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REGULAR ARTICLE

# Association of genotypes of carcinogen-metabolizing enzymes and smoking status with bladder cancer in a Japanese population

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**Abstract**
*Objectives* Arylamines are considered to be the primary causative agent of bladder cancer in tobacco smokers. To test the hypothesis that variation in the genes that metabolize tobacco carcinogens contribute to bladder cancer, we examined the effects of single nucleotide polymorphisms in the genes of four key enzymes: *cytochrome P450 1A2*, *N-acetyltransferase* (*NAT*) *2*, *sulfotransferase 1A1*, and *UDP-glucuronosyltransferase* (*UGT*) *2B7*.
*Methods* In this study, 282 Japanese patients with transitional cell carcinoma, the most common bladder cancer, and 257 healthy controls were surveyed and compared for frequencies of the genotypes of the four enzymes. Genotypes were determined using PCR-restriction fragment length polymorphism and TaqMan assays. Smoking information was collected by personal interview. Logistic regression analysis and the chi-square test were employed as statistical methods.
*Results* The *NAT2* slow genotype was significantly associated with the risk of bladder cancer [odds ratio (OR) 3.41, 95 % confidence interval (95 % CI) 1.68–6.87; $p < 0.05$). The *NAT2* slow genotype also significantly increased the risk of bladder cancer in heavy smokers (OR 8.57, 95 % CI 1.82–40.25; $p < 0.05$). Among the different combinations of the four enzyme genotypes, the highest OR (4.20; 95 % CI 1.34–13.14; $p < 0.05$) was obtained with the *NAT2* slow genotype when present in combination with the *UGT2B7*2/*2* or **1/*2* genotype.
*Conclusions* Our results suggest that individuals with different genotypes for the enzymes involved in metabolizing carcinogenic arylamines have a different risk of developing bladder cancer. In particularly, the combination of the *NAT2* slow genotype with *UGT2B7*1/*2* or **2/*2* genotype is a high risk factor for bladder cancer.

**Keywords** Genetic susceptibility · *N-acetyltransferase* · *UDP-glucuronosyltransferase B7* · Polymorphism · Bladder cancer

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## Introduction

Bladder cancer is the seventh most common cancer in men and the 17th most common cancer in women worldwide. The risk factors for bladder cancer include exposure to arylamines in occupational settings or from cigarette smoke. There are over 60 established carcinogens in cigarette smoke [1]. Among these, arylamines, of which 4-aminobiphenyl (ABP) is an example, are hypothesized to be a major causative factor for bladder cancer [2]. Smokers show significantly higher levels of 4-ABP-DNA adducts in bladder biopsies than non-smokers [3].

Arylamines are not carcinogenic in the parent form; rather, they require metabolic activation by reactive electrophiles for their carcinogenic effects to be uncovered. It is therefore possible that genetic susceptibility to bladder cancer can be explained by the metabolic differences that lead to the bioactivation or detoxification of arylamines [4]. In the human liver, arylamines are *N*-hydroxylated by a cytochrome P450 (CYP) 1A2-catalyzed reaction to produce a substrate that can be catalyzed by sulfotransferases (SULTs) or UDP-glucuronosyltransferases (UGTs). *N*-hydroxyarylamines enter the circulation and undergo renal

filtration into the bladder lumen, where they can be reabsorbed into the bladder mucosa. In the bladder mucosa, *N*-hydroxyarylamines are activated by *N*-acetyltransferase (NAT) 1 to form highly electrophilic *N*-acetoxy derivatives that can covalently bind to the bladder epithelium. Alternatively, arylamines can be catalyzed by a competing detoxification pathway. The best established of these enzymatic pathways is *N*-acetylation, which is regulated by NAT2 activity in the liver [5]. The resulting metabolites of this pathway, *N*-arylacetamides, are excreted in the urine.

