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The Effect of *CYP1A1* and *GSTM1* Gene Polymorphisms in Bladder Cancer Development in a Turkish Population

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Abstract. *Background: The aim of this study was to investigate a possible association of the CYP1A1 Ile462Val and GSTM1 null polymorphisms with the risk of developing bladder cancer in a Turkish population. Patients and Methods: The study constituted 176 patients with bladder cancer and 97 healthy individuals. Evaluation of CYP1A1 Ile462Val gene polymorphism was performed by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). GSTM1 null gene polymorphism was exclusively determined by PCR. Our results were examined by statistical analyses. Results: There were no significant differences in CYP1A1 genotype frequencies between patients and controls. Furthermore, the frequency of GSTM1 null genotype was higher in patients compared to controls, but it did not reach significance (p=0.622 χ^2=0.243 OR=0.94 95% CI=0.75-1.18). Significance was discovered in combined analysis of CYP1A1 and GSTM1 genotypes. In the present study, GSTM1 null genotype with CYP1A1 Ile/Ile genotype combination was significantly more frequent in the patient group than in controls (p=0,04, χ^2=4.217). At the same time, possessing both GSTM1 null genotype and CYP1A1 Val variants (Ile/Val+Val/Val) were significantly higher in control group than in patients (p=0.017, χ^2=5.468). When the pathological tumor grades were assessed, the frequency of CYP1A1 Val mutant variant with GSTM1 null genotype combination was higher in patients with medium and high-grade tumors than in those with low-grade tumors (p=0.06, χ^2=3.527, OR=1.36 95% CI=1.03-1.78). Conclusion: We suggest that the CYP1A1 Ile/Ile genotype with GSTM1 null genotype combination may contribute to the development of bladder cancer in this Turkish population.*

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Bladder cancer is the seventh most common cancer in men and the 17th most common cancer in women worldwide, with the highest incidence rates in Western countries and the lowest rates in Asian countries (1). Risk factors of bladder cancer can be classified as: genetic susceptibility, chemical and environmental exposures, and chronic irritation (2). Tobacco use is considered to be the most important cause of bladder cancer, accounting for 40-70% of the cases according to the WHO (3), and investigated in various epidemiological studies (4). Over 60 tobacco carcinogens, including polycyclic aromatic hydrocarbons (PAHs) such as benzo[α]pyrene, and aromatic amines, such as 2-naphthylamine and 4-aminobiphenyl, have been associated with the induction of detoxifying enzymes (5).

Genetic differences in the detoxification metabolism of xenobiotics, is thought to play a major role in individuals susceptibility to environmentally induced cancer. Endogenous chemicals and exogenous xenobiotics are mainly activated or inactivated by phase I and phase II enzymes in two steps. The phase I enzymes include several forms of cytochrome P450 (CYP450), and microsomal epoxide hydrolyases (mEHs); the phase II enzymes include glutathione-*S*-transferases (GSTs), and *N*-acetyl-transferases (NATs).

Metabolic activation means conversion of PAHs into more hydrophilic and more chemically active derivatives, is mainly initiated by the CYP450 enzyme superfamily (6). CYP1A1 is a phase I microsomal enzyme involved in the bio-activation of several carcinogenic PAHs including benzo[α]pyrene (7). Several polymorphisms of *CYP1A1* have been found, corresponding to 15 different allelic variants believed to lead to variance of gene expression or mRNA stability (8). The relationship between different *CYP1A1* variants and multiple forms of cancer, including those of the lung, head and neck

and urinary tract, have been investigated in a number of studies (9-11). A/G single base substitution at position 2455 (rs1048943) in the heme-binding region of exon 7 of CYP1A1, causing Ile462Val amino acid substitution, also known as CYP1A1*2B or m^2, results in an increase in enzyme activity (12). This mutation is also in complete linkage disequilibrium with *CYP1A1 MspI* (m1) mutation, which was associated with increased catalytic activity in a Caucasian population (13). Positive associations between the presence of these variant alleles and increased PAH DNA adducts have been reported (14-16).

