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Genetic polymorphism of sulfotransferase 1A1, cigarette smoking, hazardous chemical exposure and urothelial cancer risk in a Taiwanese population

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Objectives: To investigate the association between genetic polymorphism of sulfotransferase1A1 (SULT1A1), cigarette smoking, hazardous chemical exposure and urothelial cancer risk in a Taiwanese population.

Methods: In a hospital-based case–control study, a total of 300 urothelial cancer (UC) cases and 300 cancer-free controls frequency-matched by age and gender were recruited from September 1998 to December 2005. The SULT1A1 arginine213histidine (Arg213His) polymorphism was genotyped using a polymerase chain reaction–restriction fragment length polymorphism method.

Results: We found that the significantly increased UC risks of ever smokers and heavy smokers (≥28 pack-years) were 2.1 (95% confidence interval [CI] = 1.4–3.3) and 2.2 (95% CI = 1.3–3.6), respectively. An increased UC risk of 1.8 (95% CI = 0.8–3.8) was observed among individuals with more than one item of hazardous chemical exposure, but it was not statistically significant. Compared with study subjects carrying the SULT1A1 Arg/Arg genotype, those with SULT1A1 Arg/His or His/His genotypes have a significantly decreased UC risk (Odds ratio [OR] = 0.5, 95% CI = 0.3–0.8). Heavy smokers carrying the SULT1A1 Arg/Arg genotype have a significantly increased UC risk (OR = 5.2, 95% CI = 2.3–11.6). Individuals who had been exposed to more than one item of hazardous chemicals and who carried the SULT1A1 Arg/Arg genotype have a significantly increased UC risk (OR = 3.7, 95% CI = 1.4–9.7). The highest significant increased UC risk (OR = 16.1, 95% CI = 2.9–87.2) was observed among ever smokers with hazardous chemical exposure and the SULT1A1 Arg/Arg genotype.

Conclusions: SULT1A1 Arg213His polymorphism is associated with the development of UC, especially among cigarette smokers exposed to hazardous chemicals.

Key words: cigarette smoking, genetic polymorphism, hazardous chemicals, sulfotransferase 1A1, urothelial cancer.

Introduction

Urothelial cancer including renal pelvis, ureter and bladder is the seventh leading incidence of male malignant neoplasm in Taiwan.[1] Cigarette smoking is generally considered to be the major risk factor for bladder cancer which, is the major site of urothelial cancer.[2,3] Cigarettes contain about fifty-five carcinogens that have been evaluated by the International Agency for Research on Cancer (IARC).[4] Among these carcinogens, polycyclic aromatic hydrocarbons (PAH), aromatic amines and *N*-nitroso compounds are thought to be major risk factors for the development of urothelial cancer. Carcinogenic chemical exposure in the occupations of painters, printers and pesticide workers, and in rubber, dye, leather and hairdressing production, inflammatory reactions to parasites or to chronic infections, and exposure to arsenic in drinking water are also well-known risk factors for bladder cancer.[5,6]

Genetic polymorphisms of enzymes that respond to catalyze xenobiotically- produced carcinogens may determine individual susceptibility to the development of cancer. Metabolism of chemical carcinogens through cigarette smoking and occupational exposure usually requires metabolic activation by phase I enzymes and detoxification by phase II enzymes. Metabolic activation of PAH by phase I enzymes may generate oxidized products including quinines and reactive oxygen species (ROS).[7] Relatively, detoxification of certain carcinogens produces less toxic or more hydrophilic derivatives, which are more readily excreted.

