Candidate Genetic Modifiers of Individual Susceptibility to Renal Cell Carcinoma: A Study of Polymorphic Human Xenobiotic-metabolizing Enzymes

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ABSTRACT

The steady increase in sporadic renal cell carcinoma (RCC) observed in industrialized countries supports the notion that certain carcinogens present in the environment (tobacco smoke, drugs, pollutants, and dietary constituents) may affect the occurrence of RCC. Many of the enzymes dealing with such environmental factors are polymorphic and may, therefore, confer variable susceptibility to RCC.

This case-control study was designed to test for an association between genetic polymorphism of enzymes involved in xenobiotic metabolism and the risk of sporadic RCC. Genomic DNA was obtained from 173 patients with RCC and 211 controls of Caucasian origin. We used PCR-RFLP to investigate polymorphism for the most common alleles at two cytochrome-P450 mono-oxygenases (CYP1A1 and CYP2D6), one NAD[P]H:quinone oxidoreductase (NQO1), three glutathione S-transferases (GSTM1, GSTT1, and GSTP1), and one N-acetyltransferase (NAT2) loci.

The CYP1A1 (m) “variant” genotype, which contains at least one copy of the CYP1A1 variant alleles, was found to be associated with a 2.1-fold [95% confidence interval (CI), 1.1–3.9] increase in the risk of RCC. There was also a higher risk of RCC for subjects with the CYP1A1 (m) variant genotype combined with any of the following genotypes: GSTT1 (+) “active” [odds ratio (OR), 2.3; 95% CI, 1.2–4.5], GSTP1 (m) variant (OR, 2.4; 95% CI, 1.0–5.4), or NAT2 (+) “slow acetylator” (OR, 2.5; 95% CI, 1.1–5.5). A significant association was also found for the GSTM1 (-) “null” and GSTP1 (m) genotypes combined with either NAT2 (-) (OR, 2.6; 95% CI, 1.2–5.8) or CYP1A1 (m) (OR, 3.5; 95% CI, 1.1–11.2). The CYP2D6 (-) “poor metabolizer” and the NQO1 (+) “defective” genotypes were not clearly associated with a higher risk of RCC.

Our data demonstrate for the first time a significant association between a group of pharmacogenetic polymorphisms and RCC risk. These positive findings suggest that interindividual variation in the metabolic pathways involved in the functionalization and detoxification of specific xenobiotics is an important susceptibility factor for RCC in Caucasians.

INTRODUCTION

RCC1 accounts for 80–85% of malignant renal tumors in adults (1). Etiological studies can be used to distinguish between common sporadic (95% of cases) and rare familial forms of RCC (2). In both cases, several chromosome abnormalities have been described (3), mostly the 3p25–26 region, in which the von Hippel-Lindau disease tumor suppressor gene resides (4).

RCC is the 8th most common tumor in men and the 11th most common tumor in women (5), and its incidence (1–12 per 100,000) is rising steadily, by about 2–3% per year in industrialized countries (1). The pathogenesis of RCC is not understood, but its increasing incidence in recent years may be related to higher levels of exposure to certain risk factors. Epidemiological studies have shown that dietary and environmental factors may be involved in the development of sporadic RCC (6). Of these, compounds from tobacco smoke (7) may be involved in one-third of all cases, but the overall impact of such factors is unclear (8). Other suspected risk factors include excess body weight (9), high-protein diet (10, 11), and the use of diuretic and antihypertensive drugs (12). Occupational factors, such as employment in the coke-oven industry or exposure to petroleum products or dry-cleaning solvents, may also increase the risk of RCC (13).

Environmental risk agents, such as chemical procarcinogenic compounds, require metabolic activation by the oxidative (Phase I) enzymes (mainly CYP enzymes) to be transformed into potentially carcinogenic forms. Furthermore, most carcinogens are detoxified by Phase II conjugating enzymes. Thus, genetic polymorphism at loci encoding these xenobiotic-metabolizing enzymes may result in interindividual variation in susceptibility to the carcinogenic effects of environmental chemicals (14, 15). In this study, we tested whether polymorphism in genes encoding Phase I enzymes [such as CYP1A1 and CYP2D6 (EC 1.1.1.14.1.1) and NQO1 (EC 1.6.99.2)] and phase II enzymes [such as GSTM1, GSTT1, and GSTP1 (EC 2.5.1.18.), and NAT2 (EC 2.3.1.5.)] affected susceptibility to sporadic RCC. These enzymes, involved in the metabolism of substances that are thought to be carcinogenic, are believed to participate in a variety of tumor formation processes.

