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CYP1A2-163C/A (rs762551) polymorphism and bladder cancer risk: a case-control study

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ABSTRACT. To date, no study has investigated the association between *CYP1A2*-163C/A polymorphism and bladder cancer risk in a Chinese population. Here, we extracted genomic DNA from peripheral white blood cells, and differentiated *CYP1A2* alleles by polymerase chain reaction-based restriction fragment length polymorphism methods. Differences in genotype frequencies between the cases and controls were evaluated using a chi-square test. The odds ratio (OR) and its 95% confidence interval (CI) were calculated using an unconditional logistic regression model. This revealed that the -163A allele was present at a significantly increased frequency in bladder cancer patients compared to healthy controls (44.10 *vs* 22.25%, $P < 0.001$). The prevalence of CC genotype, CA genotype, and AA genotype was 34.91, 41.98, and 23.11% in bladder cancer patients, and 64.00, 27.50, and 8.5% in the controls, respectively. Therefore, significant differences in the frequencies of -163 genotypes were found between bladder cancer patients and controls ($P < 0.001$). We found that the AA genotype was significantly associated with increased bladder cancer risk (OR = 3.72; 95%CI = 1.55-7.16; P = 0.02), and the -163A carriers were at increased risk of bladder cancer in a multivariate COX regression model (OR = 4.89, 95%CI = 2.78-10.87, P = 0.01). We conclude that the *CYP1A2*-

163C/A polymorphism is associated with increased susceptibility to bladder cancer in the Chinese population.

Key words: CYP1A2; Polymorphism; Bladder cancer; Risk

INTRODUCTION

Globally, bladder cancer is the seventh most common cancer in men and the seventeenth most common in women. It has a higher incidence in Western countries compared with Asian countries (Murta-Nascimento et al., 2007; Kakehi et al., 2010). Despite the role that environmental factors play in the development of bladder cancer, genetic factors are also closely related to the pathophysiology of the disease (Lin et al., 2006).

Human cytochrome P4501A2 (CYP1A2) is one of the major CYPs in the human liver (Nebert and Dalton, 2006). CYP1A2 is a critical enzyme for the catalysis of 2- and 4-hydroxylations of estrogens (Nebert and Dalton, 2006) and for the metabolism of carcinogens (Eaton et al., 1995). Previously, increased CYP1A2 activity has been associated with a number of environmental factors, including tobacco smoking, occupational exposure, diet, and coffee intake (Chung et al., 2000; Djordjevic et al., 2008). More recently, however, the increased activity has been attributed to specific polymorphisms in the *CYP1A2* gene (Pavanello et al., 2005; Gunes et al., 2009).

Previously, many studies have investigated the association between CYP1A2-163C/A polymorphism and cancer risk. Sun et al. (2014) performed a meta-analysis to investigate the association between the *CYP1A2*-163C/A polymorphism and cancer risk under different inheritance models. Overall, a significant association was observed between *CYP1A2*-163C/A polymorphism and cancer risk when all the eligible studies were pooled into the meta-analysis (dominant model: OR = 1.08, 95%CI = 1.02-1.15; heterozygous model: OR = 1.06, 95%CI = 1.01-1.12; additive model: OR = 1.07, 95%CI = 1.02-1.13). In further stratified and sensitivity analyses, a significantly increased lung cancer risk and significantly decreased bladder cancer risk were associated with the *CYP1A2*-163C/A polymorphism in Caucasians. However, there has been no study investigating the association between the *CYP1A2*-163C/A polymorphism and bladder cancer risk in a Chinese population.

MATERIAL AND METHODS

Study subjects

The study was approved by the Review Board of Qilu Hospital, Shandong University. Written informed consent was obtained from each participant. The study population consisted of 212 patients with bladder cancer. Patients who had previous cancer, metastasized cancer from other or unknown origin, previous radiotherapy or chemotherapy were excluded. All cases of pathological diagnosis for tumor stage were according to the 2002 International Union Against Cancer tumor-nodes-metastasis classification, and for grade as the World Health Organization in 1973 grading of urothelial papilloma: well differentiated (grade 1, G1), moderately differentiated (grade 2, G2), or poorly differentiated (grade 3, G3).

The control group included 200 healthy subjects who came to the hospital for general health exams. The controls were genetically unrelated cancer-free individuals living in the

same residential areas, and were frequency matched to the cases on age, gender, smoking status, and alcohol use.