GSTs are a family of phase II enzymes that catalyze the conjugation of many compounds and products of phase I reactions to glutathione. In humans, GSTA, GSTM, GSTT, and GSTP enzymes and their subfamilies are encoded by eight distinct gene families (17). The genetic polymorphisms of *GSTT1, GSTM1* and *GSTP1* have been studied extensively in the determination of individual cancer risks (18). Recent meta-analyses of GSTs and bladder cancer revealed increased risk associated with *GSTM1* null, and a modest increase in risk with *GSTT1* null and *GSTP1* Ile105Val polymorphisms (19, 20). Three alleles have been described for the *GSTM1* gene localized on chromosome 1 (1p13.3). The null allele, the result of a deletion, leads to the complete loss of enzyme activity/expression in the homozygous form (21). In literature various studies associate the *GSTM1* null allele with gastric, colorectal, lung, breast, and head and neck cancer (22-25). A relationship between GSTM1 deficiency and bladder cancer was first reported in 1993 by Bell *et al.*; several studies have since appeared supporting these findings in literature (26-28).

In the present study, we aimed to investigate a possible association of the *CYP1A1* Ile462Val and *GSTM1* null polymorphisms with the risk of developing bladder cancer in a Turkish population.

Patients and Methods

Participants. A total of 273 unrelated individuals were included in this study; 176 bladder cancer patients and 97 controls. The patients were selected from the Department of Pathology, Faculty of Cerrahpasa Medicine, Univesity of Istanbul. The control group was selected from healthy blood donors. A detail medical history was recorded and phsycial and pathological examinations were performed for all patients in the study.

DNA extraction. The patients' DNA was extracted from paraffin-embedded tissue with use of a method taken from Greer *et al*. (29). For genotyping, the DNA extracted from blood of the controls and from the paraffin-embedded samples from non-tumoral neighboring bladder tissue was used. The controls' genomic DNA was isolated from venous whole blood samples (from leukoyctes) by a method based on sodium dodecyl sulphate lysis, ammonium acetate extraction and ethanol precipitation (30).

Methods of genotyping. *CYP1A1* Ile462Val polymorphism was determined by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis. *GSTM1* null polymorphism was exclusively determined by PCR.

The polymorphic site at position 2455 of the *CYP1A1* gene was amplified with use of forward (5'-AAA GGC TGG GTC CAC CCT CT-3') and reverse (5'-CCA GGA AGA GAA AGA CCT CCC AGC GGG CCA-3') primers. PCR was performed with Taq polymerase; the cycling conditions were 94˚C for 2 min followed by 35 cycles of 94˚C for 1 min, 60˚C for 1 min, and 72˚C for 2 min. The amplified 263 bp PCR product was directly digested by the restriction enzyme *NcoI* (MBI Fermentas, Vilnius, Lithuania) to 231 bp and 32 bp. DNA from the homozygote mutant (Val/Val) variant was unrestricted by *NcoI* (single 263 bp band). *NcoI*-restricted homozygote wild (Ile/Ile) variant had a single 231 bp band. The heterozygote mutant (Ile/Val) variant had both 263 and 231 bp bands.

The absence of GSTM1 activity is caused by inheritance of two null alleles (alleles that have a deletion of the *GSTM1* gene). Primers used for *GSTM1* null polymorphism were as follows: P1: 5'-CGC CAT CTT GTG CTA CAT TGC CCG-3', P2: 5'-ATC TTC TCC TCT TTC TGT CTC-3', P3: 5'-TTC TGG ATT GTA GCA GAT CA-3'. PCR was performed with Taq polymerase; the cycling conditions were 94˚C for 2 min followed by 35 cycles of 94˚C for 1 min, 60˚C for 1 min, and 72˚C for 2 min. Both of the amplified 157 and 230 bp PCR products arose from the *GSTM1*+ genotypes (non-null), whereas only the 157 bp PCR product was produced from the *GSTM1* null genotype.

PCR products were seperated in 2% agarose gel in 1X Trisborate EDTA buffer and DNA was visualized by ethidium bromide staining. Both of the two gene polymorphisms were typed by visualization under ultraviolet light and were photographed with a KODAK Gel Logic 100 Imaging System.

Statistical analysis. Statistical analyses were performed using SPSS version 11.5 (SPSS Inc, Chicago, IL, USA) including the Chi-square (χ^2) test, Fisher's exact test and the Pearson correlation test, and odds ratio (OR) and 95% confidence intervals (Cl) were calculated. Mean values were compared between patients and controls by unpaired Student's *t*-test. Values of $p<0.05$ were considered statistically significant.

Results

Each group was compared with regards to age, gender and smoking habit in study groups. The mean ages of bladder cancer patients and controls were 61.47±13.28 and 56.64±12.81 years, respectively. There were significant differences in gender and smoking habit between bladder cancer patients and controls, as expected. The frequency distribution of men and women was considerably different for the patients (10.2% females, 89.8% males). There were significantly more smokers in the patient group ($p=0.001$, $\chi^2=11.995$).

