Sulfotransferase 1A1 Arg^{213}His polymorphism and prostate cancer risk

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Abstract. Sulfotransferase 1A1 (SULT1A1) is a member of the sulfotransferase family that plays an important role in the biotransformation of numerous carcinogenic and mutagenic compounds through sulfation. A transition, G to A at position 638, in the SULT1A1 gene, results in the Arg^{213}His change. This single nucleotide polymorphism reduces the activity and thermostability of the SULT1A1 enzyme. In the present study, the relationship between the SULT1A1 Arg^{213}His polymorphism and prostate cancer was investigated using PCR-RFLP. No significant difference in genotype and allele distribution was noted between the prostate cancer and control populations (P=0.072; P=0.099, respectively). The risk of prostate cancer in individuals carrying the SULT1A1*2 allele (His^{213} allele) was determined by combining the SULT1A1*1/SULT1A1*2 (Arg/His^{213}) and SULT1A1*2/SULT1A1*2 (His/His^{213}) genotypes. No association was observed between SULT1A1 Arg^{213}His polymorphism and prostate cancer incidence (P=0.24; OR, 1.36; 95% CI, 0.84-2.25). However, the His^{213} allele was found to increase the risk of prostate cancer by 1.36-fold. In smoker and non-smoker populations, no significant relationship was determined between the prostate cancer and control population (P=0.45; P=0.34, respectively).

Introduction

Human sulfotransferases (SULTs) catalyze the conjugation of sulfate groups to a variety of endogen and exogenous substrates, including many drugs, neurotransmitters, thyroid and steroid hormones and pro-carcinogenic agents (1,2). SULTs are genetically polymorphic and are expressed in a wide variety of tissues, such as the liver, lung, brain, kidney, and platelets (3). To date, 13 human cytosolic SULT isoforms have been identified and grouped as four major families: SULT1, SULT2, SULT4 and SULT6 (4). The SULT1A1 gene mapped to chromosome 16p12.1-p11.2 encodes four different allozymes: SULT1A1*1 (wild-type), SULT1A1*2, SULT1A1*3 and SULT1A1*4. The SULT1A1 enzyme catalyzes the sulfation of certain carcinogenic and mutagenic compounds including heterocyclic and aromatic amines, and polycyclic aromatic hydrocarbons (2). A genetic polymorphism in exon 7 of the SULT1A1 gene at the nucleotide of 638 (codon 213), results in a substitution of histidine by arginine (Arg^{213}His). SULT1A1*2 (His^{213} allele) is associated with less enzymatic activity and thermal stability compared with the wild-type allele (Arg^{213} allele) in platelets (5,6).

Prostate cancer, a serious health problem in the Western world and Turkey, has shown an increasing incidence over the last decade (7). Some reports suggest that the risk of prostate cancer development is influenced by both genetic and environmental factors, such as diet, hormone levels, drinking habit, ethnicity and genetic background (8). It has been suggested by researchers that the SULT1A1 Arg^{213}His polymorphism may affect an individual's capacity in the metabolism of numerous endogenous and exogenous compounds consequently resulting in the susceptibility of an individual to cancer (2). Studies have demonstrated the relationship between genetic polymorphisms of SULT1A1 Arg^{213}His and several cancer types including prostate cancer (9-16). On the other hand, the findings of these studies remain controversial. This study investigated, for the first time, the relationship between the SULT1A1 Arg^{213}His polymorphism and prostate cancer susceptibility in a Turkish population.

Materials and methods

Study population. The study population consisted of a total of 255 Turkish men (104 cases and 151 controls). The prostate cancer patients were treated at the Urology Department, Cumhuriyet University Hospital (Central Anatolia) during the year 2004. The patients were newly diagnosed and histologically confirmed to have prostate cancer and were previously untreated (by radiotherapy or chemotherapy). The prostate cancer patients had elevated serum levels of prostate-specific antigen (PSA). The controls were selected randomly from healthy individuals without a history of cancer and having serum levels of PSA <4 ng/ml. Members of the study populations were informed in regards to the aim of this study. During

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the study period, critical information, such as age and smoking habit were collected from the members using a standardized questionnaire. This study was approved by the Ethics Committee of Cumhuriyet University.

