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Sulfotransferase 1*A*1 G638A polymorphism, cigarette smoking and bladder cancer risk in Taiwan

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ABSTRACT

Cigarette smoking is a major risk factor for bladder cancer (BC). *Sulfotransferase* 1*A*1 (*SULT*1*A*1), a phase II enzyme, plays an important role in the metabolism of several carcinogens contained in cigarettes. The aim of this study was to investigate the relationship between *SULT*1*A*1 G638A polymorphism, cigarette smoking and bladder cancer risk in Taiwan. A total of 150 BC patients and 150 cancer-free controls were recruited from February 2002 to February 2009. Genotyping of the *SULT*1*A*1 G638A polymerphism was determined using the polymerase chain reaction-restricted fragment length polymorphism (PCR-RFLP) method. The odds ratio (OR) and 95% confidence interval (CI) were calculated as a measure of the combined effect of cigarette smoking and the *SULT*1*A*1 G638A polymorphism on BC risk. In the present study, we found that study subjects with the G/G genotype of the *SULT*1*A*1 gene had a significantly higher BC risk of 1.7 (95% CI = 1.3 - 3.2) compared with those carrying the combination of G/A and A/A genotypes. Moreover, ever smokers who carried the G/G genotype of the *SULT*1*A*1 gene had a significantly increased UC risk of 3.5 (95% CI = 2.5 - 10.2) compared with never smokers who carried the G/A and A/A genotypes as the reference group. In conclusion, our findings suggest that *SULT*1*A*1 G638A polymorphism is associated with the development of BC, especially among cigarette smokers.

Keywords: Bladder Cancer; Cigarette Smoking; Polymorphism; *SULT*1*A*1

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1. INTRODUCTION

Bladder cancer (BC) is the eighth most common diagnosed malignancy among men in Taiwan [1]. Cigarette smoking is considered to be the key risk factor for bladder cancer [2,3]. Cigarettes contain about fifty-five carcinogens that have been evaluated by the International Agency for Research on Cancer [4]. Among these carcinogens, polycyclic aromatic hydrocarbons (PAHs), aromatic amines and *N*-nitroso compounds are considered to be major risk factors for the development of urothelial cancer. Additional risk factors include occupational exposure to carcinogenic chemicals, inflammatory reactions to parasites (such as schistosomiasis) or other chronic infections, and exposure to arsenic in drinking water are known risk factors for bladder cancer [5,6].

Genetic polymorphisms of the enzymes that catalyze xenobiotically-produced carcinogens may determine individual susceptibility to cancer. Most chemical carcinogens in cigarettes require metabolic activation by phase I enzymes and detoxification by phase II enzymes. Metabolic activation of PAHs by phase I enzymes leads to oxidized products, including quinones, resulting in reactive oxygen species (ROS) [7]. In contrast, detoxification of certain carcinogens leads to less toxic and more hydrophilic derivatives, which are more readily excreted.

Sulfotransferases (SULTs), a family of multifunctional enzymes, catalyze sulfonate conjugation. This is an important pathway in the metabolism of several chemicals that are exogenous (e.g. mutagens from diet and environment) or endogenous (e.g. hormones and neurotransmitters). In addition to its important role in metabolic detoxification, *SULT*1*A*1 may act to bioactivate dietary and environmental procarcinogens and promutagens [8,9]. In particular, *SULT*1*A*1 appears to be a key phenol *SULT* because of its abundance and distribution in a wide range of tissues [10]. The common polymorphism of *SULT*1*A*1

involves a single nucleotide G to A transition at nucleotide 638 (codon 213) in exon 7, which results in an arginine (Arg) to histidine (His) amino acid substitution and this polymorphism may lead to a lower enzyme activity and thermostability [11]. Epidemiological studies have shown inconsistent results for the association between the *SULT*1*A*1 G638A polymorphism and several malignancies including bladder cancer, lung cancer and breast cancer [12-15]. Moreover, due to its abundance in human tissues and significantly different expression among various genetic polymorphisms, *SULT*1*A*1 is considered to be a potentially cancer-predisposing gene.

Therefore, we conducted a hospital-based case-control study to explore the role of *SULT*1*A*1 G638A polymorphism in the development of bladder cancer in Taiwan. We also sought to investigate the combined effect of the *SULT*1*A*1 G638A polymorphism and cigarette smoking.

2. MATERIALS AND METHODS

2.1. Study Population

We studied a total of 150 histologically confirmed patients with bladder cancer (BC), diagnosed at the Chi-Mei Medical Center and the Department of Urology of the Shin Kong Wu Ho-Su Memorial Hospital between February 2002 and February 2009. A total of 150 cancer-free controls, frequency-matched with BC patients for age (±3 years) and gender, were selected from individuals who admitted to the same hospitals for a health examination and had no history of urological malignancies. All subjects were given an explanation of the present study, and then informed consents were obtained. All participants were interviewed by a well-trained interviewer using a structured questionnaire to collect information including a history of cigarette smoking. The study protocol was approved by the institutional review board at both collaborated hospitals.