Genotype frequencies for *CYP1A1* Ile462Val and *GSTM1* null polymorphisms are given in Table I. There were no significant differences in *CYP1A1* genotype frequencies between patients and controls. The frequency of *GSTM1* null genotypes was higher in patients compared to controls, but

Table I. *Genotype frequencies of CYP1A1 Ile462Val and GSTM1 null polymorphisms among bladder cancer patients and controls.*

Genotype	Patients n (%)	Controls n (%)	*p*-Value	OR (95% CI)
CYP1A1				
Ile/Ile	118 (67)	56 (57.1)	0.153	
Ile/Val	52 (29.5)	40 (40.8)		
Val/Val	6 (3.4)	2 (2)		
GSTM1				
Non-null (+)	78 (44.3)	46 (47.4)	0.622	0.882 (0.537-1.451)
Null	98 (55.7)	51 (52.6)		

n: Number of individuals; Chi-square test; OR: Odds ratio.

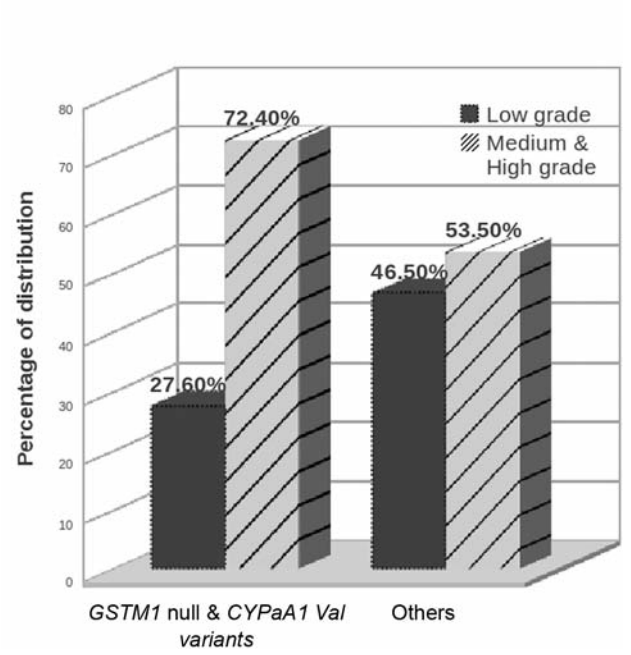

Figure 1. *Comparison of pathological tumor grades associated with CYP1A1 Val mutant variants and GSTM1 null genotype, and other haplotypes.*

this did not reach significance (p=0.622). When *CYP1A1* and *GSTM1* genotypes and gender were compared, there were no significant differences in the patient group (p>0.05). The association of genotypes and the histopathological parameters were also assessed in the study groups. *GSTM1* null genotype was more frequent in patients with medium and high-grade tumors compared to patients with the *GSTM1* non-null genotype but there were no statistically significant differences (p>0.05, OR=1.19, 95% CI=0.91-1.56).

Combined analysis was conducted to assess the cumulative effects of possible risk and protective attributes of the alleles. Significance reached statistical importance in combined analysis of *CYP1A1* and *GSTM1* genotypes (Table II). Firstly, the *GSTM1* null genotype with *CYP1A1* Ile/Ile genotype combination was significantly more frequent in the patient group than in controls (p=0.017). The *GSTM1* null genotype with *CYP1A1* Val variants (Ile/Val+Val/Val) were significantly more frequent in the control group than in patients (p=0.04). When the pathological parameters were evaluated, *CYP1A1* Val mutant variants with *GSTM1* null genotype combination tended to be more frequent in patients with medium- and high-grade tumors than in those with low-grade tumors (p=0.06, χ^2=3.527, OR=1.36 95% CI=1.03-1.78) (Figure 1).

Discussion

Epidemiological studies suggest that genetic polymorphisms of detoxifying enzymes may have a role in individual susceptibility to bladder cancer especially when in combinational with environmental factors.

