aromatic amine phenacetin (4-ethoxyacetanilide) is a widely used analgesic that has been found to be carcinogenic for the kidney (7, 21). It appears that chronic administration of phenacetin (or aspirin) damages the loop of Henle in the medulla of the kidney. Slow acetylators deactivate phenacetin at a decreased rate (34, 35), which could explain the observed increased risk among slow acetylators. NAT2 is also involved in the metabolism of diuretics such as minoxidil, hydralazine, and the arylamine-containing sulfonamide, and the slow acetylator phenotype has been identified as a risk factor for sulfonamide hypersensitivity (36–38). Use of diuretics has increased substantially in the United States, particularly among the elderly, and could be responsible in part for the increased incidence of RCC (39).

Limitations

Study subjects were recruited using a population-based approach (population-based cancer registry and random digit dialing). However, because biologic samples were required for genotyping, the requirement of obtaining a blood sample could have negatively affected our participation rates and thus could have resulted in selection bias. Because women were more likely than men to participate as controls, we performed a stratified analysis by sex. Among female and male controls, the frequencies of rapid and slow acetylator genotypes were found to be the same; it is therefore also likely that genotyped and nongenotyped subjects were similar with regard to genotype distribution, although these two populations could have differed with regard to other variables (such as exposure). During the collection of questionnaire data, every attempt was made to minimize recall bias, but nevertheless, it is possible that this case-control study could suffer from information bias.

We are currently in the process of analyzing other genes that encode carcinogen-metabolizing enzymes from the glutathione *S*-transferase and cytochrome P-450 families with the goal of complementing this analysis with gene-gene interactions.

Conclusion

Our data suggest that NAT2 is an underlying susceptibility marker for RCC that can exacerbate the cancer risk in combination with other risk factors. There is a strong interaction between the NAT2 genotype and both tobacco use and high blood pressure, possibly due to tobacco carcinogen and drug metabolism by NAT2.

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