Cigarette smoking has been associated with significantly increased phase I enzyme CYP1A2 activity [6]. Variant *CYP1A2* gene alleles are associated with differential activity of the CYP1A2 enzyme. *CYP1A2*1C* and *CYP1A2*1F* alleles have been demonstrated to be associated with decreased and increased inducibility of the *CYP1A2* gene, respectively [7]. We have previously analyzed the *CYP1A2*1C* single-nucleotide polymorphism (SNP) in a case–control study which proved that smokers with a higher CYP1A2 activity are at an elevated risk of bladder cancer [8]. Based on this result, we have selected this SNP as a candidate marker in the study reported here [9].

NATs are coded by the genes *NAT1* and *NAT2*, and human NAT2 has a much higher affinity than NAT1 for urinary bladder carcinogens such as 4-ABP [10]. NAT2 phenotypes are classified as rapid, intermediate, or slow acetylators, respectively, according to their acetylation activity [11]. The *NAT2* slow genotype is homozygotic for mutated *NAT2* genes and is characterized by decreased acetylation activity. Genotype studies show that the *NAT2* slow genotype is associated with an increased risk of bladder cancer [12].

SULTs catalyze the sulfonation of a wide range of promutagens and procarcinogens. SULT1A1, in particular, appears to be an important phenol SULT because of its abundance and distribution in a wide range of tissues [13]. *SULT1A1* has been conjectured to be a potentially important low-penetrance cancer-predisposing gene [14]. Different alleles of *SULT1A1* have been reported, such as **2* (213Arg → His), **3* (223Met → Val), and **4* (37Arg → Gln) [15]. The authors of an earlier study reported that the *SULT1A1*2* allele is associated with low activity and low thermal stability [16]. Epidemiologic studies have shown a range of effects associated with *SULT1A1* polymorphisms, including both an increased and decreased risk of bladder cancer [17].

UGTs belong to a superfamily of major phase II drug metabolism enzymes that catalyze the glucuronidation of numerous endobiotics and xenobiotics. UGTs are divided into three subfamilies: UDP-glucuronyltransferase 1A (UGT1A), UDP-glucuronyltransferase 2A (UGT2A), and UDP-glucuronyltransferase 2B (UGT2B) [18]. UGT2B7 is a major isoform of UGT2B in humans [19]. A C-to-T transition at nucleotide 802 that gives rise to enzymes with either histidine (H) or tyrosine (Y) at amino acid 268 (*UGT2B7* $H_{268}Y$) has been reported, leading to individual *UGT2B7* genotype variants, *UGT2B7*1* and *UGT2B7*2*. A recent study in workers exposed to benzidine showed an elevated cancer risk in those with the *UGT 2B7*2* allele [20].

Different mechanisms have been proposed to play a role in the individual susceptibility to arylamine-related bladder cancer in various races or ethnic groups [21]. Tsukino et al. [8] demonstrated that several genetic polymorphisms in genes such as *NAT2*, *SULT1A1,* and *CYP1A2* modulate the risk of arylamine-related bladder cancer in cigarette smokers in the Japanese population. Here, we expand on that previous research and include UGT2B7 as an additional candidate SNP to investigate the associations with arylamine metabolism and bladder cancer risk.

## Materials and methods

### Subjects

Overall, 282 consecutive patients histologically diagnosed with bladder cancer and treated at the University of Occupational Health Hospital, Miyazaki University Hospital and Miyazaki Prefecture Hospital between 1993 and 2003 were enrolled in this study. The control group comprised 257 subjects who had visited local medical clinics in Kitakyushu and Miyazaki City between 1993 and 2003 for medical health check-ups. All study subjects completed a questionnaire administered by a trained interviewer to acquire information on medical, residential, and occupational exposure to arylamines, as well as smoking history. There was no restriction in the recruitment of case and control with respect to age, sex, and smoking history. The amount of tobacco smoke was calculated as pack-years [number of pack years = (packs smoked per day) × (years as a smoker)]. The median value of smoking amounts among controls who had ever smoked was 37.4 pack-years. We used the term "light smokers" for subjects who consumed ≤37.4 pack-years and "heavy smokers" for subjects who consumed >37.4 pack-years.