CYP1A1 polymorphism is involved in the detoxifying metabolism of polycyclic aromatic hydrocarbons. The *CYP1A1* Ile462Val polymorphism is a result of A to G substitution in exon 7 causing an amino acid change in the heme-binding region. The protein of the Val variant shows an almost 2-fold higher catalytic enzyme activity than that of the Ile form (12, 31, 32). However, the *in vitro* kinetic parameters of the two forms varies against different substrates (33). U.S. smokers with the Ile462Val polymorphism had higher levels of PAH DNA adducts than those of U.S. smokers without the variant (34). Interestingly, the Val/Val genotype is strongly correlated with lung cancer incidence in Japanese, corroboratively in one of our previous studies we found similar results in a Turkish population. In contrast, in the present study, the Ile/Ile genotype tended to be more frequent in bladder cancer patients compared to controls. Brockmoller *et al*. did not find any relation between *CYP1A1* and bladder cancer; similarly Katoh *et al*. found no association with urothelial cancers in Japanese population (35, 36). Accordingly, studies of Houlston and Johns suggest a non-significant role for *CYP1A1* in cancer susceptibility (37). The accumulated data suggest that *CYP1A1* Ile462Val polymorphism may play different roles in different tumor types, since different active carcinogenic chemicals may be causing these different tumors. However, the possibility of different roles of the polymorphisms in cancer development may also exist among different populations. Therefore, parallel studies are worthwhile in order to evaluate the role of the *CYP1A1* polymorphism in the development of different tumor types in certain populations.

GSTM1 is an enzyme able to detoxify reactive intermediates of PAHs, preventing them from becoming carcinogens. The *GSTM1* locus is entirely absent from approximately 50% of Caucasian populations. The lack of

Table II. *The frequencies of haplotypes of CYP1A1 and GSTM1 genes in bladder cancer patients and controls.*

Haplotype associations	Patients n (%)	Controls n (%)	χ^2	*p*-Value	OR (95% CI)
GSTM1 null: CYP1A1 Ile/Ile					
Other genotypes	68 (38.6)	24 (24.5)	5.468	0.017	1.941 (1.119-3.370)
	108 (61.4)	74 (75.5)			
GSTM1 non-null: CYP1A1 Ile/Ile					
Other genotypes	50 (28.4)	31 (32)	0.378	0.539	0.845 (0.493-1.447)
	126 (71.6)	66 (68)			
GSTM1 null: CYP1A1 Ile/Val +Val/Val:					
Other genotypes	30 (17)	27 (27.6)	4.217	0.04	0.54 (0.299-0.977)
	146 (83)	71 (72.4)			
GSTM1 non-null: CYP1A1 Ile/Val +Val/Val					
Other genotypes	28 (15.9)	15 (15.5)	0.009	0.923	1.034 (0.523-2.047)
	148 (84.1)	82 (84.5)			

n: Number of individuals, OR: odds ratio.

GSTM1 locus appears to be common in Asian populations as well, and this genotype shows obvious ethnic variation. *GSTM1* deletion associated with increased risk of certain types of cancer (38). Multiple epidemiological studies have suggested GSTM1 as a risk factor for bladder cancer (21, 36, 39), and the deletion has been of this gene was reported to modulate the level of mutations in suppressor genes as *TP53* in bladder cancer patients (35). However, recent studies, such as that of Sobti *et al*. found no correlation between *GSTM1* null genotype and bladder cancer (40). Similiarly, our findings also corroborate those of Sobti *et al*. Cigarette smoking and occupational exposure to arylamines are well-established factors in bladder cancer etiology (41). Several studies have suggested that *GSTM1* null genotype carriers have an increased risk for tobacco-related bladder cancer (42) but some studies have observed no association between smoking habit and bladder cancer. In our population-based case control study, we found no statistically significant relation between *GSTM1* null polymorphism and smoking status supporting the findings of Salagovic *et al*. (21).

Multifarious studies also suggested allelic combinations of the *CYP1A1* Val variants with the *GSTM1* null allele clearly correlate with an increased cancer incidence (43, 44). On the contrary, haplotype association of *GSTM1* null genotype with *CYP1A1* Ile/Ile genotype was significantly higher in the patient group than in controls in the present study (p=0.017). The *GSTM1* null genotype with *CYP1A1* Val variant combinations were significantly more frequent in the control group than in patients (p=0.04). Furthermore, we also compared pathological parameters and combined analysis of *CYP1A1* and *GSTM1* alleles; *CYP1A1* Val genotypes with *GSTM1* null genotype combination was more frequent in medium- and high-grade tumors compared to the low-grade tumors in the patient.

As a conclusion, our results suggest that the *CYP1A1* Ile/Ile genotype with *GSTM1* null genotype combination may be associated with an increased risk of bladder cancer. Although *CYP1A1* Val genotype with *GSTM1* null genotype combinations are not directly associated with risk of bladder cancer, they might be associated with higher grade tumor in patients (p=0.06). Epidemiological studies show that the cooperative effect of polymorphisms in detoxification mechanisms with environmental factors is much more significant than the solitary effects of polymorphisms. In this respect, findings will be more conclusive when the data is expanded to include polymorphisms and haplotypes of other detoxification enzymes.

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