Lack of Association between the MDM2-SNP309 Polymorphism and Breast Cancer Risk

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Abstract. *Background: A T-to-G polymorphism (SNP309) at the promoter region of MDM2 has been recently reported to extend the Sp1 binding site that positively regulates the MDM2 transcription level and consequently, its expression level. MDM2 is the negative regulator of p53 tumor suppressor protein and elevated levels of MDM2 hamper the stress response driven by the p53 pathway. Whether MDM2-SNP309 was associated with breast cancer as a predisposing factor was investigated. Patients and Methods: A case-control study of 223 females diagnosed with breast cancer and 149 female controls sampled from the Turkish population was carried out and the T/G MDM2-SNP309 genotype of participants was determined. Results: There was no significant association of the G/G or G/T genotypes with breast cancer risk (odds ratio (OR) 1.14, 95% confidence interval (CI) 0.59-2.22, and OR 1.20, 95% CI 0.67-2.12, respectively). Stratification of the data for onset age or for menopausal status at the time of diagnosis also revealed no association for either group.*

It has been well established that the *p53* tumor suppressor gene, a principal mediator of multiple functions, plays a vital role in the positive regulation of apoptosis, cell cycle arrest and cellular senescence in response to DNA damage or various types of stress (1).

Bond *et al.* reported a T-to-G polymorphism at nucleotide 309 in the promoter region of MDM2 that increases the MDM2 transcription levels due to formation of an improved binding site for the transcription activator Sp1 (2). MDM2 is an oncoprotein that negatively regulates the stability of p53 through binding at the p53 transcriptional activation domain and promoting its degradation through ubiquitination. MDM2 is also overexpressed in many human tumors, including breast cancer (2, 3) raising the possibility that the G allele of SNP309 could be a strong cancer-predisposing allele.

Whether the G allele of SNP309 acted as a breast cancer predisposition factor was investigated in a case-control study among Turkish women, taking into account the menopausal status and age of onset of the disease.

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Patients and Methods

Study participants. Our case group consisted of 223 females who were diagnosed with breast cancer yet with no apparent family history of breast cancer (ductal breast carcinoma, mean age: 51, standard deviation: 13.2, age range: 20-80). Histopathology of the tumor and age at diagnosis were obtained through medical records. The age-matched control group consisted of 149 apparently healthy Turkish women with no history of cancer (mean age: 47, standard deviation: 15.6, age range: 15-83). A standardized questionnaire including information on age, menopausal status, family history of breast cancer, smoking history, and height and weight was completed for each individual at the Ankara Numune Research and Teaching Hospital, Turkey. Informed consent was obtained from all participants.

Genotyping. Genomic DNA was isolated from 200 μl peripheral blood using a DNA mini kit (Macherey-Nagel, Germany) according to the manufacturer's instructions. PCR and RFLP analysis were used to genotype the T/G MDM2-SNP309 polymorphism. PCR primers used were 5'-GCTTTGCGGAGGTT TTGTT-3' (sense) and 5'-CGGAACGTGTCTGAACTTGA-3' (antisense). PCR was done under standard conditions using 20 ng of genomic DNA and an annealing temperature of 55˚C. The resulting 304 bp PCR product was digested by *s*A1I. The fragments resolved on a 3% agarose gel containing ethidium bromide gave three distinct patterns for SNP309: T/T, G/T and G/G genotypes. The wild-type T allele produced 193 and 111 bp fragments, whereas G/T alleles produced one more restriction site resulting in 193, 147, 111 and 46 bp fragments.

Statistical analysis. The genotype frequencies were compared using Pearson's Chi-squared and Fisher's exact test. Odds ratios (OR) and 95% confidence intervals (CI) were also calculated using 2x2 contingency tables. All the calculations were conducted with R 2.2.1 statistical computing software (4).

Table I. *Allele and genotype frequencies, ORs and 95% CI for MDM2-SNP309 for patient and control groups.*

	Patients N=223 (CI)	Controls N=149 (CI)	OR (95% CI)
Alleles (χ^2=0.9, p=0.34)			
T	0.47 (0.42-0.52)	0.48 (0.42-0.54)	
G	0.53 (0.48-0.58)	0.52 (0.46-0.58)	
Genotypes (χ^2=0.07, p=0.79)			
G/G	57 (26%)	38 (26%)	1.14 (0.59 -2.22)
G/T	124 (55%)	79 (53%)	1.20 (0.67-2.12)
T/T	42 (19%)	32 (21%)	

Table II. *Genotype frequencies, ORs and 95% CI for MDM2-SNP309 for stratified patient and control groups.*

	Patients	Controls	OR (95% CI)
Age at diagnosis >40	N=86	N=93	
G/G	20	21	1.31 (0.49-3.50)
G/T	50	50	
T/T	16	22	
Age at diagnosis <40	N=27	N=42	
G/G	9	13	0.81 (0.17-4.04)
G/T	12	22	
T/T	6	7	
Pre-menopausal status	N=82	N=73	
G/G	21	19	1.04 (0.36-2.93)
G/T	45	39	
T/T	16	15	
Post-menopausal status	N=123	N=63	
G/G	31	15	1.44 (0.52-4.00)
G/T	72	34	
T/T	20	14	

Results and Discussion

Allele and genotype frequencies of the MDM2-SNP309 polymorphisms for the breast cancer patients and control groups are summarized in Table I. Neither the patient nor the control groups showed significant deviation from the Hardy-Weinberg Equilibrium (control group p=0.5, patient group p=0.14). There was no significant association of the G/G or G/T genotypes with breast cancer (OR 1.14, 95% CI 0.59-2.22 and OR 1.20, 95% CI 0.67-2.12, respectively, Table I).

In a recent study by Bond *et al.* (5), it was indicated that female breast cancer patients, who were below the average age of menopause, had a higher frequency of G/G genotype for SNP309 and were affected differently by estrogen signalling. However, stratification of the data according to age and menopausal status in this Turkish population did not change the results (Table II). There was no significant difference between the allelic frequencies of the control group (n=298 alleles) and those of the patient group (n=446 alleles) (χ^2=0.9, df=1, p=0.34). No evidence of a higher G/G distribution (25% of 82 pre-menopausal patients *vs.* 25% of 123 post-menopausal patients) was found among the breast cancer patients who were at their pre-menopausal period at the time of diagnosis.

Several studies have demonstrated that SNP309 accelerates tumor formation in patients with hereditary Li-Fraumeni Syndrome (2) and in various cancer types including colorectal cancer (6), bladder cancer (7) and non-small cell lung cancer (8). In contrast to these findings, other studies have shown that the T309-G polymorphism was not associated with ovarian and sporadic breast cancer (10) or with hereditary breast cancer (10).

In a study by Boersma *et al.* (11), the unstratified data and the stratified data by race did not reveal any association of SNP309 with breast cancer risk. Another study by Ma *et al.*, reported that this polymorphism did not play any verifiable role in breast cancer development in the Chinese population (12).

Conclusion

These results together with our own findings suggest that although the role of the MDM2-SNP309 polymorphism reveals discordant results among different types of cancer in various ethnic groups, there seems to be no association between the G allele and breast cancer risk among the different ethnic groups so far examined. Additionally, no association was found between the risk-defining G allele and menopausal status or age of onset of the disease.

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