Sulfotransferases (SULT), a family of multifunctional enzymes, play a key role in catalyzing sulfonate conjugation and in metabolizing several endogenous and exogenous chemicals including hormones, neurotransmitters, and mutagens from diet and environment.[8] Recently, at least eleven different SULT enzymes have been identified in human beings.[9] Among them, SULT1A1 appears to be a key phenol SULT due to its abundance and distribution in a wide range of tissues.[10] In addition to its important role in metabolic detoxification, SULT1A1 may also act to bioactivate dietary and environmental procarcinogens and promutagens.[11] The SULT1A1 gene, located on chromosome 16p12.1-p11.2, contains several genetic polymorphisms that would affect the individual susceptibility to cancer. The most common polymorphism of SULT1A1 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 (Arg213His) may lead to a lower enzyme activity and thermostability.[12] Several epidemiological studies have shown inconsistent results for the association between the SULT1A1 Arg213His polymorphism and several malignancies including cancers of the urinary tract, prostate, lung and breast.[13–16] Moreover, due to its abundance in distinct human tissues and significantly different expression among various genetic polymorphisms, SULT1A1 is considered to be a potentially low-penetrance cancer- predisposing gene.

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Therefore, we conducted a hospital-based case–control study to investigate the association between SULT1A1 Arg213His polymorphism and urothelial cancer risk in a Taiwanese population. In addition, we also evaluated the joint effects of cigarette smoking, hazardous chemical exposure and SULT1A1 Arg213His polymorphism on the risk of development of urothelial cancer.

Methods

Study subjects and biospecimen collection

We recruited a total of 300 histologically confirmed urothelial cancer (UC) cases, diagnosed at the Department of Urology of the Shin Kong Wu Ho-Su Memorial Hospital and the ChiaYi Christian Hospital between September 1998 and December 2005. Pathological confirmation of UC was performed by regular urological practice including endoscopic biopsy and surgical resection of urinary tract tumors. Staging and grading of tumors was carried out according to the criteria of the tumor-node- metastasis (TNM) staging system and the World Health Organization International Society of Urological Pathology.[17] Clinical stage was then classified into two subgroups, including superficial (≤T1) and invasive (T2-T4). Pathological grade was also recorded as three subgroups (G1, G2 and G3). A total of 300 cancer-free controls, frequency-matched with UC cases on age and gender, were recruited from individuals who were admitted to the same hospitals for a health check-up and had no history of urological neoplastic diseases or any other malignancy. All study subjects received a detailed description of this study and signed informed consents. All participants were interviewed by a well-trained interviewer using a structured questionnaire to collect information including demographic characteristics, exposure to hazardous chemicals from hair dye, paint, pesticide and printing ink, and history of cigarette smoking and alcohol consumption. This study protocol was approved by the institutional review board at both collaborated hospitals.

DNA extraction and determination of SULT1A1 polymorphism

A venous blood sample (10 mL) was drawn into an ethylenediaminetetraacetic acid vial for each participant. Genomic DNA was extracted from peripheral lymphocytes by proteinase K digestion and phenol/chloroform method, which was stored in a −80°C refrigerator for further genotyping assay. We used a polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) method to determine the genotypes of the SULT1A1 Arg213His polymorphism at codon 213 of exon 7, which has been published previously.[13] In brief, the SULT1A1 gene was amplified by PCR using primers: SULT1A1F (5′-GTT GGC TCT GCA GGG TTT CTA GGA-3′) and SULT1A1R (5′-CCC AAA CCC CCT GCT GGC CAG CAC CC-3′). The 333 bp amplified fragment was then digested with a restriction enzyme, HaeII (New England Biolabs). The frequent homozygous genotype (Arg/Arg) yielded two band (168 and 165 bp), the heterozygous genotype (Arg/His) yielded three bands (333, 168 and 165 bp), and the variant homozygous genotype (His/His) yielded one band (333 bp). To ensure the quality of genotyping, 10% of random samples were repeated for genotyping with 100% concordance.