### Genotyping

Genomic DNA was isolated from peripheral leukocytes by proteinase K digestion and phenol/chloroform extraction. The genetic polymorphism in the 5′-flanking region of *CYP1A2* (*rs20695140*) was determined by PCR amplification, followed by digestion with the *Dde*I restriction enzyme, using a previously described method [7]. A *NAT2* high-activity allele, *NAT2*4,* and *NAT2* low-activity

alleles, *NAT2*5*, *NAT2*6*, and *NAT2*7*, were determined according to a previously published PCR-restriction fragment length polymorphism (RFLP) method [22]. A *NAT* variant allele (*NAT2*14*), which is very rare (<1 % frequency) in the Japanese population, was not considered [23]. The *NAT2* genotypes were categorized as rapid, intermediate, and slow genotype, depending on whether an individual was homozygous, heterozygous, or null for the NAT2*4 allele, respectively. Genotyping of *SULT1A1* for a polymorphism at codon 213 (Arg/His) (*rs9282861*) was performed by PCR–RFLP [16].

The *UGT2B7* polymorphism at locus C802T (His268Tyr) (*rs7439366*) was genotyped by real time-PCR as follows. In one TaqMan® Array Plate, the total reaction solution was 440 μL and composed of 242 μL Genotyping Master Mix (TaqMan® Universal PCR Master Mix II), 12.1 μL primer (forward primer: CAATGGGGAAAGCTGACG; reverse primer: AGTCCTCCAACAAAATCAACA), and 185.9 μL nuclease-free water. The reaction solution (9 μL) was placed into each well of a 48-well reaction plate (the remainder of the reaction solution was used to prevent experimental errors), and 1 μL DNA sample or water control was added to each tube. The homozygous mutant **1/*1 UGT2B7* genotype DNA sample was set as a control sample in each array plate. The standard mode reaction time was 90 min.

### Statistical analyses

The chi-square test and Student's *t* test were carried out on both the patients and control subjects. Relative associations between the two groups were assessed by calculating odds ratios (ORs) from contingency tables. In logistic regression analysis, the OR with corresponding 95 % confidence intervals (95 % CI) were calculated. ORs were adjusted for age and gender. All statistical tests were based on two-tailed probability, and $p$ values of <0.05 were considered to be significant. Statistical analyses were carried out using SPSS ver. 19 (SPSS Inc., Chicago, IL).

### Ethical considerations

The Ministry of Education, Culture, Sports, Science and Technology, the Ministry of Health, Labor and Welfare, and the Ministry of Economy, Trade and Industry have jointly established general ethical guidelines for analytical research on the human genome/genes (2008). The Ethics Review Board of Kumamoto University approved this study (No. 181, March 16, 2011) based on the "Ethical Guidelines for Analytical Research on the Human Genome/Genes" (2008). All participants were given detailed explanations of the objectives of this study and their informed consent was obtained. They were assured that personal data provided on the written forms would be fully protected.

## Results

The study involved 282 patients with pathologically confirmed bladder cancer and 257 healthy controls (Table 1). Gender distribution in the two groups was approximately equal. In addition, smoking status did not appear to affect the risk for bladder cancer.

The relationship between the *CYP1A2*, *NAT2*, *SULT1A1*, and *UGT2B7* genotypes and bladder cancer risk is shown in Table 2. After adjusting for age and sex, only those who had the *NAT2* slow genotype were associated with increased bladder cancer risk (OR 3.41, 95 % CI 1.69–6.87; $p < 0.05$). Although the ORs of the *CYP1A2*1C/*1C* genotype, *SULT1A1*2/*2* genotype, and *UGT2B7 *2/*2* genotype were higher in the case group than in the control group, the association was not significant.