SULT1A1 genotyping. Genomic DNA of the study populations was extracted from blood leukocytes using the standard phenol-chloroform method (17). SULT1A1 $Arg^{213}His$ genotypes were determined using polymerase chain reaction (PCR)-based restriction fragment length polymorphism (RFLP) assay. The PCR reaction was carried out in a total volume of 25 μl containing ~100 ng genomic DNA, 200 mM deoxynucleotide triphosphates (dATP, dCTP, dGTP and dTTP), 0.2 m of each SULT1A1 primer (forward, 5'-GGG TCT CTA GGA GAG GTG GC-3'; reverse, 5'-GCT GTG GTC CAT GAA CTC CT-3'), 1X reaction buffer [75 mM Tris-HCI pH 8.8 at 25˚C, 20 mM $(NH_4)_2SO_4$, 0.01% Tween-20, MBI Fermentas], 1.5 mM $MgCl_2$, and 2 units Taq polymerase (MBI Fermentas) in a thermal cycler (Techne, UK). PCR conditions consisted of 94˚ for, 5 min, followed by 35 cycles of 30 sec at 94˚C, 30 sec at 62˚C, 30 sec at 72˚C, with a final extension step at 72˚C for 5 min. Amplification products (333 bp) were observed in a 2% agarose gel. The PCR product (10 μl) was digested with 10 units of *Hae*II restriction enzyme (New England Biolabs, Beverly, MA) overnight. The fragments were separated in a 2.5% agarose gel. The SULT1A1*1/SULT1A1*1 (Arg/Arg^{213}) genotype yielded two distinct digestion products (168 and 165 bp), the SULT1A1*1/SULT1A1*2 (Arg/His^{213}) genotype yielded three distinct digestion products (333, 168 and 165 bp), and the SULT1A1*2/SULT1A1*2 (His/His^{213}) genotype yielded no digestion products (333 bp) (Fig. 1).

Statistical Package for the Social Sciences (SPSS) release 10.0.1 software was used to perform the statistical analyses. Hardy-Weinberg equilibrium, genotype frequencies and allele frequencies were tested by the Pearson's χ^2 test. The statistical significance of the differences in SULT1A1 $Arg^{213}His$ genotypes among the cases and controls was determined by the χ^2 test. Probability values <0.05 were regarded as statistically significant. Odds ratios and 95% confidence intervals (CIs) for prostate cancer were calculated by using a multivariate logistic regression analysis adjusting several confounding variables such as age and smoking status.

Results

Demographic characteristics of the cases and controls are summarized in Table I. Mean ages of the cases and controls were 65.2±13.2 years (range, 42-88) and 61±11.4 years (range, 41-82), respectively. No significant relationship was found between the cases and controls in terms of smoking status (P=0.83). Mean PSA levels were 3.4±0.5 and 30.6±11.2 ng/ml in the controls and patients, respectively.

SULT1A1 $Arg^{213}His$ allele and genotype frequencies are indicated in Table II. The genotype and allele frequencies were found to be in Hardy-Weinberg equilibrium. In the cases, the frequencies of the homozygous wild-type genotypes (Arg/Arg^{213}), the heterozygous genotype (Arg/His^{213}) and the homozygous variant genotype (His/His^{213}) were 52.8, 36.6 and 10.6%, respectively; in the controls, these frequencies were 60.3, 35.8 and 3.9%, respectively.

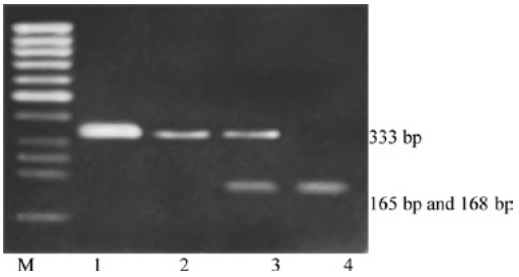

Figure 1. SULT1A1 genotypes determined by PCR-RFLP analysis. Lanes 1 and 2, SULT1A1*2/SULT1A1*2 genotype; lane 3, SULT1A1*1/SULT1A1*2 genotype; lane 4, SULT1A1*1/SULT1A1*1 genotype; and M, molecular weight marker (50 bp DNA ladder, Fermentas).

Table I. Characteristics of the prostate cancer patients and controls.

	Controls		Patients
Sample size (n)			
Males	151		104
Age (year)			
Range	41-82		42-88
Mean ± SD	61±11.4		65.2±13.2
Smoking history, n (%)			
Smokers	79 (52.3)		53 (50.9)
Non-smokers	72 (47.7)		51 (49.1)
χ^2		0.045	
P-value[a]		0.830	
PSA (ng/ml)	3.4±0.5		30.6±11.2

[a]P-values were calculated using the χ^2 test.

The risk of prostate cancer in individuals carrying the His^{213} allele was determined by combining Arg/His^{213} and His/His^{213} genotypes. No statistically significant difference was found between the cases and controls in comparison of the genotype combination (P=0.24; OR, 1.36; 95% CI, 0.84-2.25). Concerning the smoker and non-smoker populations, no significant relationship was evident between the case and control groups regarding genotypic combinations (P=0.45; OR, 1.32 and P=0.34; OR, 1.39, respectively) (Table III).