CYP1A1 activates polycyclic aromatic hydrocarbons, such as those found in cigarette smoke. Four-point mutations have been detected in the CYP1A1 gene, resulting in four variant alleles: CYP1A1*2A, CYP1A1*2B, CYP1A1*3, and CYP1A1*4. Two alleles (CYP1A1*2A and CYP1A1*2B) are associated in vitro with a highly inducible phenotype and have been identified as risk factors for lung cancer in Japanese (16) and, to a lesser extent, in Caucasian individuals (17).

CYP2D6 metabolizes many clinically important drugs and procarcinogens, such as a tobacco-specific nitrosamine, 4-(methylnitrosamo)-1-(3-pyridyl)-1-butane. The CYP2D6 locus is highly polymorphic with PM and extensive metabolizer phenotypes. The PM phenotype is inherited as an autosomal recessive trait and occurs in 5–10% of Caucasians. It is not clear whether there is an association between the CYP2D6 polymorphism and higher risk of developing various diseases, including lung and bladder cancers (18, 19).

NQO1 catalyzes the two-electron reduction of quinoid compounds to hydroquinones, thereby providing protection against a variety of xenobiotics while activating certain antitumor quinones. The NQO1 locus is polymorphic, with an allele coding for an inactive enzyme (20). Low levels of NQO1 activity have been associated with carcinogenesis and resistance to chemotherapy agents (21).

The ubiquitous GSTs metabolize a large variety of substrates, including a number of electrophilic molecules, such as arylepoxides.
There is a large overlap in the substrate specificities of GSTs. The \( \mu \) class GSTM1 enzyme detoxifies reactive metabolites such as smoke-derived epoxides of polycyclic aromatic hydrocarbons, including BPDE. Forty to 50% of Caucasians have a homozygous deletion of \( \text{GSTM1} \) (\( \text{GSTM1}^{\ast 0/\ast 0} \)) genotype. Conflicting data have been obtained, but the \( \text{GSTM1} \) deficiency seems to be associated with the susceptibility to certain cancers (22, 23). The \( \theta \) class GSTT1 enzyme catalyzes the detoxification of ethylene oxide and methyl bromide and activates halogenated metabolites, such as dichloromethane and TRI. The enzyme conjugates a variety of aromatic amines, including procarcinogens such as benzidine and \( \beta \)-naphthylamine. The polymorphic \( \text{NAT2} \) locus is responsible for the slow and rapid acetylator phenotypes, each occurring with a frequency of about 50% in Caucasians. Slow and rapid acetylator phenotypes are associated with susceptibility to certain malignancies (28) including, respectively, bladder (18) and colon (29) cancers.

A case-control study (173 RCC cases and 211 controls) was designed to test for an association between polymorphism of the genes encoding \( \text{CYP1A1}, \text{CYP2D6}, \text{NQO1}, \text{GSTM1}, \text{GSTT1}, \text{GSTP1}, \) and \( \text{NAT2} \) and the susceptibility to sporadic RCC. We also investigated the potential contribution of specific interlocus allelic combinations in conferring susceptibility to RCC, due to the redundancy of metabolic pathways and the possible synergistic/antagonist effects of allelic variants from different loci.

### MATERIALS AND METHODS

#### Subjects

All subjects included were unrelated French Caucasians. One hundred seventy-three sporadic RCC patients were recruited at Necker-Enfants Malades Hospital (Paris, France) and Edouard Herriot Hospital (Lyon, France) from 1989 through 1998. A group of healthy controls (\( n = 211 \)) was recruited at the Centre de Médecine Préventive (Vandoeuvre-lès-Nancy, France). Appropriate informed consent was obtained in accordance with local ethical committee guidelines. Age and gender were recorded for each study subject. The cases had histologically confirmed clear cell type of RCC according to the classification Thoenes et al. (30). The mean age of RCC patients was 60.8 ± 11.6 years (120 men and 53 women), and that for controls was 43.8 ± 6.1 years (106 men and 105 women).

#### Genotype Determination

DNA was extracted from the normal kidney of RCC patients, as described previously (31). DNA was extracted from the blood samples of controls using surplus material acquired during routine laboratory measurements. Genotypes were analyzed using PCR-based methods as described below.