In order to check the *SULT1A1*, *NAT2*, *UGT2B7*, and *CYP1A2* genotypes in patients with bladder cancer in combination with their smoking status, we classified all individuals in this study group into non-smokers, light-smokers, and heavy-smokers, respectively. Only the *NAT2* slow genotype significantly increased bladder cancer risk in heavy smokers (OR 8.57, 95 % CI 1.82–40.25; $p < 0.05$; Table 3). This result may indicate that the *NAT2* slow acetylator genotype is not only an independent risk factor for bladder cancer, but also plays a modulatory role in the metabolism of tobacco smoke components (possibly arylamines).

Considering that the most significant OR in increasing bladder cancer risk was observed in *NAT2* genotypes, we analyzed combinations of other enzyme genotypes with *NAT2* slow, intermediate, and rapid genotypes, respectively (Table 4). This analysis showed that *UGT2B7*2/*2* or *UGT2B7*1//*2* genotypes occurring together with the *NAT2* slow genotype have the highest OR (4.2) after age and gender were adjusted for. This results indicates that the *NAT2* slow genotype in combination with the *UGT2B7*2* allele is most probably associated with an increased risk of bladder cancer. Furthermore, the *NAT2* slow genotype in combination with *CYP1A2*1C/*1A* and *A/*A*, *SULT1A*1/*1*, or *UGT2B7*1/*1* had an adjusted OR (95 % CI) of 2.82 (95 % CI 1.42–5.60), 3.18 (95 % CI 1.46–6.94), and 2.85 (95 % CI 1.21–6.71), respectively, compared with the non-at-risk genotypes.

## Discussion

To the best of our knowledge, our study is the first to report a significant effect of the interactions of the *NAT2* gene and *UGT2B7* SNPs on the risk of bladder cancer. We confirmed that among our study cohort the *NAT2* slow genotype is associated with a significantly increased risk of bladder

**Table 1** Characteristics of bladder cancer patients and control individuals

| Variables | Patients ($n = 282$) | Controls ($n = 257$) | $p$ value |
|---|---|---|---|
| Age at reference (years) | | | |
| <50 (%) | 15 (5.3) | 22 (8.6) | |
| 50–<60 (%) | 33 (11.7) | 37 (14.4) | |
| 60–<70 (%) | 84 (29.8) | 68 (26.5) | |
| ≥70 (%) | 150 (53.2) | 130 (50.6) | 0.32[a] |
| Smoking status | | | |
| Non-smokers (%) | 85 (30.1) | 81 (31.5) | |
| Light smokers (%) | 94 (33.3) | 99 (38.5) | |
| Heavy smokers (%) | 103 (36.5) | 77 (30.0) | 0.25[a] |
| Sex | | | |
| Male (%) | 225 (79.8) | 203 (79.0) | |
| Female (%) | 57 (20.2) | 54 (21.0) | 0.82[a] |
| Mean age (years), mean ± SD | 69.5 ± 10.6 | 67.5 ± 12.0 | 0.05[b] |
| Mean pack-years of smokers, mean ± SD | 40.0 ± 21.7 | 37.4 ± 23.2 | 0.27[b] |

Data are presented as the number of patients/controls, with the percentage given in parenthesis, unless indicated otherwise