Discussion

The incidence of prostate cancer displays large ethnic variations worldwide. While the lowest incidence rate of prostate cancer is observed for Chinese men, African-American men have the highest rate of incidence (7). It is believed that advanced age, an intact androgen metabolism, ethnicity and genetic background are risk factors for prostate cancer development (8). The majority of studies suggest that genetic polymorphisms in xenobiotic metabolizing enzymes may play an important role in the susceptibility of individuals to cancer (18). In our previous study, we found that the GSTM1

Table II. Genotype and allele frequencies for SULTA1 locus in cases and controls.

Genotype	Controls (n=151)	Cancer patients (n=104)	P-value	χ^2
Allele frequency, n (%)			0.072	3.23
Arg allele	236 (78.1)	148 (71.1)		
His allele	66 (21.9)	60 (38.9)		
Genotype frequency, n (%)			0.099	4.62
Arg/Arg	91 (60.3)	55 (52.8)		
Arg/His	54 (35.8)	38 (36.6)		
His/His	6 (3.9)	11 (10.6)		

Table III. Risk estimates for SULTA1 genotypes categorized according to total cases and smoking status.

Variable	Genotype combinations	Controls n (%)	Cancer patients n (%)	χ^2	P-value	[a]OR (95% CI)
Total	Arg/Arg	91 (60.2)	55 (52.8)	1.37	0.24	1.36 (0.84-2.25)
	Arg/His + His/His	60 (39.8)	49 (47.2)			
Smoking status						
Smokers	Arg/Arg	44 (29.1)	26 (25)	0.56	0.45	1.32 (0.68-2.65)
	Arg/His + His/His	35 (23.17)	27 (25.9)			
Non-smokers	Arg/Arg	47 (31.12)	29 (27.8)	0.89	0.34	1.39 (0.62-2.91)
	Arg/His + His/His	25 (16.55)	22 (21.1)			

[a]Adjusted for age and smoking status, where appropriate.

null genotype may play an important role as a risk factor for prostate cancer development in the Turkish population (14).

Allelic frequencies of the His^{213} allele differ ranging from 5 to 32% among ethnic populations (5,19,20). The frequency of the His^{213} allele was reported to be 18.5 and 22% in a study of a Turkish population (21,22). In the present study, the frequency of this allele in the control population was determined to be 21.9% which was higher than the frequency in Chinese, Taiwanese and Koreans while lower than the frequency reported for Caucasian and Nigerian populations (5,19,20). Distributions of the SULT1A1 $Arg^{213}His$ genotypes and alleles were also not significantly different between the cases and controls in the present study.

In many studies, a significant relationship has been demonstrated between the His^{213} allele and various cancer types, including gastric, lung, colorectal, and breast (9,10,13,21). In addition, a statistical significant association was noted between the His^{213} allele and primary brain tumor and lung cancer incidence in our previous studies (21,22). However, in the present study, no significant relationship was determined between the His^{213} allele and prostate cancer although His^{213} allele frequencies were higher in the patients than in the controls. Another study which is in agreement with ours was reported by Steiner *et al* (15). In contrast, Nowell *et al* (11) found a positive association between the Arg^{213} allele (rapid sulfation allele) and prostate cancer risk. These controversial results may be due to the dual role (bioactivation and detoxification) of SULT1A1 in the metabolism of various carcinogens (23). Although SULT1A1 is considered as a phase II enzyme, it has been demonstrated that this enzyme acts to bioactivate various pro-carcinogens and pro-mutagens such as dietary carcinogen 2-amino-1-methyl-6-phenyl-imidazo (4,5-b) pyridine which induces prostate tumors in rats (24). Chou *et al* (25) and Ozawa *et al* (26) found that SULT1A1 catalyzes the sulfation of N-hydroxy derivates of arylamines and heterocyclic amines, to form more reactive DNA adduct-forming compounds. In this context, differing environmental parameters may also influence the function of a given allele. Thus, a different genetic background and different carcinogen exposure may play an important role in the different risk estimates associated with polymorphisms. In light of this knowledge, we believe that the influence of the SULT1A1 polymorphism on cancer development risk may differ according to the type of exposed carcinogen.

In the present study, there was no statistically significant association between the SULT1A1 $Arg^{213}His$ polymorphism and smoking status of the study population which is in accordance with previously published studies (21,22).

In summary, our results suggest that the SULT1A1 $Arg^{213}His$ polymorphism does not play a role in prostate cancer susceptibility in the Turkish population. However, since this study is the first report carried out in a Turkish population, it may contribute to the understanding of the relationship between the SULT1A1 polymorphism and prostate cancer. In order to elucidate the role of genetic polymorphisms in carcinogen metabolizing enzymes in prostate cancer development more

accurately, environmental exposure to specific carcinogens must be investigated in larger studies.

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