**\( \text{CYP1A1} \).** \( \text{CYP1A1} \) polymorphisms were detected by a PCR-RFLP method using the primers described by Bailey et al. (32).

**\( \text{CYP2D6} \).** The alleles \( \text{CYP2D6}^{\ast 3}, \text{CYP2D6}^{\ast 4}, \text{CYP2D6}^{\ast 6}, \text{CYP2D6}^{\ast 8}, \text{CYP2D6}^{\ast 10}, \text{CYP2D6}^{\ast 12}, \) and \( \text{CYP2D6}^{\ast 14} \) were identified by detecting the mutations C188T, G212A, G1846T/A, and G1934A and the deletions \( \Delta T1795 \) and \( \Delta A2637 \). A modification of a previously described method (33) was used. We omitted the first step, amplification of a 4681-bp fragment, and PCR was performed directly on genomic DNA, using primers specific to each polymorphic site studied. The PCR method used to detect the deleted \( \text{CYP2D6}^{\ast 5} \) allele has been described elsewhere (33, 34).

**\( \text{NQO1} \).** \( \text{NQO1}^{\ast 1} \) and \( \text{NQO1}^{\ast 2} \) alleles were detected by a PCR-RFLP procedure, as described by Schulz et al. (35). A recent study identified a third allele \( \text{NQO1}^{\ast 3} \), with a frequency in Caucasians of about 5% (36). \( \text{NQO1}^{\ast 3} \) distribution was not determined in this study.

**\( \text{GSTM1} \) and \( \text{GSTT1} \).** The \( \text{GSTM1} \) and \( \text{GSTT1} \) genes were identified by a multiplex-PCR procedure (37), based on that previously described by Arand et al. (38). The \( \text{GSTM1}^{\ast A/\ast A} \) or \( \text{GSTM1}^{\ast A/\ast 0} \), \( \text{GSTM1}^{\ast 0/\ast 0} \) or \( \text{GSTT1}^{\ast 0/\ast 0} \), and \( \text{GSTM1}^{\ast A/\ast B} \) or \( \text{GSTT1}^{\ast A/\ast B} \) genotypes were identified among the “non-null” \( \text{GSTM1} \) genotypes by a PCR-RFLP approach (39).

**\( \text{GSTP1} \).** The A1404G and C2294T mutations were detected by PCR-RFLP using the primers \( \text{GSTP1-F} \) (\( 5' \)-ACCCAACCCCGGCTCTAT-3') and \( \text{GSTP1-R} \) (\( 5' \)-AATGAGGCTTTGCCTCCT-3'). The PCR conditions were as described previously (27). The 1074-bp PCR product was digested with NcoII. A sample of the resulting fragments was separated by electrophoresis to detect the C2294T mutation, which resulted in polymorphic fragments of 80 and 30 bp (C2294) or 110 bp (T2294). The rest of the digestion mixture was then cleaved with BamHI to detect the A1404G mutation with polymorphic bands of 279 bp (A1404) or 243 and 36 bp (G1404).

**\( \text{NAT2} \).** A PCR-RFLP method was used to detect \( \text{NAT2} \), essentially as published (40). The mutations G191A, G590A, and G857A were detected by the loss of MspI, TaqI, and BamHI restriction sites, respectively. TaqI-digested and nondigested DNA were subjected to electrophoresis in a 2.5% (w/v) low-melting point agarose gel. The T341C substitution was detected using a previously described allele-specific reamplification (40). We also used a novel approach to detect the T341C polymorphism. The first amplification product (1072 bp) was used for a nested PCR to amplify a 243-bp fragment using the forward primer \( \text{VT341C} \) (\( 5' \)-CTTCTCCTCGAGGTGCTA-3')3', and in which A337T and C339T mismatches (italicized) create a partial AccI restriction site, and the reverse primer \( \text{VT341C} \) (\( 5' \)-TGTGGTTGTTTCTTCTGGC-3'). The T341C substitution was detected by AccI cleavage of the 243-bp product into 227 and 16 bp fragments.

All PCR were performed using a GeneAmp 9600 thermocycler. Restriction enzymes were obtained from New England Biolabs. Unless otherwise stated, both digested and nondigested DNA samples were separated by electrophoresis in a polyacrylamide gel at the appropriate concentration.