[a] Pearson's $\chi^2$ test

[b] Student's $t$ test

**Table 2** Odds ratios of bladder cancer patients for the *CYP1A2*, *NAT2*, *SULT1A1*, and *UGT2B7* genotypes[a]

| Genotype | Patients ($n = 282$) | Control ($n = 257$) | Crude OR (95 % CI) | Adjusted OR[b] (95 % CI) |
|---|---|---|---|---|
| *CYP1A2* | | | | |
| **1A/*1A* | 169 (59.9) | 147 (57.2) | 1[c] | 1[c] |
| **1A/*1C* | 94 (33.3) | 99 (38.5) | 0.83 (0.58–1.18) | 0.82 (0.57–1.17) |
| **1C/*1C* | 19 (6.7) | 11 (4.3) | 1.50 (0.69–3.26) | 1.45 (0.67–3.16) |
| *NAT2* | | | | |
| Rapid acetylator | 123 (43.6) | 139 (54.1) | 1[c] | 1[c] |
| Intermediate acetylator | 123 (43.6) | 106 (41.2) | 1.31 (0.92–1.87) | 1.34 (0.94–1.92) |
| Slow acetylator | 36 (12.8) | 12 (4.7) | 3.40 (1.69–6.81)[d] | 3.41 (1.69–6.87)[d] |
| *SULT1A1* | | | | |
| **1/*1* | 218 (77.3) | 201 (78.2) | 1[c] | 1[c] |
| **1/*2* | 59 (20.9) | 52 (20.2) | 1.05 (0.69–1.59) | 1.03 (0.68–1.57) |
| **2/*2* | 5 (1.8) | 4 (1.6) | 1.15 (0.31–4.35) | 1.23 (0.32–4.68) |
| *UGT2B7* | | | | |
| **1/*1* | 141 (50.0) | 137 (53.3) | 1[c] | 1[c] |
| **1/*2* | 116 (41.1) | 100 (38.9) | 1.13 (0.79–1.61) | 1.13 (0.79–1.62) |
| **2/*2* | 25 (8.9) | 20 (7.8) | 1.22 (0.65–2.29) | 1.20 (0.63–2.26) |

Data are presented as the number of patients/controls, with the percentage given in parenthesis unless indicate otherwise

*OR* Odds ratio, *CI* confidence interval

[a] *CYP1A2*, *Cytochrome P450 1A2*; *NAT2*, *N-acetyltransferase*; *SULT1A1*, *sulfotransferase 1A1*; *UGT2B7*, *UDP-glucuronosyltransferase 2B7*

[b] Odds ratios were adjusted for age and gender

[c] Reference category

[d] $p$ value $< 0.05$

cancer, especially among heavy smokers. We have also shown that the *CYP1A2*1A* allele and the *SULT1A1*1/*1* genotype in combination with the *NAT2* genotype were positively associated with an increased risk of bladder cancer. These observations support our hypothesis that variations in the genes involved in the metabolism of tobacco carcinogens, such as arylamines, modify the risk for developing bladder cancer.

**Table 3** Odds ratios of bladder cancer patients for the *CYP1A2*, *NAT2*, *SULT1A1*, and *UGT2B7* genotypes stratified by smoking status

| Genotype | Non-smokers | | Smokers | | | |
|---|---|---|---|---|---|---|
| | | | Light smokers (≤37.4 pack-years) | | Heavy smokers (>37.4 pack-years) | |
| | No.[a] Patient/ control | OR[b] (95 % CI) | No.[a] Patient/ control | OR[b] (95 % CI) | No.[a] Patient/ control | OR[b] (95 % CI) |
| All | 85/81 | 1[c] | 94/99 | 0.89 (0.55–1.43) | 103/77 | 1.27 (0.76–2.13) |
| *CYP1A2* | | | | | | |
| **1A/*1C* or **1A/*1A* | 81/78 | 1[c] | 87/96 | 0.86 (0.53–1.41) | 95/72 | 1.30 (0.76–2.21) |
| **1C/*1C* | 4/3 | 1.16 (0.25–5.40) | 7/3 | 2.07 (0.50–8.58) | 8/5 | 1.47 (0.44–4.94) |
| *NAT2* | | | | | | |
| Rapid or intermediate acetylator | 74/76 | 1[c] | 85/94 | 0.97 (0.59–1.61) | 87/75 | 1.31 (0.76–2.27) |
| Slow acetylator | 11/5 | 2.07 (0.68–6.31) | 9/5 | 2.06 (0.62–6.87) | 16/2 | 8.57 (1.82–40.25)[d] |
| *SULT1A1* | | | | | | |
| **1/*1* | 63/62 | 1[c] | 70/82 | 0.86 (0.52–1.40) | 85/57 | 1.40 (0.81–2.42) |
| **1/*2* or **2/*2* | 22/19 | 1.15 (0.56–2.35) | 24/17 | 1.30 (0.58–2.91) | 18/20 | 0.84 (0.37–1.94) |
| *UGT2B7* | | | | | | |
| **1/*1* | 38/41 | 1[c] | 51/59 | 0.87 (0.50–1.51) | 52/37 | 1.56 (0.83–2.95) |
| **1/*2* or **2/*2* | 47/40 | 1.23 (0.66–2.27) | 43/40 | 1.29 (0.63–2.64) | 51/40 | 1.37 (0.64–2.95) |