Statistical analysis

A χ^2 test was used to test Hardy–Weinberg equilibrium by comparing the observed genotype frequencies with the expected value among controls. Study subjects who consumed more than 100 cigarettes during their lifetime were defined as ever smokers, while those who consumed less than 100 cigarettes in their lifetime were defined as never smokers. The number of pack-years was calculated using the following formula: pack-years = (cigarettes/day ÷ 20) × (smoked years). For alcohol consumption, ever drinkers were recognized as those who had consumed alcohol three days or more per week and continued for at least six months, while the others were regarded as never drinkers. In addition, study subjects who had been exposed to hazardous chemicals including hair dye, paint, pesticide and printing ink seven times or more per week and continued for at least one month were classified as ever exposed, while the others without the exposed history were regarded as never exposed. To investigate the association between SULT1A1 Arg213His polymorphism and UC risk, the odds ratio (OR) and its 95% confidence interval (CI) was calculated using a multivariate-adjusted unconditional logistic regression model. To examine the joint effect between SULT1A1 Arg213His polymorphism and cigarette smoking on UC risk, a stratified analysis was carried out. SAS version 6.12 (SAS Institute, Cary, NC) was used for all analyses with two-tailed probabilities. The differences between compared groups were considered significant if the *P*-values were less than 0.05.

Results

Comparisons of characteristics between UC cases and controls

The distribution of basic characteristics for UC cases and controls is shown in Table 1. Among UC cases, 187 were male (mean age ± standard deviation [SD], 63.5 ± 12.2 years) and 113 were female (65.4 ± 12.4 years). Among controls, 194 were male (mean age ± SD, 63.6 ± 11.9 years) and 106 were female (60.9 ± 11.2 years). There were no significant differences in the distribution of age ($X^2 = 3.19$, $P = 0.20$) and gender ($X^2 = 0.35$, $P = 0.55$) between the UC case and the control groups. We found a significantly increased UC risk in ever smokers (OR = 2.1, 95% CI = 1.4–3.3) and also in alcohol drinkers (OR = 1.9, 95% CI = 1.2–3.2). The median value of pack-years among controls who had smoked in their lifetime was 28 pack-years; however, there were still four UC cases without this data. Compared with never smokers, significantly increased UC risks of 1.9 (95% CI = 1.1–3.2) and 2.2 (95% CI = 1.3–3.6) were observed for light (1–27 pack-years) and heavy smokers (≥28 pack-years), respectively. We found a higher UC risk of 1.8 (95% CI = 0.8–3.8) for those who had ever been exposed to more than one item of hazardous chemicals. Regarding clinical stage and pathological grade, higher proportions of UC cases were determined as superficial stage (≤T1, 60.7%) and high grade (≥G2, 84%). We evaluated the associations between SULT1A1 Arg213His polymorphism and clinical stage and pathological grade, respectively. The frequencies of superficial (≤T1) and invasive (T2-T4) tumors were 53.9% and 46.1% for Arg/His and His/His genotypes, and 61.7% and 38.3% for the Arg/Arg genotype. The frequencies of pathological grade (G1, G2 and G3) were 10.3%, 61.5% and 28.2% for Arg/His and His/His genotypes, and 16.9%, 42.5% and 40.6% for the Arg/Arg genotype. We found no significant associations between SULT1A1 Arg213 His polymorphism and clinical stage ($X^2 = 0.87$, $P = 0.349$) and pathological grade ($X^2 = 4.95$, $P = 0.084$), respectively.

Comparisons of SULT1A1 Arg213His polymorphism between UC cases and controls

Observed genotype frequencies of the SULT1A1 gene were in the Hardy–Weinberg equilibrium ($P = 0.828$). Among UC cases, the fre-

Table 1 Distribution of characteristics for urothelial cancer cases and controls

Characteristic	UC cases	Controls	OR (95% CI)
	n (%)	*n* (%)	
Age (years)			
<55	66 (22.0)	78 (26.0)	
55–69	121 (40.3)	129 (43.0)	
≥70	113 (37.7)	93 (31.0)	
Gender			
Female	113 (37.7)	106 (35.3)	
Male	187 (62.3)	194 (64.7)	
Cigarette smoking			
Never smokers	153 (51.0)	189 (63.0)	1.0[c]
Ever smokers	147 (49.0)	111 (37.0)	2.1 (1.4–3.3)**
Cigarette smoking (pack-years)[a]			
Never smokers	153 (51.7)	189 (63.0)	1.0[c],†
1–27	66 (22.3)	58 (19.3)	1.9 (1.1–3.2)*
≥28	77 (26.0)	53 (17.7)	2.2 (1.3–3.6)**
Alcohol consumption			
Never drinkers	235 (78.3)	264 (88.0)	1.0[d]
Ever drinkers	65 (21.7)	36 (12.0)	1.9 (1.2–3.2)**
Hazardous chemical exposure[b]			
0	143 (47.7)	150 (50.0)	1.0[e],†
1	137 (45.7)	138 (46.0)	1.0 (0.7–1.4)
≥2	20 (6.6)	12 (4.0)	1.8 (0.8–3.8)
Clinical stage			
Superficial (≤T1)	182 (60.7)	–	
Invasive (T2-T4)	118 (39.3)	–	
Pathological grade			
grade 1 (G1)	48 (16.0)	–	
grade 2 (G2)	135 (45.0)	–	
grade 3 (G3)	117 (39.0)	–	

