Polymorphisms of glutathione S-transferase genes (GSTM1, GSTP1 and GSTT1) and bladder cancer susceptibility in the Turkish population

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Abstract We investigated the effect of the GSTM1 and GSTT1 null genotypes, and GSTP1 313 A/G polymorphism on bladder cancer susceptibility in a case control study of 121 bladder cancer patients, and 121 age- and sex-matched controls of the Turkish population. The adjusted odds ratio for age, sex, and smoking status is 1.94 [95% confidence intervals (CI) 1.15–3.26] for the GSTM1 null genotype, and 1.75 (95% CI 1.03–2.99) for the GSTP1 313 A/G or G/G genotypes. GSTT1 was shown not to be associated with bladder cancer. Combination of the two high-risk genotypes, GSTM1 null and GSTP1 313 A/G or G/G, revealed that the risk increases to 3.91-fold (95% CI 1.88–8.13) compared with the combination of the low-risk genotypes of these loci. In individuals with the combined risk factors of cigarette smoking and the GSTM1 null genotype, the risk of bladder cancer is 2.81 times (95% CI 1.23–6.35) that of persons who both carry the GSTM1-present genotype and do not smoke. Similarly, the risk is 2.38-fold (95% CI 1.12–4.95) for the combined GSTP1 313 A/G and G/G genotypes and smoking. These findings support the role for the GSTM1 null and the GSTP1 313 AG or GG genotypes in the development of bladder cancer. Furthermore, gene-gene (GSTM1-GSTP1) and gene-environment (GSTM1-smoking, GSTP1-smoking) interactions increase this risk substantially.

Keywords Bladder cancer · Gene polymorphism · Glutathione transferase

Introduction

Bladder cancer is the third most common cancer in males, and the eighth most common cancer in females of the Turkish population (Öz賽和 Atasever 1997). Environmental risk factors play a substantial role in the development of this malignancy. They include cigarette smoke, chlorophenazine, phenacetin-containing analgesics, cyclophosphamide, arsenic, and occupational exposure to aromatic amines (Johansson and Cohen 1997). The type and amount of exposure to the carcinogen, and its metabolism in the body are important factors for cancer risk. Drug metabolizing enzymes carry out the metabolism of carcinogens in two phases. Phase I enzymes, such as the cytochromes P450, introduce an electrophilic centre, and hence activate the carcinogen. Phase II enzymes detoxify the activated carcinogen by introducing a hydrophilic group such as glutathione in to the metabolite (Lang and Pelkonen 1999).

Glutathione S-transferases (GSTs), which conjugate glutathione, comprise a gene super-family made up of four individual gene families called α, μ, θ and π. Allelic polymorphisms in GST genes have been defined, and disease-association studies were conducted in different populations for various cancers (Strange and Fryer 1999). Among them, the most extensively studied are the GSTM1 null, the GSTP1 313 A/G and the GSTT1 null polymorphisms. The functional consequences of the GSTM1 and the GSTT1 null genotypes are obvious in terms of enzyme activity: no gene, no enzyme, and no activity. The GSTP1 313 A/G polymorphism at the nucleotide level leads to an amino acid variation of isoleucine/valine at codon 105 in the protein. The valine
amino acid results in decreased enzyme activity (Ali-Osman et al. 1997). Despite this neat theoretical framework, the results of association studies between GST genotypes and bladder cancer are discordant in different populations (Bell et al. 1993; Zhong et al. 1993; Lin et al. 1994; Anwar et al. 1996; Brockmoller et al. 1996; Kempkes et al. 1996; Okkels et al. 1996; Golka et al. 1997; Harries et al. 1997; Katoh et al. 1999; Salagovic et al. 1999; Georgiou et al. 2000; Mungan et al. 2000; Steinhoff et al. 2000). Although methodological differences might be partially responsible for this discrepancy, the risk might be specific for the studied population because of differences in environmental factors, polymorphism frequencies and gene-gene interactions.