#### Statistical Analysis

The relative risk associated with certain genotypes was estimated by calculating crude ORs with 95% CIs. Homogeneity was tested as described by Mantel and Haenszel (see Ref.41). Subsequent analysis included logistic regression analyses, adjusting for the potential confounding factors: age and gender. The level of significance was set at \( P = 0.05 \). All statistical analyses were performed using Statistica software (StatSoft, 1995).

#### RESULTS

**Allele Frequencies.** The polymorphic \( \text{CYP1A1}^{\ast}, \text{CYP2D6}^{\ast}, \text{NQO1}^{\ast}, \text{GSTP1}^{\ast}, \) and \( \text{NAT2}^{\ast} \) allele distributions were determined for 173 RCC cases and 211 controls. The allele frequencies in control individuals were consistent with those of previous studies (Table 1). Moreover, the allele frequency distributions in RCC patients did not differ significantly from that of the control group (\( \text{CYP1A1} \)) \( \chi^2 = 3.03; \) df = 3; \( P = 0.39 \) with \( \text{CYP1A1}^{\ast 2B} \) and \( \text{CYP1A1}^{\ast 3} \) alleles combined; \( \text{CYP2D6} \) \( \chi^2 = 5.12; \) df = 5; \( P = 0.40 \) with \( \text{CYP2D6}^{\ast 3}, \text{CYP2D6}^{\ast 8}, \text{CYP2D6}^{\ast 12}, \) and \( \text{CYP2D6}^{\ast 14} \) alleles combined; \( \text{NQO1} \) \( \chi^2 = 1.97; \) df = 1; \( P = 0.16 \); \( \text{GSTP1} \) \( \chi^2 = 1.11; \) df = 1; \( P = 0.29 \) with \( \text{GSTP1}^{\ast 1} \) and \( \text{GSTP1}^{\ast 1} \) alleles combined; \( \text{NAT2} \) \( \chi^2 = 1.42; \) df = 3;
The frequencies obtained are for: 210–211 controls and 171–173 RCC cases for CYP1A1, CYP2D6, NQO1, GSTM1, and GSTT1: 211 controls and 159 RCC patients for NAT2; and 189 controls and 160 RCC patients for GSTP1.

Reference data are taken from studies with European Caucasians, restricted to those with similar distributions of age and sex, as the control subjects: 880 individuals (42) for CYP1A1, 784 individuals (33, 43) for CYP2D6; 235 individuals (20, 44) for NQO1; 95 individuals (18, 45) for GSTM1; 967 individuals (18, 45) for GSTT1; 180 individuals (40) for GSTP1; and 1128 individuals (18, 47–49) for NAT2.

Nomenclature of alleles is as described by Cascorbi et al. (42).

CYP2D6 (wt) represents the alleles associated with normal or ultrarapid CYP2D6 activity; the CYP2D6*10 allele has also been excluded from the PM alleles (33, 50).

No data have been reported with regards to GSTP1*C allele frequency in Caucasians.

P = 0.70 with NAT2*7 and NAT2*4 alleles combined). The PCR amplification of GSTM1 and GSTT1 genes cannot discriminate heterozygous from homozygous nondeleterious genotypes, so it was not possible to assess allele frequency for these loci.

**Genotype Frequencies.** At five loci, the genotypes could be assigned to three functional classes with none (wt/wt), one (wt/m) or two (m/m) variant alleles associated with changes in phenotypic activity (variant CYP1A1* alleles, PM CYP2D6* alleles, *defective* NQO1* allele, *variant* GSTP1* alleles, and *slow acetylator* NAT2* alleles; Table 2). All subjects were assigned a genotype, based on this classification. Observed and expected genotype frequency distributions, according to the Hardy-Weinberg equation, were similar in the control group (CYP1A1: $\chi^2 = 0.00004$; df = 2; $P > 0.9$; CYP2D6: $\chi^2 = 1.2$; df = 2; $P = 0.55$; NQO1: $\chi^2 = 0.0001$; df = 2; $P > 0.9$; GSTP1: $\chi^2 = 0.97$; df = 2; $P = 0.62$; NAT2: $\chi^2 = 0.78$; df = 2; $P = 0.68$).

As stated above, only two genotypes (‘null’ and ‘non-null’) were distinguished for the GSTM1 and GSTT1 loci, which made it impossible to determine the Hardy-Weinberg distribution.