*P < 0.05, **P < 0.01, †P for trend <0.05. [a]Pack-years of 4 UC cases were missing. [b]Item(s) of hazardous chemicals including hair dye, paint, pesticide and printing ink. [c]Adjustment for age, gender, alcohol consumption and hazardous chemical exposure. [d]Adjustment for age, gender, cigarette smoking and hazardous chemical exposure. [e]Adjustment for age, gender, cigarette smoking and alcohol consumption. CI, confidence interval; OR, odds ratio; UC, urothelial cancer.

quencies of Arg/His and His/His genotypes combined, and the Arg/Arg genotype were 14.2% and 85.8% for female and 12.3% and 87.7% for male, respectively. In addition, 25.5% and 74.5% for female and 17.0% and 83.0% for male were observed in the controls, respectively. We found no significant association between SULT1A1 Arg213His polymorphism and gender in UC cases ($X^2 = 0.22$, $P = 0.64$) and controls ($X^2 = 3.07$, $P = 0.08$), respectively. Compared with subjects carrying the SULT1A1 Arg/Arg genotype, individuals who carried SULT1A1 Arg/His or His/His genotypes have a significantly decreased UC risk of 0.5 (95% CI = 0.3–0.8) (Table 2).

Joint effects of cigarette smoking, hazardous chemical exposure and SULT1A1 Arg213His polymorphism on UC risk

We further investigated the joint effects of cigarette smoking, hazardous chemical exposure and the SULT1A1 Arg213His polymorphism

Table 2 Distribution of sulfotransferase1A1 arginine213histidine polymorphism in urothelial cancer cases and controls

SULT1A1 genotypes	UC cases	Controls	OR[a] (95% CI)
	n (%)	*n* (%)	
Arg/Arg	261 (87.0)	240 (80.0)	1.0†
Arg/His	37 (12.3)	54 (18.0)	0.5 (0.3–0.9)**
His/His	2 (0.7)	6 (2.0)	0.3 (0.1–1.6)
Arg/Arg	261 (87.0)	240 (80.0)	1.0
Arg/His + His/His	39 (13.0)	60 (20.0)	0.5 (0.3–0.8)**

**P < 0.01, †P for trend <0.05. [a]Adjustment for age, gender, cigarette smoking, alcohol consumption and hazardous chemical exposure. Arg, arginine; CI, confidence interval; His, histidine; OR, odds ratio; SULT1A1, sulfotransferase1A1; UC, urothelial cancer.

on UC risk (Table 3). Compared with never smokers carrying the SULT1A1 Arg/His or His/His genotypes as the reference group, we found that never smokers with the SULT1A1 Arg/Arg genotype have a significantly increased UC risk (OR = 2.5, 95% CI = 1.3–4.7). Moreover, light and heavy smokers carrying the SULT1A1 Arg/Arg genotype have significantly increased UC risks of 4.6 (95% CI = 2.1–10.4) and 5.2 (95% CI = 2.3–11.6), respectively (P for trend = 0.0003). In addition, a significantly increased UC risk of 3.6 (95% CI = 1.3–11.4) was observed among heavy smokers with SULT1A1 Arg/His and His/His genotypes. As shown in Table 3, we observed that individuals who carried the SULT1A1 Arg/Arg genotype have significantly increased UC risks of 2.5 (95% CI = 1.3–4.9) and 2.4 (95% CI = 1.2–4.6) for those who had ever been exposed to zero and one item of hazardous chemicals, respectively. In addition, a significantly higher UC risk of 3.7 (95% CI = 1.4–9.7) was observed for those who carried the same genotype of SULT1A1 and also had ever been exposed to more than one item of hazardous chemicals, showing a borderline joint effect between hazardous chemical exposure and SULT1A1 genotypes (P for trend = 0.092).

