Genetic Polymorphisms of *CYP2A6* and *CYP2E1* with Tobacco Smoking is not Associated with Risk of Urothelial Cancer

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Abstract

Objectives: To elucidate the association between genetic polymorphisms of *CYP2A6* and *CYP2E1* and urothelial cancer susceptibility.

Methods: A total of 137 Japanese patients with urothelial cancer and 217 Japanese healthy controls, frequency-matched for age and gender, were selected. The polymorphisms of *CYP2A6* and *CYP2E1* were analyzed by PCR-RFLP, and cigarette smoking histories were obtained through interviews.

Results: The frequency of *CYP2A6* homozygote deletion genotype was 2.9% in the patients, compared with 3.2% in the controls (OR=0.84, 95% CI 0.24–2.96). The frequencies of *CYP2E1* C1/c2 and C2/c2 were 27.7% and 4.4% in the patients, compared with 35.5% and 6.0% in the controls (OR=0.68, 95% CI 0.42–1.09, OR=0.67, 95% CI 0.24–1.84, respectively). No statistically significant differences were observed when the *CYP2A6* homozygote deletion genotype and the *CYP2E1* genotypes were examined relative to smoking status.

Conclusions: Our data indicate that neither a relationship between genetically impaired nitrosamine metabolism and tobacco-smoking consumption, nor urothelial cancer risk related to the *CYP2A6* deletion genotype and *CYP2E1* *Rsa* I genotype was found in Japanese population.

Key words: CYP2A6, CYP2E1, urothelial cancer, smoking, Japanese

Introduction

There is considerable evidence to support the view that carcinogenic *N*-nitrosamine derivatives are important factors in human cancer. Human CYP2A6 and CYP2E1 are enzymes known to be involved in the metabolic activation of *N*-nitrosamines (1–3). Recently, deletion-type alleles of the *CYP2A6* (*CYP2A6**4) have been identified (4), and a high frequency of the gene deletion among Asian populations has been reported (4, 5). Diminished CYP2A6 activity might decrease the production of genotoxic metabolites of these nitrosamines and potentially reduce the risk of tobacco-smoking related cancer by this mechanism. The

CYP2E1 gene is also present in the population in various polymorphic forms. The variant detectable by *Rsa* I digestion (called the C2 variant) contains polymorphic base substitution sites in a region of the gene that is not transcribed but that appears to be involved in the transcriptional regulation of CYP2E1 expression. In this study, we examine whether *CYP2A6* deletion and *CYP2E1* *Rsa* I polymorphism combined with tobacco smoking are associated with urothelial cancer risk in a Japanese study population.

Material and Methods

Blood samples were taken from 137 urothelial cancer patients (107 men, 30 women, mean age 69.3 years; 95 cases of bladder cancer, 14 cases of renal pelvic cancer, 16 cases of ureter cancer and 12 overlapping cases) and 217 healthy controls, all of whom were Japanese. Patients were treated at the University of Occupational and Environmental Health Hospital and had been newly histologically diagnosed with urothelial cancer during the period from September 1992 to June 1995. A total of 217 controls (169 men, 48 women, mean age 68.5 years), frequency-matched with cases for age and gender, were selected from the people who visited a medical

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Abbreviations: CYP, cytochrome P450; CYP2A6, cytochrome P4502A6; CYP2E1, cytochrome P4502E1; PCR, polymerase chain reaction, OR; odds ratio, 95% CI; 95% confidence interval.

Table 1 Relationship between CYP2A6 and CYP2E1 genotype and urothelial cancer

	Controls (n=217) %(n)	Patients (n=137) %(n)	Crude OR (95% CI)	OR* (95% CI)
CYP2A6 genotype				
*1/*1 or *1/*4	96.8% (210)	97.1% (133)	1	1
*4/*4	3.2% (7)	2.9% (4)	0.90 (0.26–3.14)	0.84 (0.24–2.96)
CYP2E1 genotype				
C1/c1	58.5% (127)	67.9% (93)	1	1
C1/c2	35.5% (77)	27.7% (38)	0.64 (0.40–1.02)	0.68 (0.42–1.09)
C2/c2	6.0% (13)	4.4% (6)	0.63 (0.23–1.72)	0.67 (0.24–1.84)
Risk for any C2 allele vs C1/c1	41.5% (90)	32.1% (44)	0.67 (0.43–1.05)	0.67 (0.43–1.05)

*ORs and 95% CI were adjusted for age, gender and smoking status.

institution located in Kitakyushu City for a general health check-up between September 1993 and April 1995. All study subjects completed a questionnaire administered by a trained interviewer, covering medical, residential, occupational and smoking history. "Smoking" was summarized as smoker (those who had smoked) or non-smoker (those who had never smoked). All participants were given an explanation of the nature of the study, and informed consent was obtained. This study was approved by the ethics committee of University of Occupational and Environmental Health.