The frequencies of genotypes in RCC cases and controls were not significantly different (CYP1A1: $\chi^2 = 2.72$; df = 2; $P = 0.62$; CYP2D6: $\chi^2 = 1.36$; df = 2; $P = 0.51$; NQO1: $\chi^2 = 2.06$; df = 2; $P = 0.36$; GSTTI1: $\chi^2 = 1.37$; df = 1; $P = 0.24$; GSTP1: $\chi^2 = 1.02$; df = 2; $P = 0.6$; NAT2: $\chi^2 = 0.41$; df = 2; $P = 0.81$). Similar results were obtained for the GSTM1 locus whether the wild-type allele was treated as a non-null allele (df = 1; $P = 0.43$) or as a “non-A” allele ($\chi^2 = 2.65$; df = 2; $P = 0.27$).

**Association between Genotypes and Risk of RCC.** Homozygous variant NAT2, CYP2D6, and NQO1 genotypes with a “low” level or a complete lack of enzyme activity (-) were pooled and distinguished from those with “rapid” or normal activity (+). For CYP1A1 and GSTP1 genotypes, subjects were classed into presumed variant (m) and normal (+) classes, the former containing at least one variant allele. Risk estimates were made by calculating crude ORs, as described by Mantel and Haenszel (see Ref. 41). Similar trends were observed for NQO1 (-) defective genotype (OR = 1.71; CI = 0.67–4.36; $P = 0.25$; CYP2D6 (-) PM genotype (OR = 1.47; CI = 0.66–3.27; $P = 0.34$), CYP1A1 (m) variant genotype (OR = 1.44; CI = 0.93–2.25; $P = 0.10$), GSTTI1 (+) active genotype (OR = 1.38; CI = 0.8–2.39; $P = 0.24$), GSTP1 (m) variant genotype (OR = 1.21; CI = 0.8–1.85; $P = 0.37$), and NAT2 (-) slow acetylator genotype (OR = 1.13; CI = 0.74–1.71; $P = 0.58$). For the GSTM1 genotype, a similar trend was obtained by considering a “GSTM1 (A)” group, including all combinations in which at least one GSTM1*A allele was present (OR = 1.43; CI = 0.93–2.22; $P = 0.10$).

Some subgroups were significantly heterogeneous. There were differences between men and women in the relative risk associated with the CYP1A1 (m), CYP2D6 (-), and GSTP1 (m) genotypes [CYP1A1 (m): $\chi^2_{hom} = 12.1$; $P < 0.001$; CYP2D6 (-): $\chi^2_{hom} = 4.71$; 0.02 $P < 0.05$; GSTP1 (m): $\chi^2_{hom} = 4.75$; 0.02 $P < 0.05$]. ORs among gender groups were between 1.15 and 1.71 (not significant).

Two age groups (50 years and >50 years) also differed in the relative risk associated with the CYP1A1 (m) genotype [CYP1A1 (m): $\chi^2_{hom} = 28.43$; $P < 0.001$] and the GSTTI1 (+) genotype (GSTTI1 (+): $\chi^2_{hom} = 16.61$; $P < 0.001$). A significant risk was observed for the CYP1A1 (m) genotype among the group >50 years of age (OR = 5.87; CI = 1.30–25.9; $P = 0.01$). A similar trend was observed for the GSTTI1 (+) genotype among the group <50 years of age (OR = 3.62; CI = 0.83–15.9; $P = 0.07$). Therefore, age and gender were considered to be potential confounding factors.

ORs adjusted for age and gender are shown in Fig. 1. The dichotomous variable for age (≤50 years or >50 years) might not be a good indicator because about 87.5% of control subjects were 50 years of age or younger. Therefore, age was considered as a continuous variable in the logistic regression analysis. Combinations of genetic polymorphisms were systematically explored to link to risk of RCC.

The risk of RCC was significantly higher for subjects with the CYP1A1 (m) variant genotype considered individually (OR = 2.06; CI = 1.08–3.94; $P = 0.03$) or combined with any of the following genotypes: GSTTI1 (+) active (OR = 2.30; CI = 1.17–4.49; $P = 0.01$), GSTP1 (m) variant (OR = 2.36; CI = 1.04–5.36; $P = 0.04$), or NAT2 (-) slow acetylator (OR = 2.51; CI = 1.15–5.49; $P = 0.02$). A similar trend was observed for CYP1A1 (m) combined with other genotypes: GSTTI1 (+) GSTP1 (m) (OR = 2.56; 0.95–7.74).