Combination analysis of SULT1A1 Arg213His polymorphism, cigarette smoking, and hazardous chemical exposure on UC risk

The combination analysis of SULT1A1 Arg213His polymorphism, cigarette smoking, and hazardous chemical exposure on UC risk is shown in Table 4. Compared with never smokers with less than two items of hazardous chemical exposure and SULT1A1 Arg/His or His/His genotypes as the referent group, we found a strikingly increased UC risk of 16.1 (95% CI = 2.9–87.2) for ever smokers who had been exposed to greater than and equal to two items of hazardous chemicals and carried the SULT1A1 Arg/Arg genotype. For study subjects with either a cigarette smoking habit (+) or a hazardous chemical exposure history (≥2), we found significantly increased UC risks of 3.1 (95% CI = 1.3–7.5) and 4.5 (95% CI = 2.2–9.2) for individuals carrying the SULT1A1 Arg/His or His/His genotypes and those with the SULT1A1 Arg/Arg genotype, respectively. Furthermore, study subjects with the SULT1A1 Arg/Arg genotype and without either a cigarette smoking habit or a hazardous chemical exposure history had a 2.5-fold risk (95% CI = 1.3–4.8) of urothelial cancer.

Table 3 Joint effects of cigarette smoking, hazardous chemical exposure and sulfotransferase1A1 arginine213histidine polymorphism on urothelial cancer risk

	SULT1A1 Arg213His polymorphism			
	Arg/His + His/His		Arg/Arg	
	Cases/controls	OR[c] (95% CI)	Cases/controls	OR[c] (95% CI)
Cigarette smoking[a]				
Never smokers	16/37	1.0† (reference)	137/152	2.5 (1.3–4.7)**
1–27	8/11	2.7 (0.8–8.5)	58/47	4.6 (2.1–10.4)***
≥28	14/12	3.6 (1.3–11.4)*	63/41	5.2 (2.3–11.6)***
Hazardous chemical exposure[b]				
0	16/31	1.0 (reference)	127/119	2.5 (1.3–4.9)**
1	22/29	1.5 (0.6–3.4)	115/109	2.4 (1.2–4.6)*
≥2	1/0	–	19/12	3.7 (1.4–9.7)**

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, †P for trend <0.05. [a]Pack-years of 4 UC cases were missing. [b]Item(s) of hazardous chemicals including hair dye, paint, pesticide and printing ink. [c]Adjustment for age, gender, alcohol consumption and hazardous chemical exposure. Arg, arginine; CI, confidence interval; His, histidine; OR, odds ratio; SULT1A1, sulfotransferase1A1.

Table 4 Combination analysis of cigarette smoking, hazardous chemical exposure and sulfotransferase1A1 arginine213histidine polymorphism on urothelial cancer risk

Cigarette smoking		Hazardous chemical exposure[a]		SULT1A1 Arg213His polymorphism			
				Arg/His + His/His		Arg/Arg	
Never (–)	Ever (+)	<2 (–)	≥2 (+)	Cases/controls	OR[b] (95% CI)	Cases/controls	OR[b] (95% CI)
–			–	16/37	1.0 (reference)	127/142	2.5 (1.3–4.8)**
–			+	22/23	3.1 (1.3–7.5)*	125/96	4.5 (2.2–9.2)***
+			–				
+			+	1/0	–	9/2	16.1 (2.9–87.2)***

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. [a]<2: study subjects with less than two items of hazardous chemicals; ≥2, those with greater than or equal to two items of hazardous chemicals. [b]Adjustment for age, gender and alcohol consumption. Arg, arginine; CI, confidence interval; His, histidine; OR, odds ratio; SULT1A1, sulfotransferase1A1.

