MDM2 T309G Polymorphism is Associated with Bladder Cancer

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Abstract. *Recently, a functional T to G polymorphism at nucleotide 309 in the promoter region of the MDM2 gene (rs: 2279744, SNP 309) has been identified. This polymorphism has an impact on the expression of the MDM2 gene, which is a key negative regulator of the tumor suppressor molecule p53. The effect of T309G polymorphism of the MDM2 gene on bladder cancer susceptibility was investigated in a case-control study of 75 bladder cancer patients and 103 controls from Turkey. The G/G genotype exhibited an increased risk of 2.68 (95% CI, 1.34-5.40) for bladder cancer compared with the combination of low-risk genotypes T/T and T/G at this locus. These results show an association between MDM2 T309G polymorphism and bladder cancer in our study group. To the best of our knowledge, this is the first study reporting that MDM2 T309G polymorphism may be a potential genetic susceptibility factor for bladder cancer.*

Bladder cancer is a major cause of morbidity and mortality. In the Turkish population, it is the third most common cancer in men and the eighth in women (1). Although multiple environmental and host genetic factors are known to be important in bladder cancer development, the exact molecular mechanisms of genetic susceptibility and molecular changes during malignant transformation are still under investigation.

Recently, a functional T to G polymorphism at nucleotide 309 in the promoter region of the *MDM2* gene (rs: 2279744) has been identified (2). We hypothesized that this gene polymorphism might be a critical predisposition factor for bladder cancer, as the MDM2 molecule is an important player in bladder cancer pathogenesis, evidenced by its over-expression in 30% of urothelial carcinoma (3). This oncoprotein attenuates p53 activity by promoting ubiquitin-mediated degradation (4). In addition to functional inactivation by *MDM2*, structural *TP53* mutations have been observed in 50% of urothelial cancer and these mutations were associated with poor prognosis, advanced stage and higher grade of the bladder cancer (3).

MDM2 T309G polymorphism is a functional polymorphism having an impact on the p53 protein level in the cell. The G allele confers an increased binding affinity to the Sp1 transcriptional activator, hence increased transcription of the *MDM2* gene. Eventually, the relative increase in the level of MDM2 protein causes a relative decrease in the level of the p53 protein (2).

It is recognized that host genetic factors modifying the genotoxicity of carcinogens are important for the genetic susceptibility to bladder cancer. For example, gene polymorphisms decreasing the carcinogen detoxification activity of glutathione S-tranferases and N-acetyl transferases are established predisposition factors for this cancer (5). The p53 molecule is considered to be the guardian of the genome, since it plays a vital part in various antineoplastic mechanisms such as cell cycle arrest, senescence and apoptosis, preventing the carcinogenic effect of mutagens (6). Therefore, it is conceivable that *MDM2* SNP 309, which has an effect on the level of p53, may also be a genetic predisposition factor for bladder cancer.

In order to investigate the role of MDM2 T309G polymorphism in bladder cancer, a case-control study was performed with 75 patients and 103 controls. Our results indicated an association between bladder cancer risk and *MDM2* SNP309 polymorphism in the group indicated.

Patients and Methods

Peripheral blood samples were collected from 75 bladder cancer patients and 103 age-matched controls (non-cancer) diagnosed at Hacettepe University Medical School, and Ankara Numune Hospital, Turkey. The mean age of the bladder cancer patients was 59.87 years, with a standard deviation of 12.54, range 25-87; the mean age of the control group was 59.33 years, with a standard deviation of 13.58, range 23-79. Genomic DNA was isolated from

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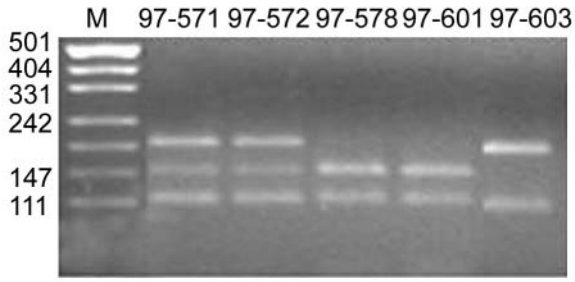