**Table 2. Distribution of genotypes in RCC and control populations**

<table>
<thead>
<tr>
<th>Locus</th>
<th>wt/wt (%)</th>
<th>wt/m (%)</th>
<th>m/m (%)</th>
<th>Number of subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A1 Controls</td>
<td>156 (74.3)</td>
<td>50 (23.8)</td>
<td>4 (1.9)</td>
<td>210</td>
</tr>
<tr>
<td>CYP1A1 Cases</td>
<td>114 (66.7)</td>
<td>52 (30.4)</td>
<td>5 (2.9)</td>
<td>171</td>
</tr>
<tr>
<td>CYP2D6 Controls</td>
<td>110 (52.1)</td>
<td>89 (42.2)</td>
<td>12 (5.7)</td>
<td>211</td>
</tr>
<tr>
<td>CYP2D6 Cases</td>
<td>93 (54.1)</td>
<td>65 (37.8)</td>
<td>12 (6.1)</td>
<td>172</td>
</tr>
<tr>
<td>NQO1 Controls</td>
<td>136 (64.8)</td>
<td>66 (31.4)</td>
<td>8 (3.8)</td>
<td>210</td>
</tr>
<tr>
<td>NQO1 Cases</td>
<td>102 (58.9)</td>
<td>60 (34.7)</td>
<td>11 (6.4)</td>
<td>173</td>
</tr>
<tr>
<td>GSTP1 Controls</td>
<td>93 (49.2)</td>
<td>75 (39.7)</td>
<td>21 (11.1)</td>
<td>189</td>
</tr>
<tr>
<td>GSTP1 Cases</td>
<td>71 (44.4)</td>
<td>67 (41.9)</td>
<td>22 (13.7)</td>
<td>160</td>
</tr>
<tr>
<td>NAT2 Controls</td>
<td>16 (7.6)</td>
<td>75 (35.5)</td>
<td>120 (56.9)</td>
<td>211</td>
</tr>
<tr>
<td>NAT2 Cases</td>
<td>10 (6.3)</td>
<td>54 (34.0)</td>
<td>95 (59.7)</td>
<td>159</td>
</tr>
</tbody>
</table>

$a$ m. allele carrying at least one nucleotide change associated with altered phenotypic activity (variant CYP1A1* alleles; PM CYP2D6* alleles, defective NQO1* alleles; variant GSTP1* alleles, and slow acetylator NAT2* alleles), wt. allele with no such nucleotide changes. For distribution of GSTM1 and GSTT1 genotypes, see Table 1.
CI = 1.09–5.98; \( P = 0.03 \), GSTTL1 (+) vs NAT2 (-) (OR = 2.85; CI = 1.26–6.44; \( P = 0.01 \), GSTTI1 (-) vs NAT2 (-) (OR = 3.05; CI = 1.12–8.32; \( P = 0.03 \), and GSTD1 (m)/GSTM1 (A) (OR = 3.91; CI = 1.06–14.5; \( P = 0.04 \)).

Comparison of combinations of these “at risk” genotypes with “mirror” genotypes increased the strength of the observed association in comparison with all other genotypes: CYP1A1 (m)/NAT2 (-)/GSTTI1 (+) versus CYP1A1 (m)/NAT2 (-) (OR = 3.47; \( P = 0.04 \), Fig. 1).

There was also a significant association if GSTD1 (-) and GSTD1 (m) genotypes were combined with either NAT2 (-) (OR = 2.59; CI = 1.16–5.79; \( P = 0.02 \)) or CYP1A1 (m) (OR = 3.47; CI = 1.07–11.2; \( P = 0.04 \)).

The risk associated with CYP1A1 (m)/GSTM1 (A)/GSTTI1 (+) (OR = 2.88; CI = 1.0–8.31; \( P = 0.05 \)) was not significant, but the observed trend was reinforced by comparison of CYP1A1 (m)/GSTTI1 (+) vs CYP1A1 (m)/GSTTI1 (-) (OR = 3.54; CI = 1.03–12.2; \( P = 0.04 \)).

Another combination gave a significant association only if mirror genotypes were considered: CYP1A1 (m)/GSTM1 (-) vs CYP1A1 (m)/GSTM1 (-) (OR = 3.0; CI = 1.12–8.0; \( P = 0.03 \)) compared to CYP1A1 (m)/GSTM1 (m) versus other genotypes (OR = 2.08; CI = 0.94–4.59; \( P = 0.07 \); Fig. 1).

DISCUSSION

This is the first epidemiological study to test whether there is an association between the polymorphism of genes encoding several xenobiotic-metabolizing enzymes and the risk of sporadic RCC.

