

Investigation of the association between the *MDM2* *T309G* polymorphism and gastric cancer

AYCA TAS¹, MUSTAFA ATABEY², GULCIN CAGLAYAN³, MERIC EMRE BOSTANCI⁴,
SERAP SAHIN BOLUKBASI⁵, OMER TOPCU² and YAVUZ SILIG³

¹Department of Nutrition and Diet, Cumhuriyet University, Faculty of Health Sciences; Departments of ²General Surgery and ³Biochemistry, Cumhuriyet University, Faculty of Medicine, 58140 Sivas; ⁴Clinic of General Surgery, Sivas Sample Hospital, 58060 Sivas; ⁵Department of Biochemistry, Cumhuriyet University, Faculty of Pharmaceutics, 58140 Sivas, Turkey

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Abstract. Murine double minute clone 2 oncoprotein (MDM2) is a key component in the regulation of the tumour suppressor p53. The association between the *MDM2* polymorphism and gastric cancer (GC) has been investigated in Turkish population. In the present case-control study, the aim was to investigate the association between genetic polymorphisms of the *MDM2* gene (a major regulator of p53 function) and primary GC risk in a Turkish population. The polymorphism, *T309G* (rs2279744) in the *MDM2* gene was determined in patients with GC (n=65) and in healthy control subjects (n=67) using the polymerase chain reaction-restriction fragment length polymorphism method. The findings were evaluated using logistic regression and χ^2 tests. No statistically significant differences were observed between the control subjects and patients with GC regarding smoking status. A comparison between GC cases and control subjects indicated a statistically significant difference for family history of cancer [odds ratio (OR)=0.17; 95% confidence interval (CI), 0.05-0.56; $\chi^2=0.19$; P=0.01]. A significant difference was identified in the GG genotype distribution between GC patients and control subjects (OR=4.58; 95% CI, 1.18-17.79; P=0.022). Thus, the results of the present study indicate that the *MDM2* gene *T309G* intron (GG) genotype may be an important risk factor for GC development in the Turkish population.

Introduction

Genetic factors that may alter the repair of damaged gastric and colonic epithelial cell DNA, resulting in their transformation into cancer cells remain poorly understood. Accumulation of permanently generated reactive oxygen species during

cellular metabolism and extracellular processes may lead to carcinogenesis caused by oxidative DNA damage. p53, a tumor suppressor protein encoded in humans by the *p53* gene, regulates the cell cycle and preserves the stability of the human genome to prevent cancer initiation (1,2). Gastric cancer (GC) is the fourth most common malignancy and the second primary cause of cancer mortality, resulting in >800,000 mortalities worldwide annually (3,4). In addition to environmental factors, genetic variants are significant. As a crucial tumor suppressor gene, *p53* is critical in cell-cycle regulation, DNA repair, cellular differentiation and apoptosis in various types of cancer. Furthermore, variants of *p53* gene exert an important effect on tumor development and cancer risk (5-7).

Recently, numerous studies have evaluated the interaction between *p53* and its regulators in carcinogenesis. Of these regulators, the murine double minute clone 2 (*MDM2*) proto-oncogene, an important negative regulator of the *p53* gene, has become a point of interest (8). *MDM2* is a major regulator of p53 function. *p53* and *MDM2* act in a feedback cycle whereby p53 activates *MDM2* at the transcriptional level, while *MDM2* binds, obstructs and degrades the *p53* protein through E3 ligase activity (9-11). The human *MDM2* gene is located on chromosome 12q13-14 (genomic size, 34 kb) and contains two promoters, the constituent promoter and the *p53*-responsive intronic promoter (12). The inherently occurring sequence variation in the *MDM2* promoter results in a changed *MDM2* protein expression leading to the alteration in *p53* mediated tumor suppression activity. A single nucleotide polymorphism (SNP; *T309G*) located in the first intron of the core promoter region of the *MDM2* gene influences binding of the transcription factor, Sp1. Sp1 binds with higher affinity to the G allele than to the T allele, which results in increased transcription of the *MDM2* gene and higher expression levels of *MDM2* protein, thereby obstructing the tumor suppressor function of *p53*. This SNP leads to increased *MDM2* mRNA and protein expression levels and, as a result, *p53* inhibition. Additionally, there is a disposition, which demonstrated earlier cancer inception in certain groups of patients harboring the GG or TG genotype for *MDM2 T309G* (13). The *MDM2 T309G* polymorphism has been reported to be associated with certain tumors, such as colon, gastric and hepatocellular cancer (14-16).

Correspondence to: Dr Yavuz Silig, Department of Biochemistry, Cumhuriyet University, Faculty of Medicine, 58140 Sivas, Turkey
E-mail: ysilig@cumhuriyet.edu.tr

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In the present study, the aim was to investigate the association between genetic polymorphisms of the *MDM2* gene, a major regulator of *p53* function, and primary GC risk in a Turkish population.