Figure 1. *MDM2 T309G polymorphism genotyping. MspAI1 was used to digest PCR products and the products were electrophoresed on 3% agarose gel. T309G polymorphism produces one more restriction site (147 bp, 111 bp, 46 bp), whereas the wild-type T allele produces two fragments (193 bp, 111 bp). 97-571 and 97-572 are examples of G/T heterozygotes; 97-578 and 97-601 are G/G homozygotes; and 97-603 is a T/T homozygote. M is the pUC mix 8 (MBI Fermentas).*

200 μl blood by standard phenol-chloroform extraction. *MDM2* T309G polymorphism was determined by polymerase chain reaction (PCR) and restriction digestion. The PCR amplification was carried out using primers: MDM2F (5'-GCTTTGCGGAGGTTTTGTT-3') and MDM2R (5'-TCAAGTTCAGACACGTTCCG-3'). After confirming the presence of the 304-bp amplicon on 2% agarose test gel, the PCR products were digested with MspA1I and electrophoresed in 3% agarose gel for SNP 309 genotyping. The T allele had a constitutional restriction site, which also served as an internal control for restriction digestion. The G allele had an additional restriction site to the constitutional restriction site. After digestion, T allele yielded two fragments (193 bp and 111 bp), where as the G allele yielded three fragments (147 bp, 111 bp and 46 bp) (Figure 1).

The G/G genotype was defined as the risk group for statistical analysis. Odds ratio (OD) tests with 95% confidence interval (CI) and χ^2 analysis were performed with the GraphPad Prism4 statistical software.

Results and Discussion

The genotype frequencies of *MDM2* T309G polymorphism in the bladder cancer patients and control groups are summarized in Table I. The genotype frequency values for the control group closely resembled the results from other Caucasian populations (7-9) and were in Hardy Weinberg equilibrium. The comparison of the high-risk genotype (G/G) with the combination of the two low-risk alleles (G/T and T/T) revealed that the G/G genotype conferred a risk of 2.68 (95% CI 1.34-5.40) relative to the low-risk genotypes (Table I). The G allele frequency in the patient group was 0.58 (T allele: 0.42), the control group it was 0.44 (T allele: 0.56). There was a significant difference between the allelic frequencies of the control (n=150 alleles) and patient groups (n=206 alleles) (χ^2: 6.76, df: 1, p=0.0093). Odds ratio analysis revealed that the G allele resulted in a 1.72-fold risk increase (95% CI 1.14-2.60) compared to the T allele.

Table I. *Distribution of the MDM2 SNP 309 genotypes in the bladder cancer patient and control group.*

Genotype	Patient group N=75 (100%)	Control group N=103 (100%)	Odds ratio (95% Cl) G/G *vs.* T/T+T/G	*p* value
T/T	13 (17.33)	29 (28.16)		
G/T	36 (48.00)	57 (55.34)		
G/G	26 (34.66)	17 (16.50)	2.68 (1.34-5.40)	0.0075

After the initial discovery of *MDM2* T309G polymorphism, several reports were published with discordant results regarding the impact of this polymorphism on cancer risk. In two separate studies, it was shown that G/G genotype caused a reduction in the age of onset of cancer in Li-Fraumeni syndrome patients (2, 10). However, no age of onset reduction was observed for Lynch syndrome (7). The case-control studies on colorectal cancer (9), squamous cell carcinoma of the head and neck (9), uterine leiomyosarcoma (9), breast (8, 11) and ovarian cancer (8) did not show an association. Interestingly, two lung cancer studies in the Chinese population reported discordant results: in one study an association was observed (12), while in the other it was not (13).

Issues with sampling and population stratification have always been cited for the lack of reproducibility between different case-control studies (14), but p53-related factors might also have contributed to such problems. It is intriguing that *MDM2* T309G polymorphism had an impact on a hereditary cancer syndrome (2, 10) characterized by germ line p53 mutations (*i.e.*, Li-Fraumeni syndrome), but had no effect on another hereditary cancer such as lynch syndrome (7) with relatively rare somatic p53 mutations (15).

In conclusion, this study showed an association between *MDM2* T309G polymorphism and bladder cancer in the Turkish population. The small sample size was a limitation of the study and the results should definitely be validated on larger bladder cancer cohorts in different populations. That said, to our knowledge, the study is the first study to indicate that *MDM2* T309G polymorphism could be a potential genetic susceptibility factor for bladder cancer.

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