Genotyping

Venous blood (10 mL) was collected from each patient into tubes containing 50 mM EDTA, and genomic DNA was isolated with a DNA blood Mini kit, according to the manufacturer instructions. Quality control measures included validation of results by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) genotyping and blind repeat of 10% of samples for the *CYP1A2*-163C/A polymorphic site. Briefly for the RFLP analysis, all PCRs (25 μL) were performed on a GeneAmp PCR System 9700, with each mastermix comprised of 0.2 mM dNTPs, 1 U Taq polymerase, the appropriate concentration of $MgCl_2$ (1.75 and 1.25 mM), and 0.4 mM of each primer. About 10% of the samples were randomly selected to perform repeat assays, and the results were 100% concordant. Two researchers, blinded to the clinical data, scored the genotypes independently.

Statistical analysis

Hardy-Weinberg equilibrium (HWE) was carried out by a goodness-of-test for the distribution of genotypic frequencies among the controls. Differences in the distributions of demographic characteristics, selected variables and genotype frequencies between the cases and controls were evaluated using the chi-square test (for categorical variables). The odds ratio (OR) and its 95% confidence interval (CI) were calculated using an unconditional logistic regression model. All analyses were performed using the SPSS 18.0 software. All statistical tests were two sided and $P < 0.05$ was considered statistically significant.

RESULTS

Characteristics of the study population

The key demographic and clinical information for 212 bladder cancer cases and 200 healthy controls are presented in Table 1. No significant difference in age or gender distribution was observed between cases and controls (χ^2 test, $P = 0.844$ and $P = 0.125$, respectively). Moreover, no significant difference in smoking status or drinking status distribution was observed between cases and controls (χ^2 test, $P = 0.599$ and $P = 0.367$, respectively, shown in Table 1).

CYP1A2-163C/A genotype frequencies in bladder cancer cases and controls

The genotype distributions for the *CYP1A2*-163C/A SNP in controls were in accordance with those predicted from the HWE model ($P > 0.05$). The allele and genotype frequencies of *CYP1A2*-163C/A for the cases and controls are presented in Table 2. Frequency of the -163A allele was significantly increased in bladder cancer patients compared to healthy controls (44.10 *vs* 22.25%, $P < 0.001$). The prevalence of CC genotype, CA genotype, and AA genotype was 34.91, 41.98, and 23.11% in bladder cancer patients, and 64.00, 27.50, and 8.5% in the controls, respectively. Therefore, significant differences in the frequencies of

-163 genotypes were found between bladder cancer patients and controls (P < 0.001, shown in Table 2).

Table 1. Characteristics of the study population.

Variables	Bladder cases (N = 212)		Healthy controls (N = 200)		P value
	N	%	N	%	
Age (years)					
≤65	89	41.98	91	45.50	0.844
>65	123	58.02	119	59.50	
Gender					
Male	127	59.91	135	67.50	0.125
Female	85	40.09	65	32.50	
Smoking status					
Never smoker	13	6.13	17	8.50	0.599
Former smoker	21	9.91	17	8.50	
Current smoker	178	83.96	166	83.00	
Alcohol drinking					
Never drinker	34	16.04	41	20.50	0.367
Former drinker	33	15.57	35	17.50	
Current drinker	145	68.40	124	62.00	
Tumor grade					
Low grade	87	41.04	-	-	
High grade	125	58.96	-	-	
Tumor stage					
NMIBC	123	58.02	-	-	
MIBC	89	41.98	-	-	

NMIBC = non-muscle-invasive bladder cancer; MIBC = muscle invasive bladder cancer.

Table 2. *CYP1A2*-163C/A genotype frequencies in bladder cancer cases and controls.

	Bladder cancer cases (N = 212)		Healthy controls (N = 200)		P value
	N	%	N	%	
CYP1A2 genotype					
CC	74	34.91	128	64.00	<0.001
CA	89	41.98	55	27.50	
AA	49	23.11	17	8.50	
Allele					
C	237	55.90	311	77.75	<0.001
A	187	44.10	89	22.25	

CYP1A2-163C/A polymorphisms and susceptibility to bladder cancer

We found that the AA genotype was significantly associated with increased bladder cancer risk (adjusted OR = 3.72; 95%CI = 1.55-7.16; P = 0.02). However, the CA genotype was not significantly associated with bladder cancer risk (adjusted OR = 1.84; 95%CI = 0.79-3.28; P = 0.16). We also found that -163A carriers were at increased risk of bladder cancer in a multivariate COX regression model, which was adjusted for age, gender, alcohol intake, and smoking status (OR = 4.89, 95%CI = 2.78-10.87, P = 0.01). There was no significant association between *CYP1A2*-163C/A polymorphisms and susceptibility to bladder cancer under both dominant and recessive models (OR = 2.97, 95%CI = 0.89-5.11, P = 0.11 and OR = 2.45, 95%CI = 1.55-4.58, P = 0.09, respectively, shown in Table 3).