Materials and methods

Study population. The study was approved by the local ethic committee and informed consent was obtained from all patients. All subjects agreed to participate in the study and completed a short questionnaire, which included questions about their occupation, tobacco use, alcohol consumption and family history of cancer. In the present study, a total of 132 individuals (65 GC patients and 67 control subjects) were investigated. Blood samples were collected from the 65 patients who were diagnosed with GC at the Department of General Surgery, Cumhuriyet University, Faculty of Medicine (Sivas, Turkey). The diagnosis of GC was histologically confirmed and the tumor types were classified according to WHO guidelines, (<https://www.iarc.fr/en/publications/pdfs-online/pat-gen/bb2/bb2-chap3.pdf>) No age and sex restrictions were applied for the selection of healthy volunteers, who were free of any chronic diseases, lived in the same geographic area, and had no history of cancer. All cases and controls were born and lived in Turkey.

DNA isolation. Peripheral blood samples (2 ml) were obtained and collected into citrate-containing tubes from all subjects. The DNA was extracted from whole blood using the salting out procedure as soon as the samples reached the laboratory (17).

***MDM2* genotyping.** The *MDM2 T309G* polymorphism was analyzed using the polymerase chain reaction (PCR)-restriction fragment length polymorphism method. The following primers were used: Forward, 5'-CGC GGG AGT TCA GGG TAA AG-3' and reverse, 5'-CTG AGT CAA CCT GCC CAC TG-3' to amplify the *MDM2* polymorphism. Amplification was performed using the following: 25 pmol each primer, 200 mM total dNTP, 1.5 mM MgCl₂, 1X PCR buffer and 2.5 U Taq DNA polymerase and 50-100 ng DNA in a total volume of 50 μ l. The PCR program was initiated with denaturation at 95°C for 5 min, followed by 30 cycles of 94°C for 60 sec, 55°C for 60 sec (annealing) and 72°C for 60 sec (extension). The PCR was completed with a final extension cycle at 72°C for 5 min. Following confirmation of PCR amplification by 1.5% agarose gel electrophoresis, the amplified product was digested overnight with *MspAII* restriction enzyme at 37°C and electrophoresed on 3% agarose gel stained with ethidium bromide and visualized under UV light. Genotypes were identified for the polymorphism as TT (157 bp), TG (157, 110 and 47 bp), or GG (110 and 47 bp) (18).

Statistical analysis. All statistical analyses were performed using SPSS version 11 (SPSS, Inc., Chicago, IL, USA). Genotype-associated odds ratios (ORs), their corresponding 95% confidence intervals (CIs), and associated P-values were estimated via unconditional logistic regression. Differences in the distributions of demographic characteristics between the cases and control subjects were evaluated using Student's t-test. χ^2 or Fischer's exact test (two-sided) were used to compare the sex distribution, to test the association between the

Table I. Demographics of control subjects (n=67) and gastric cancer cases (n=65) recruited in the present study.

Variable	Control, n (%)	Gastric cancer, n (%)
Sex		
Males	53 (79.1)	53 (81.5)
Females	14 (20.9)	12 (18.5)
Age (years)		
Range	40-85	40-85
Means \pm SD	62.34 \pm 10.54	62.25 \pm 10.95
Males	62.58 \pm 9.86	62.32 \pm 10.55
Females	61.43 \pm 13.19	61.92 \pm 13.08
Smoking history		
Smoker	31 (46.3)	30 (46.2)
Males	30 (56.6)	28 (52.8)
Females	1 (7.1)	2 (16.7)
Alcoholic drink consumption		
Smoker	7 (10.4)	14 (21.5)
Males	7 (13.2)	11 (20.8)
Females	0	3 (25.0)
Family history of cancer	18 (26.9)	4 (6.2)

genotypes and alleles in relation to the controls, and to test for deviation of the genotype distribution from Hardy-Weinberg equilibrium. Pearson's χ^2 test was used to determine whether there were any significant differences in allele and genotype frequencies between patients and control subjects. Logistic regression procedures were performed to assess the interaction between age, sex and all genotypes. In addition, the recessive or dominant effect of the *MDM2* genotype on risk was estimated, and statistical analysis was performed on the relative risk of the GG genotype against the TG + TT genotype or the TG + GG genotype against the TT genotype.

Results

In the present study, the association between the *MDM2* polymorphism and GC was investigated in a Turkish population. The polymorphism in the *MDM2* gene, *T309G* has been determined in 65 patients with GC and in 67 healthy control subjects using the PCR-RFLP method.