[a] Number of patients/controls

[b] Odds ratios were adjusted for age and gender

[c] Reference category

[d] $p$ value $< 0.05$

**Table 4** Odds ratios of bladder cancer patients for the *NAT2* genotype combined with the *CYP1A2*, *SULT1A1*, and *UGT2B7* genotypes

| Genotype | *NAT2* rapid or intermediate | | | *NAT2* slow | | |
|---|---|---|---|---|---|---|
| | OR[a] (95 % CI) | No.[c] | $p$ value | OR[a] (95 % CI) | No. | $p$ value |
| *CYP1A2*1 C/*1A* or **1A/*1A* | 1[b] | 230/234 | | 2.82 (1.42–5.60) | 33/12 | <0.01 |
| *CYP1A2 *1C/*1C* | 1.42 (0.65–3.15) | 16/11 | 0.382 | NC | 3/0 | NC |
| *SULT1A1*1/*1* | 1[b] | 190/192 | | 3.18 (1.46–6.93) | 28/9 | <0.05 |
| *SULT1A1*2/*2* or **1/*2* | 1.06 (0.69–1.63) | 56/53 | 0.791 | 2.72 (0.71–10.44) | 8/3 | 0.145 |
| *UGT2B7 *1/*1* | 1[b] | 120/129 | | 2.85 (1.21–6.71) | 21/8 | <0.05 |
| *UGT2B7 *2/*2* or **1/*2* | 1.16 (0.82–1.66) | 126/116 | 0.404 | 4.20 (1.34–13.14) | 15/4 | <0.05 |

*NC* Not calculated due to the small number of control subjects

[a] Odds ratios were adjusted for age and sex

[b] Reference category

[c] Number of patients/controls

NATs detoxify various tobacco toxicants, including arylamines. The results of 22 cooperative studies, which included a total of 4,306 cases, clearly demonstrate an association between the *NAT2* slow genotype and higher ORs for bladder cancer in patients who were smokers. These studies generated a summary OR of 1.2 (95 % CI 1.1–1.5; $p = 0.009$) [24]. The relationship of the *NAT2* slow genotype and smoking status was also confirmed in our previous study on bladder cancer in which the slow or intermediate acetylator genotype of *NAT2* among smokers showed the highest OR of 4.28 (95 % CI 1.96–9.36; $p < 0.05$) [25]. The *NAT2* slow genotype among heavy smokers was found to have the highest and significant OR of 7.31 (95 % CI 1.90–28.05; $p < 0.005$) [8]. The findings of this study are congruent with those of previous studies, indicating that the *NAT2* slow acetylator genotype can decrease the detoxification of tobacco mutagens, such as arylamines, and be synergistic with other metabolizing enzymes.

