

Short Communication

Phenol Sulfotransferase *SULT1A1*2* Allele and Enhanced Risk of Upper Urinary Tract Urothelial Cell Carcinoma

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Abstract

Cytosolic sulfotransferases (SULT) are involved in detoxification pathways. A functional polymorphism in the *SULT1A1* gene, leading to an Arg213His substitution (*SULT1A1*2*), is thought to confer susceptibility to various types of cancer. Upper urinary tract urothelial cell carcinomas (UUT-UCC) are rare (5% of all urothelial carcinomas). We genotyped 268 patients with UUT-UCC and 268 healthy controls matched for age, gender,

tobacco consumption, and ethnicity. His213 (*SULT1A1*2*) allele frequency was significantly higher in patients than in controls (37.1% versus 28.9%; $P = 0.004$). The His/His genotype corresponding to low-activity *SULT1A1* enzyme conferred a significantly higher risk of UUT-UCC (odds ratio, 2.18; 95% confidence interval, 1.28-3.69; $P = 0.004$). (Cancer Epidemiol Biomarkers Prev 2007; 16(11):2500-3)

Introduction

Urothelial cancer of the bladder and upper urinary tract (UUT) represents the fourth most common malignancy worldwide (1, 2). However, UUT-urothelial cell carcinomas (UUT-UCC) are located either in the renal pelvis or in the ureter and account for only 5% of all urothelial carcinomas. The annual incidence of UUT-UCCs is 1 to 2 cases per 100,000 inhabitants (2). Known environmental risk factors for UUT-UCC are tobacco consumption, occupational carcinogens, and abuse of analgesics or Chinese herbs (2-4). Patients with Balkan endemic nephropathy or human nonpolyposis colorectal carcinoma syndrome are susceptible to UUT-UCC, suggesting complex causal interrelationships between nongenetic and genetic factors (5).

Sulfation is an important step not only in the detoxification of many environmental chemicals but also in the bioactivation of dietary and other mutagens. Cyto-

solic sulfotransferases (SULT) catalyze the conjugation of sulfo group to these substrates (6), the two major gene families being the phenol (*SULT1A1*) and hydroxysteroid (*SULT2A*) sulfotransferases. The *SULT1A1* gene is located on chromosome 16p12.1-p11.2 and is expressed in several tissues including liver, lung, and kidney (6). A polymorphism (G→A) in exon 7 of the gene results in substitution of an arginine residue by histidine, decreased enzymatic activity, and changes in mutagen and procarcinogen detoxification and bioactivation rates (6-8). The variant allele *SULT1A1*2* with reduced sulfotransferase activity might enhance the risk of cancer (6). The association between the *SULT1A1* gene and bladder urothelial cancer has already been investigated (8-10). Although more and more lines of evidence underline that histologically identical urothelial tumors may arise through different molecular mechanisms (bladder versus upper tract tumors; refs. 5, 11, 12), this association has never been explored specifically in UUT-UCCs. Therefore, the purpose of this study was to determine whether the Arg213His polymorphism (*SULT1A1*2*) is associated with enhanced susceptibility to UUT-UCC in a population of French Caucasian patients.

Materials and Methods

We recruited 268 patients (White Caucasian profile) with a histologically confirmed diagnosis of UUT-UCC in our database (six academic institutions among France). All

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patients and controls gave their informed consent to undergo screening for identification of urothelial cancer susceptibility genes. No patient had a familial history of human nonpolyposis colorectal carcinoma syndrome-related tumors or evidence of "analgesics abuse" or an occupational risk for urothelial cancer. Mean age at diagnosis was 68.6 years (range, 34-93 years). Controls were 268 healthy subjects from the same database (French population), who came in our hospitals for noncancerous urological disease such as lithiasis or benign prostatic hyperplasia. Controls had no personal history of any cancer and matched with cases for gender, age, smoking habits (13), and ethnicity. A patient who smoked at least 20 cigarettes per day for 5 years was defined as a smoker as described previously (14). Their mean age was 68.6 years (range, 34-92 years; see Table 1).