Table 3. *CYP1A2*-163C/A polymorphism and susceptibility to bladder cancer.

	Patients	Controls	OR (95%CI)[1]	P value
General genotype				
CC	74	128	1.00 (Reference)	
CA	89	55	1.84 (0.79-3.28)	0.16
AA	49	17	3.72 (1.55-7.16)	0.02
Dominant genotype				
CC	74	128	1.00 (Reference)	
CA+AA	138	72	2.97 (0.89-5.11)	0.11
Recessive genotype				
CC+CA	163	183	1.00 (Reference)	
AA	49	17	2.45 (1.55-4.58)	0.09
Allele frequency				
C	237	311	1.00 (Reference)	
A	187	89	4.89 (2.78-10.87)	0.01

[1]Adjusted for gender, age, smoking status, and drinking status.

DISCUSSION

Epidemiologic studies have emphasized the significant contribution of food and lifestyle to bladder cancer risk (He and Shui, 2014; Liang et al., 2014). High-fat and low-fiber diets, as well as alcohol, tobacco, and red or processed meat consumption, produce high levels of polycyclic aromatic hydrocarbons and heterocyclic aromatic amines. These procarcinogenic agents are potentially very harmful and may play a key role in the malignant transformation of cells by interacting with DNA (Steinmaus et al., 2000). This risk may be due to carcinogenic polycyclic aromatic hydrocarbons and heterocyclic amines produced when meat is cooked at high temperatures (Wang and Jiang, 2012).

Environmental and genetic factors influence the activity of CYP1A2. Tobacco smoking and consumption of fried and grilled food, coffee and cruciferous vegetables increases CYP1A2 activity in humans (Djordjevic et al., 2008). However, intake of apiaceous-like vegetables and the use of oral contraceptives decrease this activity (Lampe et al., 2000). CYP1A2 activity is also modulated by specific polymorphisms in the *CYP1A2* gene (Dobrinas et al., 2011). Polymorphisms located in the 5'-non-coding promoter region [-3860G/A (rs2069514), -2467T/delT (rs3569413)] and in intron 1 [-163C/A (rs762551)] of the *CYP1A2* gene modified CYP1A2 activity of smokers, measured by the urinary caffeine metabolic ratio (Ghotbi et al., 2007; Gunes et al., 2009).

Previously, many studies have investigated the association between *CYP1A2*-163C/A polymorphism and the risk of cancer. Sun et al. (2014) performed a meta-analysis to investigate whether *CYP1A2*-163C/A polymorphism was associated with cancer risk under different inheritance models. Overall, a significant association was observed between *CYP1A2*-163C/A polymorphism and cancer risk when all the eligible studies were pooled into the meta-analysis (dominant model: OR = 1.08, 95%CI = 1.02-1.15; heterozygous model: OR = 1.06, 95%CI = 1.01-1.12; additive model: OR = 1.07, 95%CI = 1.02-1.13). In the further stratified and sensitivity analyses, for *CYP1A2*-163C/A polymorphism, significantly increased lung cancer risk and significantly decreased bladder cancer risk were observed in Caucasians. However, there have been no studies investigating the association between *CYP1A2*-163C/A polymorphism and bladder cancer risk in a Chinese population.

Here, we found that frequency of the -163A allele was significantly increased frequency in bladder cancer patients compared to healthy controls. The prevalences of CC,

CA, and AA genotypes were 34.91, 41.98, and 23.11% in bladder cancer patient; this was significantly different to the frequencies in controls (64.00, 27.50, and 8.5%, respectively). We found that the AA genotype was significantly associated with increased bladder cancer risk. We also found that -163A carriers were at increased risk of bladder cancer in a multivariate COX regression model, which was adjusted for age, gender, alcohol intake, and smoking status. We suggest that an important next step is to broaden this type of study to larger patient cohorts in order to strengthen our conclusions.

Conflicts of interest

The authors declare no conflict of interest.

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