2.2. Genotyping of the *SULT*1*A*1 G638A Polymorphism

A venous blood sample (6 - 8 ml) was drawn into an EDTA vial for each participant. Genomic DNA was extracted from peripheral lymphocytes by proteinase K digestion and phenol/chloroform method, which was stored at a –80˚C for further genotyping. We used a previously described polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method to determine the genotypes of *SULT*1*A*1 G638A polymerphism at codon 213 of exon 7 was also performed according to a previously published PCR-RFLP method [12]. The frequent homozygous genotype (G/G) yielded two band (168 and 165 bp), the heterozygous genotype (G/A) yielded three bands (333, 168 and 165 bp), and the variant homozygous genotype (A/A) yielded one band (333 bp). To ensure the quality, positive and negative tests were used in each experiment.

2.3. Statistical Analysis

The Hardy-Weinberg equilibrium (HWE) test was evaluated by comparing the observed with the expected genotype frequencies among controls using a Chi-square test. Study subjects who consumed more than 100 cigarettes during their lifetime were defined as ever smokers, while those who consumed less than 100 cigarettes were defined as never smokers. The number of pack-years was calculated using the formula: pack-years = (cigarettes per day/20) × (smoked years). The odds ratio (OR) and 95% confidence interval (CI) were calculated as a measure of the effect of the *SULT*1*A*1 G638A polymorphism on BC risk. Furthermore, the combined effect of the *SULT*1*A*1 G638A polymorphism and cigarette smoking on BC risk was estimated by a multivariate-adjusted logistic regression. Statistical Analysis Software (SAS, Version 9.1; SAS Institute, Cary, NC) was used for all analyses. The differences between BC cases and controls were considered significant if the p-values were less than 0.05.

3. RESULTS

3.1. The Distribution of Basic Characteristics

The distribution of basic characteristics for BC cases and controls is shown in **Table 1**. Among BC cases, 94 were male (mean age ± SD, 63.5 ± 12.3 years) and 56 were female (64.5 ± 12.3 years). Among controls, 97 were male (mean age ± SD, 63.6 ± 11.6 years) and 53 were female (61.9 ± 11.3 years). There were no significant differences in the distribution of age and gender between BC cases and controls. We found a significantly increased BC risk in ever smokers (OR = 1.8; 95% C = 1.4 - 3.2). The median value of pack-years among controls who had smoked was 30 pack-years. A significantly increased BC risk was also found in study subjects who smoked more than 30 pack-years (OR = 1.8; 95% CI = 1.3 - 3.7).

3.2. Comparison of *SULT*1*A*1 G638A Polymorphism

The distribution of the observed genotype frequencies was in Hardy-Weinberg equilibrium for *SULT*1*A*1 (P = 0.728). Study subjects who carried the G/G genotype of *SULT*1*A*1 gene have a non-significant higher BC risk (OR = 1.7; 95% CI = 0.8 - 16.4), compared with those with the A/A genotype. However, compared with individuals who carried the combination of *SULT*1*A*1 A/A and G/A genotypes, those with the G/G genotype had a significantly higher BC risk of 1.7 (95% CI = 1.3 - 3.2)

(**Table 2**).

3.3. Combination Analysis of *SULT1A1* G638A Polymorphism and Smoking

Because we hypothesized that *SULT1A1* G638A polymorphism would modulate the effect of chemical carcinogens in cigarettes on BC, we examined the combined effect of *SULT1A1* G638A polymorphism and cigarette smoking. **Table 3** outlines the relationship between the *SULT1A1* G638A polymorphism and BC risk by stratification of cigarette smoking. Comparing with never smokers who carried the G/A and A/A genotypes of the *SULT1A1* gene as the reference group, significantly increased BC risks of 2.1 (95% CI = 1.2 - 5.0), 2.4 (95% CI = 1.3 - 8.5) and 3.5 (95% CI = 2.5 - 10.2) were found for never smokers with the G/G genotype, ever smokers with the G/A and A/A genotypes and ever smokers with the G/G genotype, respectively.

Table 1. Distribution of basic characteristics for BC cases and controls.

	BC cases	Controls	OR[a] (95% CI)
	n (%)	n (%)	
Age (years), Mean ± SD	64.2 ± 12.3	63.3 ± 11.8	
Gender			
Female	56 (37.3)	53 (35.3)	
Male	94 (62.7)	97 (64.7)	
Cigarette smoking			
Never	76 (50.7)	94 (62.7)	1.0
Ever	74 (49.3)	56 (37.3)	1.8 (1.4 - 3.2)**
Cigarette smoking[b] (pack-years)			
0	79 (52.7)	95 (63.3)	1.0†
1 - 30	35 (23.3)	31 (20.7)	1.4 (1.1 - 2.9)*
30+	36 (24.0)	24 (16.0)	1.8 (1.3 - 3.7)**

*$P < 0.05$, **$P < 0.01$, †P for trend < 0.05; [a]OR adjusted for age and gender.