The formalin-fixed, paraffin-embedded primary UUT-UCCs of all patients were retrieved and genomic DNA was extracted from 40- μ m normal tissue (nonurothelial) sections as described previously (5). DNA extraction was then done using the QIAmp DNA Mini Kit (Qiagen) according to the manufacturer's instructions. For controls, DNA was extracted from blood using a standardized protocol (15). *SULT1A1* genotyping was done by the 5' nuclease PCR method using the ABI/PE Biosystems TaqMan system. The sequences of the primers and probes were SULT1A1-213F: GGGAGATTCAA-AGATCCTGGAGTT, SULT1A1-213R: CGTGTGCTGA-ACCATGAAGTC, Vic-AGGGAGCGCCCCACA, and Fam-CAGGAGTGCCCCACA. The PCR amplification cycles were 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Levels of Fam and Vic fluorescence were determined and allelic discrimination was carried out using the ABI 7000 Sequence Detector. SULT1A1 genotyping was confirmed on several samples by PCR combined with restriction enzyme digestion. PCR was carried out in a final volume of 20 μ L containing 50 ng of genomic DNA, 800 nmol/L of each primer (GTTGGCTCTGCAGGGTTTCTAGGA and CC-CAAACCCCTGCTGGCCAGCACCC), 250 μ mol/L of

each deoxynucleotide triphosphate, 1 \times PCR buffer containing 1.5 mmol/L MgCl₂, and 1 unit of AmpliTaq Gold (Applied Biosystems). After PCR amplification, 10 μ L of PCR products were digested overnight at 37°C with 2 units of *Hae*II (New England Biolabs) in a final volume of 20 μ L. The products were separated on a 2% agarose gel and stained with ethidium bromide to identify the base pair change. Results obtained by the two PCR methods were in agreement for 98% of the 48 samples tested. To run the genotyping assay, we used one well filled in by water instead of DNA on each plate as negative control. As positive controls, six wells were filled in by DNA from individuals with an already known genotype (two A1/A1, two A2/A2, and two A1/A2) on each plate we used to run an assay. The size of the *SULT1A1* alleles was determined after digestion and migration of fragments obtained from both methods on agarose gels, as described previously (16). Allelic and genotypic frequencies between cases and controls, and correlations with clinical and pathology data, were assessed by χ^2 analysis. The odds ratio (OR) and 95% confidence intervals were calculated using unconditional logistic regression models (Statview, Abacus Concepts, Inc.). In addition, the false positive report probability was assessed as described previously (17). It represents the probability of no true association between a genetic variant and disease given a statistically significant finding.

Results

Patient characteristics are given in Table 1; allele distribution and genotype frequencies are given in Table 2. Genotypes were in Hardy-Weinberg equilibrium for both patient cases and healthy controls. The variant allele frequency for controls (28.9%) agrees with reported values for a Caucasian population (6, 7, 18). The frequency of the variant was significantly higher in UUT-UCC cases than in controls (χ^2 test, $P = 0.004$; Table 2). The OR suggested a significantly increased risk of UUT-UCC in individuals carrying the variant His/His genotype (OR, 2.18; $P = 0.0046$), but not the Arg/His genotype (OR, 1.15; $P = 0.45$), compared with individuals with the wild Arg/Arg genotype. The OR for this variant was also significantly higher than for the combined wild allele genotypes (Arg/Arg + Arg/His), suggesting a recessive model of inheritance. Assuming that the variant confers an OR of 2 to 2.2 to UUT-UCC cancer risk, the calculated range of false positive report probability was below the suggested criterion of 0.2 (17). There was no association between tumor aggressiveness and allele or genotype distribution, whether for grade (G1-G3) or stage (superficial versus invasive, T_a versus T₁, T₂ versus T₃ + T₄).

Discussion

Results on the effect of SULT1A1 polymorphisms on bladder cancer risk are conflicting. Studies of UUT-UCCs in more than 50 patients are scarce (2, 5). Although our results need to be confirmed in a larger population-based study, to our knowledge, the current study is the first to report that the variant His/His genotype in phenol sulfotransferase is associated with an increase risk of developing UUT-UCC. Our findings on UUT-UCC do not confirm the marginally protective effect of the

Table 1. Patient characteristics and distributions of selected variables by case status

	UUT-UCC cases (N = 268), n (%)	Controls (N = 268), n (%)
Gender		
Male	189 (70.5)	189 (70.5)
Female	79 (29.5)	79 (29.5)
Age (y), mean \pm SD	68.6 \pm 11	68.6 \pm 14
Smoking status		
Never	102 (38)	100 (37.3)
Current/former*	166 (62)	168 (62.7)
Tumor stage		
Superficial		
T _a	53 (19.7)	
T _{is}	7 (2.6)	
T ₁	63 (23.5)	
Invasive		
T ₂	50 (18.6)	
T ₃	71 (26.4)	
T ₄	24 (8.9)	
Tumor grade		
G1	36 (13.4)	
G2	115 (42.9)	
G3	117 (43.7)	

*At least 20 cigarettes a day for 5 y (14).