Genomic DNA was isolated from peripheral leukocytes by proteinase K digestion and phenol/chloroform extraction. The single PCR and RFLP methods were used to identify the *CYP2A6* wild and variant alleles described, using the method described previously (4). A *CYP2A6* – specific PCR reaction was accomplished with the primer pair Kd1F (forward): 5' CCT GAT CGA CTA GGC GTG GTA 3' and E3R (reverse): 5' TCG TCC TGG GTG TTT TCC TTC 3', yielding a single 215-base pair (bp) product. One hundred nanograms of DNA were amplified in a total volume of 40 µl containing 70 pmol of each primer, 1U of Taq polymerase, 2.2 mM MgCl₂, and PCR buffer. The amplification was performed by denaturing at 94°C for 3 min, and annealing and extending at 94°C for 30s, 56°C for 30s, and 72°C for 45s for 35 cycles. Ten µl each of the PCR products were then digested without further purification by the restriction enzymes *Msp* I, *Xcm* I and *Dde* I in a total volume of 15 µl. The digested PCR products were identified in 3% agarose gels with ethidium bromide. The fragment patterns determined the presence of the *CYP2A6**1, *CYP2A6**2 and *CYP2A6**3. The genetic polymorphism in the 5'-flanking region of *CYP2E1* was determined by PCR amplification followed by digestion with *Rsa* I, using the method described previously (6). The predominant allele (*C1*) was sensitive to *Rsa* I digestion, and the *C2* allele was resistant to *Rsa* I digestion.

Crude odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated for *CYP2A6* and *CYP2E1* genotypes. Odds ratios were adjusted for age, gender and smoking status, using multiple logistic regression analysis by SPSS Medical Pack for Windows.

Results and Discussion

The frequencies of both *CYP2A6* deletion and *CYP2E1*

Rsa I genotype for patients with urothelial cancer and controls, and their relation to urothelial cancer risk, are shown in Table 1. The frequencies of *CYP2A6* *1/*1 or *1/*4 and *4/*4 were 97.1% and 2.9% in the patients, compared with 96.8% and 3.2% in the controls. There were no significant differences in these frequencies between the cancer cases and controls. The adjusted odds ratio of the *CYP2A6* was 0.90 (95% CI 0.26–3.14). There were no *CYP2A6**2 or *CYP2A6**3 alleles among our samples. The population of the individuals with mutant *CYP2E1* was higher in the controls (35.5% vs. 27.7% for the heterozygote *C1/c2* genotype; 6.0% vs. 4.4% for the homozygote *C2/c2* genotype), the adjusted odds ratios being 0.68 and 0.67, respectively (95% CI 0.42–1.09 and 0.24–1.84). A comparison between individuals with homozygous *C1* alleles and those with *C2* alleles (*C1/c2* and *C2/c2*) indicated an odds ratio of 0.67 (95% CI 0.43–1.05). In the present study, we had 80% power (two-sided test of significance, $\alpha=0.05$) to detect an OR of 3.0 for *CYP2A6* deletion type. For *CYP2E1*, we had 80% power (two-sided test of significance, $\alpha=0.05$) to detect an OR of 1.9 (*C1/c2*), 3.3 (*C2/c2*) and 1.9 (any *C2* allele) relative to the *C1/c1* genotype, respectively.

In this study, we did not find any relationships between *CYP2A6* deletion genotype and urothelial cancer development. Recently a novel mutation in exon 9 of the *CYP2A6* gene was reported (7). This variant allele decreased CYP2A6 activity and was contained in the *CYP2A6**1 allele. There is the possibility that this variant allele modifies the relationship between the *CYP2A6* deletion genotype and cancer susceptibility.

Our study is the first to investigate the relationship between *CYP2A6* deletion and urothelial cancer development. Our data indicate that neither a relationship between genetically impaired nitrosamine metabolism and tobacco-smoking consumption, nor urothelial cancer risk related to the *CYP2A6* deletion genotype and *CYP2E1* *Rsa* I genotype was found.

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