Table 2. Distribution of *SULT1A1* G638A polymorphism in BC cases and controls.

SULT1A1 polymorphism	BC cases	Controls	OR[a] (95% CI)
	n (%)	n (%)	
A/A	2 (1.3)	3 (2.0)	1.0†
G/A	18 (12.0)	27 (18.0)	1.0 (0.3 - 9.3)
G/G	130 (86.7)	120 (80.0)	1.7 (0.8 - 16.4)
A/A + G/A	20 (13.3)	30 (20.0)	1.0
G/G	130 (86.7)	120 (80.0)	1.7 (1.3 - 3.2)**

*$P < 0.05$, **$P < 0.01$, †P for trend < 0.05; [a]OR adjusted for age, gender and cigarette smoking.

Table 3. The combined effect of *SULT1A1* G638A polymorphism and cigarette smoking on BC risk.

Cigarette smoking	*SULT1A1* polymorphism	BC cases	Controls	OR[a] (95% CI)
		n (%)	n (%)	
Never	A/A + G/A	8 (5.3)	19 (12.7)	1.0†
	G/G	68 (45.3)	76 (50.7)	2.1 (1.2 - 5.0)*
Ever	A/A + G/A	12 (8.0)	12 (8.0)	2.4 (1.3 - 8.5)*
	G/G	62 (41.4)	43 (28.6)	3.5 (2.5 - 10.2)**

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, †P for trend < 0.05; [a]OR adjusted for age and gender.

4. DISCUSSION

Our study evaluated whether the *SULT1A1* G638A polymorphism is associated with bladder cancer risk. We also investigated a potential interaction of the *SULT1A1* G638A polymorphism with cigarette smoking. Several studies have elucidated the association between cigarette smoking and bladder cancer [2,3,16]. We found that ever smokers have a significantly increased risk of BC, especially among those who had smoked over 30 pack-years.

Sulfonation is thought to be a detoxification pathway for various xenobiotics, and it is also involved in the bioactivation of several carcinogens by O-esterification to form DNA-damaging toxic metabolites [8,11]. The G to A polymorphism at position 638 of *SULT1A1* may result in reduced enzyme activity and reduced thermostability. Few prior studies had investigated the effect of *SULT1A1* on risk of cigarette-related cancer and no consistent results were observed [12,14,15]. We found that individuals carrying the G/G genotype of the *SULT1A1* gene have a significantly increased risk (OR = 1.7) for bladder cancer. Ever smokers with the G/G genotype of the *SULT1A1* gene have a significantly higher BC risk of 3.5. Our findings, however, differ from previous reports, which have observed an increased cancer risk or null results associated with the A allele of *SULT1A1* [13,15, 17]. Positive association was also reported for the A allele of S*ULT1A1* in esophageal cancer (OR = 3.5; 95% CI = 2.12 - 5.87) [18]. However, a Japanese study found a marginally protective effect of the A allele of the S*ULT1A1* gene on urothelial carcinoma [14]. Furthermore, a four-fold increased risk of colorectal adenomas was observed in cigarette smokers carrying the *SULT1A1* G/G genotype compared to never smokers with the G/A and A/A genotypes of *SULT1A1* [19]. The increased BC risk we observed may be due to the dual role of SULTs, such as *SULT1A1*, which are involved in both the detoxification and bioactivation of several carcinogens.

*SULT*1*A*1 activity is also known to depend upon tissue or organ specificity. *SULT*s have substrate-dependent effects and the *SULT*1*A*1 can activate both heterocyclic amines and aromatic amines to become DNA-binding species. This activity may be greatly reduced in those with the A/A phenotype [12]. Therefore, the association between *SULT*1*A*1 and the risk for cancer development may be explained by wide substrate specificity and different distribution of the enzyme within the tissue.

We noted that the divergent genetic background and differing carcinogen exposure in various populations may explain the differing risk assessments related to the genetic polymorphisms [20-22]. Our study is weakened somewhat by recall bias, in that it can be difficult to recall carcinogen exposure at time points in the past, and cases are more motivated than controls to recollect past exposures.

In conclusion, the present study provides epidemiologic evidence that the *SULT*1*A*1 G638A polymorphism can modulate individual susceptibility to BC, in combination with cigarette smoking. Further large studies are needed to estimate the effect of differing candidate alleles on carcinogens-metabolized enzymes and to provide more rigorous data on potential risk factors for BC.

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