Table 2. Frequencies and risk estimates for *SULT1A1* genotypes and alleles

	UUT-UCC cases (N = 268), n (%)	Controls (N = 268), n (%)	OR (95% confidence interval)	P
Alleles				
Arg	337 (62.9)	381 (71.1)	1	
His	199 (37.1)	155 (28.9)	1.45 (1.12-1.87)	0.0043
Genotypes (codominant model)				
Arg/Arg	119 (44.4)	140 (52.2)	1	
Arg/His	99 (36.9)	101 (37.7)	1.15 (0.78-1.67)	0.45
His/His	50 (18.7)	27 (10.1)	2.18 (1.28-3.69)	0.0046
Genotypes (recessive model for His allele)				
Arg/Arg + Arg/His	218 (81.3)	241 (89.9)	1	
His/His	50 (18.7)	27 (10.1)	2.05 (1.3-3.4)	0.0052

Arg213His polymorphism with respect to bladder cancer risk (6, 8, 19). A reduced bladder cancer risk in individuals with the *SULT1A1**2 allele genotypes (low-activity *SULT1A1**2 allozyme profile) has previously been established (OR, 0.72; 95% confidence interval, 0.54-0.97). However, it was reported in an unusual population (i.e., women and nonsmoking patients; ref. 8). Our results are more in line with the high-activity *SULT1A1**1 allozyme being associated with a reduced risk of colorectal, head and neck, and lung cancer (7, 18, 20). Although it is not unusual that a genotype confers protection for an organ and increases the risk for another, our results underline the fact that UUT-UCC may share some risk factors or molecular disruption pathways with bladder UCC, but each has its own specific features (5, 11, 12, 14). Currently, 20% to 30% of patients with a UUT-UCC have a history of bladder cancer whereas fewer than 2% of patients with bladder cancer develop a UUT-UCC; microsatellite instability is more common in UUT than bladder UCCs (5, 11, 14). The genetic risk of developing UUT-UCC may therefore depend on the presence of specific pathways for carcinogens in the UUT (5). Genetic polymorphism of enzymes metabolizing carcinogens would yield products of different activity and may determine cancer risk. In addition, difference between upper tract and lower tract urothelium has recently been described in the biological characteristics of the urothelium in the ureter and renal pelvis compared with the bladder (21).

Besides, our findings suggest that susceptibility to UUT-UCC may be linked to complex interactions between a recessive gene and the environment. Recent evidence to support the functional significance of *SULT1A1* was the finding that the enzyme sulfonates a wide variety of lipophilic compounds (22). Therefore, the altered formation of sulfo conjugate of xenobiotic and endogenous (endobiotic) small molecules by members of the *SULT* enzyme family seems to be an essential step of carcinogenesis. Because of its broad presence in human tissues and the functional significance of its frequent polymorphism, *SULT1A1* gene has been conjectured to be a potentially important low-penetrance cancer-predisposing gene. Not forgetting that the major causal factor for urothelial carcinoma remains carcinogenic aromatic amines, such as 4-aminobiphenyl detected in cigarette smoke, this process involves numerous competing metabolic steps. Carcinogenic aromatic amines are known to undergo *N*-hydroxylation by cytochrome *P*450. Subsequently, the produced *N*-hydroxy aromatic amines are further *O*-sulfated by arylamine phenol sulfotransferase to yield highly reactive intermediates capable of

binding DNA (23). We believe that the His/His genotype, corresponding to low-activity *SULT1A1* enzyme, conferred a risk of UUT-UCC due to low detoxification of phenolic xenobiotics. However, *SULT1A1* also catalyzes the sulfation of certain *N*-hydroxy heterocyclic and aromatic amines found in the environment. In these instances, instead of serving as a detoxification mechanism, sulfation generates a reactive species capable of adducting to cellular macromolecules including DNA, which, if not repaired, can initiate carcinogenesis (19, 23). Thus, differences observed among studies might also involve differences either of carcinogens or of populations in polymorphisms in other enzymes such as cytochrome *P*450s and especially *N*-acetyltransferases (9, 19, 24).

We now need to explore the relationship between the *SULT1A1* genotype and other genetic polymorphisms of detoxification/bioactivation enzymes and the differential effects of exposure to different substrates on the risk of developing UUT and/or bladder cancer.

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