Two genotypes for susceptibility to RCC have been reported elsewhere. A higher prevalence of the defective NQO1* allele has been reported for RCC patients than for controls, and a similar trend has also been observed in patients with urothelial carcinoma (35). This suggested that NQO1 activity is involved in the detoxification of carcinogens implicated in renal and urothelial tumorigenesis. Insufficient NQO1 activity may, therefore, increase susceptibility to RCC. However, as emphasized by Schulz et al. (35), the possibility of enzyme induction by a variety of compounds may reduce the association between the defective allele and actual NQOR activity. The overrepresentation of heterozygotes and defective homozygotes among RCC patients in our study (41%) is consistent with the results of Schulz et al. (35%). However, in contrast to Schulz et al. (35), the observed differences in this study were not significant (\( P = 0.24 \)). The major difference between the two studies is the distribution of NQOR genotypes in the control group. This demonstrates that association studies are very sensitive to the selection of control samples, especially if the role in the disease process of the locus studied is complex. Nevertheless, both studies suggest that a lack of NQO1 activity is involved in RCC tumorigenesis. This notion is supported by the demonstration that NQO1 prevents the formation of benzo(a)pyrene quinone-DNA adducts generated by CYP1A1 and P450 reductase activity (51).

A recent investigation has demonstrated a higher risk of RCC for GSTTI1 (+) workers exposed to high concentrations of TRI (52). These subjects have a high mutation rate at the von Hippel-Lindau tumor suppressor locus, which suggests that metabolites derived from the GST-dependent pathway of TRI are involved in the development of RCC (53).

We observed an overrepresentation of the variant CYP1A1 (m) (7.6%) genotype in RCC patients (Table 2). Because it seems reasonable to assume that each possible at risk genotype for a multifactorial
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disease has low penetrance if considered individually, combinations of such genotypes were expected to increase the association with RCC susceptibility. Moreover, analysis of combinations of genetic polymorphisms may provide data about the role of the corresponding enzymes in RCC. Nevertheless, subgroupings lead to small numbers of subjects at risk and multiple comparisons between same study subjects. It warrants caution regarding the statistical data because the possibility of false positive results cannot be excluded.

Our results were obtained by comparing the RCC patient group (mean age, 60.8; males, 69%) and a control group (mean age, 43.8; males, 50%). No report found that sex and age have a significant effect on the distribution of the genetic polymorphisms studied. Therefore, demographic parameters of patients and controls were unlikely to be responsible for differences in allele frequency for studied loci (Table 1).

The risk of RCC was higher (ORs between 2.3 and 2.5) if the CYP1A1 (m) genotype occurred in combination with the GSTT1 (+), NAT2 (-), or GSTP1 (m) genotypes (Fig. 1). The CYP1A1 (+)/GSTT1 (-) combination seemed to be particularly protective (OR = 0.59; CI = 0.22–1.53; P = 0.27) against RCC. The same trend was observed for CYP1A1 (+)/NAT2 (+) (OR = 0.53; CI = 0.27–1.04; P = 0.06). There was no association between the null GSTM1 genotype and RCC risk, whereas a nonsignificant trend toward such an association was observed with the GSTM1 (A) genotype. However, GSTM1*0/M*0 conferred a higher risk of RCC if present in combination with other genotypes (Fig. 1). These results highlight the difficulties encountered in estimating risk by studying a single locus polymorphism. They also suggest that there is functional synergy between several xenobiotic-metabolizing enzymes, especially between CYP1A1, GSTP1, GSTT1, and NAT2, as suggested in other association studies of cancer susceptibility. For example, an association between the null GSTM1 and RCC risk, whereas a nonsignificant trend toward such an association was observed with the GSTM1 (A) genotype. However, GSTM1*0/M*0 conferred a higher risk of RCC if present in combination with other genotypes (Fig. 1). These results highlight the difficulties encountered in estimating risk by studying a single locus polymorphism. They also suggest that there is functional synergy between several xenobiotic-metabolizing enzymes, especially between CYP1A1, GSTP1, GSTT1, and NAT2, as suggested in other association studies of cancer susceptibility. For example, an association between the null GSTM1 and RCC risk, whereas a nonsignificant trend toward such an association was observed with the GSTM1 (A) genotype. However, GSTM1*0/M*0 conferred a higher risk of RCC if present in combination with other genotypes.

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