Evidence linking elevated CYP1A2 activity to increased bladder cancer risk has been reported [6]. Polycyclic aromatic hydrocarbons, heterocyclic aromatic amines, and certain dietary components are known to induce the activities of this enzyme [26]. CYP1A2, which is highly expressed in the liver, has been shown to be highly relevant in terms of activating many environmental carcinogens (i.e., aromatic or heterocyclic amines) [9]. Therefore, CYP1A2 activity may be a risk factor for the development of cancers in other tissues that are targets for activated carcinogens (i.e., bladder). An increased risk of bladder cancer has been related to the *CYP1A2* genotype in smokers [27]. Cigarette smoke contains a considerable amount of arylamines, which are catalyzed by CYP1A2 in the first step, and high CYP1A2 activity has been associated with elevated bladder cancer risk in smokers [28]. To date, several variant *CYP1A2* alleles have been reported online [http://www.cypalleles.ki.se/cyp1a2.htm]. One of these, CYP1A2 −3860G>A (*CYP1A2*1C*), reduces enzyme activity in smokers [7]. In our study, a combination of *CYP1A2*1C/*1A* or **1A/*1A* genotype with the *NAT2* slow genotype was significantly associated with an increased risk of bladder cancer, demonstrating that specific genotypes may be able to affect the metabolism of chemical carcinogens. High CYP1A2 activity and slow NAT2 activity may increase the hydroxylated forms of arylamines, which are proximate carcinogens. Our study showed a positive association of increased risk of bladder cancer with high CYP1A2 enzyme activity and NAT2 slow acetylator activity.

The results of some studies in which a positive association between *SULT1A1* polymorphisms and cancer susceptibility was found suggest that SULT enzymes are involved in numerous detoxification pathways [16]. SULT1A1 is found ubiquitously in human tissues, such as the liver and urothelial mucosa. In a previous study, SULT1A1*1 demonstrated a higher in vitro catalyzing activity of 3′-phosphoadenosine 5′-phosphosulfate-dependent DNA adduct formation than did SULT1A1*2. The high activity of the SULT1A1*1 allozyme activates *N*-hydroxy arylamines and forms arylnitrenium ions, leading to DNA adducts, mutation, and neoplasia [12]. The OR of the single *SULT1A1* genotype associated with bladder cancer risk in this study was not significant. However, a significantly high OR was obtained for the *SULT1A1*1/*1* genotype in combination with the *NAT2* slow genotype. This result indicated that in individuals with this genotype combination, reactive metabolites from acetylation and sulfonation might increase bladder cancer risk.

Several studies investigating UGT have measured the *N*-glucuronidation of human and rat liver microsomes towards arylamines [29]. UGTs play a major role in the elimination of numerous carcinogens by (1) excretion via the biliary or urinary tract and (2) the sequestration of proximate carcinogens, leading to their detoxification [30]. The bladder is involved in the main excretion pathway for glucuronides and expresses all subtypes of UGT except UGT2B17 [31]. Interestingly, in a previous study, we found that *UGT2B7*2* was significantly associated with bladder cancer [32]. Here, we found a significant association for the combination of *UGT2B7*2* with the *NAT2* slow allele and bladder cancer risk. In order to gain a better understanding of the relationship of smoking habit with bladder cancer and the combination of *UGT2B7* and *NAT2* genotypes, we reclassified all individuals by smoking status. No associations of between the *NAT2* slow genotype and the *UGT2B7*1/*2* or **2/*2* genotypes in either the heavy smokers or control individuals (data not shown) were observed. The combination of *NAT2* slow genotype with *UGT2B7*1/*1* genotype was observed in two heavy smokers in the control group. Consequently, because of the limited number of heavy smokers with this genotype combination, we were unable to compare the different genotypes of *UGT2B7* combined with the *NAT2* slow genotype after stratifying by smoking status. Therefore, our observations need to be confirmed in a larger scale study and in another study population.

This hospital-based case–control study has several inherent limitations. First, selection bias could be present because we included patients from tertiary referral hospitals, and our control population was limited to local clinics. Second, recall bias could be present. Even though we used the method of direct interview to reduce information bias, the accuracy of assessments on cigarette smoking may still be subject to recall bias. Finally, we did not consider information on dietary carcinogens.

Our data suggest that individual genetic variations affecting the metabolic activity of enzymes involved in arylamine detoxification could modify the risk of bladder cancer.

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