Discussion

Cigarette smoking and occupational risk exposure are considered to be major risk factors for the development of urothelial cancer.[2,6] Sulfotransferase 1A1, a phase II enzyme, plays a role in the metabolism of carcinogens contained in cigarettes and hazardous chemicals of occupational risk exposure.[8,10] Polymorphisms of carcinogen-metabolized enzymes have been found to affect individual susceptibility to the development of cancer. In this study, we evaluated the association between SULT1A1 Arg213His polymorphism, cigarette smoking, hazardous chemical exposure and urothelial cancer risk in a Taiwanese population. We found that both ever smokers and heavy smokers (≥28 pack -years) have significantly increased risks of UC which had been confirmed in previous studies.[2,18] A significantly increased UC risk of 1.9 was observed for alcohol drinkers after adjustment for age, gender, cigarette smoking and hazardous chemical exposure. Based on a meta-analysis of sixteen epidemiological studies, it was suggested that there was a slightly increased cigarette smoking–adjusted risk of UC due to alcohol consumption among men.[19] Furthermore, we found that study subjects who had an exposed history of hazardous chemicals had a non-significantly increased UC risk. Epidemiological studies have shown that workers in high-risk occupations, including the chemical industry, hairdressing, leather and shoe manufacturing, painting, printing, agriculture, and the plastic and rubber industry had a significantly increased risk of bladder cancer.[20,21]

The differences in the distribution of SULT1A1 Arg213His polymorphism were observed in several studies carried out among various races (Table 5). The East Asian populations including Chinese, Japanese and Taiwanese have a higher frequency of the Arg/Arg genotype of SULT1A1 than that of non-East Asian populations including Caucasian, North Indian and Italian. Our study observed a consistent pattern regarding the distribution of the SULT1A1 Arg213His polymorphism. The genotype frequency of the SULT1A1 Arg213His polymorphism in our study showed a similar distribution pattern to Chinese and Japanese.

Table 5 The distribution of genotype frequencies for sulfotransferase1A1 arginine213histidine polymorphism in different populations and diseases

Populations/diseases	n^a	Genotype frequency (%)			Authors (Ref.)
		Arg/Arg	Arg/His	His/His	
Taiwanese/urothelial cancer	600	83.5	15.2	1.3	The present study
Caucasians/bladder cancer	770	46.8	42.7	10.5	Zheng *et al.*[13]
Japanese/urothelial cancer	612	78.5	19.9	1.6	Tsukino *et al.*[15]
Italian/bladder cancer	415	57.1	38.6	4.3	Hung *et al.*[22]
Japanese/urothelial cancer	380	74.2	22.4	3.4	Ozawa *et al.*[23]
Chinese/breast cancer	635	81.1	16.1	2.8	Han *et al.*[24]
Taiwanese/betel quid chewers	193	91.2	8.8	0.0	Wong *et al.*[25]
Caucasians/prostate cancer	853	42.6	44.2	13.2	Nowell *et al.*[26]
North Indian/lung cancer	225	38.2	52.5	9.3	Pachouri *et al.*[27]

[a]*n*: number of study subjects, including cases and controls. Arg, arginine; His, histidine.