In this study, we determined the genotypic frequencies of the GSTM1 null, GSTP1 313 A/G and GSTTI null polymorphisms in bladder cancer patients, age- and sex-matched controls, and randomly selected individuals to understand whether these polymorphisms are associated with bladder cancer in the Turkish population.

Materials and methods

Peripheral blood samples were collected from 121 patients with bladder cancer (transitional cell carcinoma) diagnosed at Hacettepe University Medical School, and Ankara Numune Hospital. The mean age was 60.15 years, standard deviation 11.10, range 25–87; 72.0% of the patients were smokers, and the male:female ratio was 5:1. Information about sex, age of individual patients, and histopathology of the tumours was obtained from their medical records. Non-smokers are defined as individuals who never smoked, and smokers are individuals who smoked at least one packet of cigarettes daily for 1 year (i.e. one pack-year). The age-sex-matched control group comprised of 121 individuals from Atatürk Chest Disease Research Hospital (non-cancer patients). The mean age was 59.33 years, standard deviation 13.58, range 23–79; 63.8% were smokers and the male:female ratio was 5:1. All three hospitals are in Ankara, and serve patients predominantly from Central Anatolia. Seventy-seven randomly selected Bilkent University students were also included in the study. Informed consent was obtained from all subjects. Genomic DNA was isolated from 700 μl blood by standard phenol-chloroform extraction.

GSTM1 genotyping was performed by simultaneous amplification of GSTM1 primers (Anwar et al. 1996) with CYP2E1 primers (Anwar et al. 1996) in the same polymerase chain reaction (PCR) tube. These primers were:

- G1, 5'-GAA CTC CCT GAA ACG CTA AAG C
- G2, 5'-GTT GGG CTC AAA TAT ACG GTG G
- CYP2E1F, 5'-CCA GTC GAG TCT ACA TTG TCA
- CYP2E1R, 5'-TTT ACT CTG TCT TCT AAC TGG

PCR products were electrophoresed in 2% agarose gels, and visualized by ethidium bromide staining. Null genotype was scored by the presence of a 412-bp CYP2E1 band in the absence of a 215-bp GSTM1 fragment.

A313G polymorphism in GSTP1 was analysed using a previously described PCR-restriction fragment length polymorphism (RFLP) method (Harries et al. 1997). Briefly, amplification was carried out using primers:

- p105F, 5'-ACC CCA GGG CTC TAT GGG AA
- p105R, 5'-TGA GGG CAC AAG AAC CCC CT

The 176-bp amplified product was digested with Alw26I and electrophoresed in 3% NuSieve gel. Presence of the restriction site resulted in two fragments of 91 bp and 85 bp, which is indicative of the G allele.

GSTTI genotype was determined by using the previously described primers (Bringuier et al. 1998):

- GSTT1F, 5'-AGG CAG CAG TGG GGG AGG ACC
- GSTT1R, 5'-CTC ACC GGA TCA TGG CCA GCA

in combination with the above mentioned GSTP1 primers. A GSTTI-specific 138-bp fragment was observed in positive individuals. Null genotype was scored after confirming with at least two independent experiments.

GSTM1 null, GSTP1 313 A/G or G/G, and GSTTI null are defined as the risk genotypes for statistical analyses. Odds ratio (OR) with 95% confidence intervals (CI), and χ² analyses were performed (Daniel 1998). Age-, sex- and smoking-adjusted OR with 95% CI were calculated by a multiple logistic regression model (Hosmer and Lemeshow 1989). Two-gene interactions were calculated by using a previously described model (Yang and Khoury 1997). Analyses were done by using SPSS v.10.0 software.

Results

The genotype frequencies of the GSTM1, GSTP1 and GSTTI polymorphisms in the patients, and the age-sex-matched control groups are summarized in Table 1. A group of randomly selected university students (n = 77) was also genotyped to compare with the age-sex-matched control group. In the randomly selected group, the