In this study, heavy smokers (≥28 pack -years) carrying the SULT1A1 Arg/Arg genotype had a significantly increased UC risk of 5.2. A fourfold increased risk of colorectal adenomas was observed in cigarette smokers who had smoked for more than 25 years and carried the SULT1A1 Arg/Arg genotype compared with never smokers with the SULT1A1 Arg/His and His/His genotypes as the referent group.[28] Tsukino *et al.*[15] reported that there were non-significant increased UC risks of 1.74 and 1.09 for individuals carrying the His/His genotype of SULT1A1 and those with the His-allele, respectively, which was different from our study showing a significantly decreased UC risk for those with the His-allele of SULT1A1. However, after stratified by the amounts of cigarette smoking, they found that subjects who smoked for more than 33.5 pack-years and carried the Arg/Arg genotype have a significant increased UC risk as compared to non-smokers with the same genotype. We reanalyzed our data using never smokers with the Arg/Arg genotype as the referent group. A significantly increased UC risk of 2.1 was also observed for heavy smokers (≥28 pack-years) with the Arg/Arg genotype. Furthermore, one Japanese study indicated that individuals carrying the Arg/Arg genotype of SULT1A1 had a higher UC risk as compared to those with the His-allele.[23] Comparing Tsukino's study with ours, we found that heavy smokers carrying the Arg/Arg genotype of SULT1A1 might be a high-risk group for UC. Recently, some studies showed that *SULT1A2* (*1, *2 and *3) was in linkage disequilibrium with SULT1A1 (*1, *2 and *3) and it was found in Taiwanese but not in Japanese.[29,30] The combined effect of SULT1A1 and *SULT1A2* on UC risk needed to be explored and it might be a possible reason for the inconsistence between these two studies. Moreover, different reference groups of non-smokers with the Arg/Arg genotype in Tsukino's study and never smokers with Arg/His and His/His genotypes in our study might be another reason for the differences.

Regarding the level of hazardous chemical exposure, we observed a significantly higher UC risk for those who carried the SULT1A1 Arg/Arg genotype and had ever been exposed to more than one item of hazardous chemicals. Moreover, we observed the highest significant UC risk of 16.1 for study subjects who had both exposure to cigarette smoking and more than and equal to two items of hazardous chemicals and carried the SULT1A1 Arg/Arg genotype. This finding indicated that individuals carrying the SULT1A1 Arg/Arg genotype and also having exposure of both cigarette smoking and hazardous chemicals would have a significantly increased risk of UC. In addition to traditional risk factors of UC including cigarette smoking and hazardous chemical exposure, we also observed that alcohol consumption is a possible risk factor for the development of UC after adjustment for age, gender, cigarette smoking and hazardous chemical exposure. However, the association between alcohol consumption and UC risk is still unclear.[31,32]

The present study has some possible limitations. First, because this is a hospital- based case–control study, a selection bias should be carefully considered. To avoid this bias, subjects from the same residential area were recruited and with no significant difference of SULT1A1 genotype frequencies in Hardy–Weinberg equilibrium. Second, the frequency-matching of age and gender between UC cases and controls was not exactly equal. Because UC cases were male preponderance and older, it was difficult to recruit more eligible female or older subjects as controls. These reasons caused a slight difference in the distribution of age and gender between UC cases and controls. However, we found no significant differences in the distribution of age and gender between the two groups. Third, a more exact estimation of hazardous chemical exposure was not available in our study, due to the lack of detailed information on hazardous chemical exposure. Finally, more single nucleotide polymorphisms (SNP) of SULT1A1 were recently discovered.[33,34] We should take a better approach including tagging the SNP to clarify the detailed effect of SULT1A1 polymorphisms on UC risk.

Carcinogenic aromatic amines in cigarettes and hazardous chemicals are thought to be a major risk factor of UC and are known to undergo *N*-hydroxylation by cytochrome P450s (CYP). The *N*-hydroxy aromatic amines are further catalyzed by N-acetyltransferases (NAT) and SULT to produce reactive intermediates capable of binding to DNA. Previous studies have shown that individuals who are rapid for CYP1A2 and slow for NAT2 might have a higher risk for development of arylamine-induced UC. Low-activity alleles of *NAT2* were associated with UC risk, especially among cigarette smokers. Another study found that a combination of the SULT1A1 Arg/Arg genotype and slow *NAT2* genotypes may increase the risk of UC.[23] Further study with the gene-gene interaction of SULT1A1 and cigarette smoking–related genes on UC risk is necessary to examine the association between cigarette smoking and UC.

In conclusion, our study suggests that the SULT1A1 Arg/Arg genotype can significantly increase the risk of UC. In addition, a significantly increased UC risk was also observed among study subjects with an exposure history to both cigarette smoking and hazardous chemicals.

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