The demographic characteristics of the study population are presented in Table I. The mean age of the GC patients and control subjects were 62.25 \pm 10.95 years (males, 62.32 \pm 10.55; females, 61.92 \pm 13.08) and 62.34 \pm 10.54 years (males, 62.58 \pm 9.86; females, 61.43 \pm 13.19), respectively. The percentage of males and females in the cases were 81.5 and 18.5%, respectively (Table I). No statistically significant differences were identified between the cases and control subjects by age and sex (P>0.05). Nor were there statistically significant associations between the cases and control subjects for smoking history (OR=0.99; 95% CI, 0.50-1.97; P=0.989; Table II). Furthermore, no statistically significant differences

Table II. Distribution of selected variables in gastric cancer cases and control subjects.

Variable	Gastric cancer (n=65)		Control (n=67)		P-value	Odds ratio
	n	%	n	%		
Smoking status						
No	35	53.8	36	53.7	0.989	0.99 (0.50–1.97)
Yes	30	46.2	31	46.3		
Drinking status						
No	51	78.5	60	89.6	0.082	2.35 (0.88–6.27)
Yes	14	21.5	7	10.4		
Family history of cancer						
No	61	93.8	41	73.1	0.010 ^a	0.17 (0.05–0.56)
Yes	4	6.2	18	26.9		

^aFisher's exact test.

Table III. Stratification analyses between murine double minute-2 *T309G* genotypes and gastric cancer risk.

<i>MDM2 T309G</i>	Controls (n=67)	Gastric cancer (n=65)	χ^2	P-value	Crude odds ratio
Allele frequency					
T allele	65	47	Ref.	0.042	1.66 (0.99-2.81)
G allele	69	83	4.12		
Genotype frequency					
TT	10 (14.9)	4 (6.2)	Ref.	-	-
TG	45 (67.2)	39 (60.0)	1.55	0.213 ^a	2.16 (0.62-7.45)
GG	12 (7.9)	22 (33.8)	5.21	0.022	4.58 (1.18-17.79)
TG + GG	57 (85.1)	61 (93.8)	2.67	0.102	2.65 (0.79-9.01)
TT + TG	45 (67.2)	39 (60.0)	0.73	0.392	0.73 (0.36-1.49)

^aFisher's exact test. MDM2, murine double minute-2.

were identified regarding alcohol consumption among the cases and control subjects (OR=2.35; 95% CI, 0.88-6.27; P=0.082) (Table II). Comparison between GC cases and control subjects indicated statistically significant differences in family history of cancer (OR=0.17; 95% CI, 0.05-0.56; P=0.01; Table II). Individuals with the GG genotype were associated with a higher risk for GC and the GG polymorphism was significantly associated with this disease (OR=4.58; 95% CI, 1.18-17.79; P=0.022; Table III). Analysis of the *MDM2* gene *T309G* polymorphisms is presented in Fig. 1.

Discussion

GC is the fourth most common cancer and the second leading cause of cancer-associated mortality worldwide (4). GC is a complex trait caused by genetic and environmental factors (19). Genetic polymorphisms in the carcinogen detoxification, antioxidant protection, DNA repair and cell proliferation processes are also crucial in the development of GC (20).

In the current study, of 65 GC cases and 67 control subjects, the crude OR of GC patients for family history of cancer was

0.17, indicating an association between GC incidence and family history of cancer (P=0.01; Table II). To the best of our knowledge, GC and family history of cancer association has not been proposed in previous studies. In addition, GC patients and control subjects were evaluated by logistic regression analysis for smoking habits. No statistically significant difference was identified between GC patients and smoking habits in the Turkish population investigated in the current study (P=0.989; Table II).

The *MDM2 T309G* polymorphism in GC patients has been investigated in various different regions of the world; however, to the best of our knowledge, no research has been performed in Turkey. This study was the first study to evaluate the polymorphic variants of *MDM2 T309G* polymorphism and risk of GC. The *MDM2* protein negatively regulates *p53* function via multiple independent mechanisms, such as by binding to transcription activation domains, inhibiting acetylation, promoting nuclear export and, most importantly, promoting proteasomal degradation via ubiquitination (9,11,21,22). The *MDM2 T309G* polymorphism is a single nucleotide T to G polymorphism located at the 309th nucleotide in the *MDM2* gene promoter. Over the

Table IV. Distribution of polymorphisms of murine double minute-2 *T309G* genotype frequencies in different populations.

Country	Gastric cancer (%)			Control (%)			Year	(Refs.)
	TT	TG	GG	TT	TG	GG		
Japan	23.9	45.9	30.2	22.6	55.0	22.4	2006	(15)
China	21.4	50.0	28.6	29.8	49.8	20.4	2007	(33)
Korea	26.8	46.0	27.2	20.4	50.8	28.8	2008	(28)
China	21.7	48.1	30.2	36.8	43.4	19.8	2009	(31)
China	28.5	46.2	25.4	31.5	54.3	14.2	2009	(27)
China	20.9	54.5	24.6	20.5	51.6	27.9	2011	(26)
China	30.1	45.3	24.6	34.7	51.6	13.8	2013	(29)
Turkey	6.2	60.0	33.8	14.9	67.2	17.9	2017	Current study

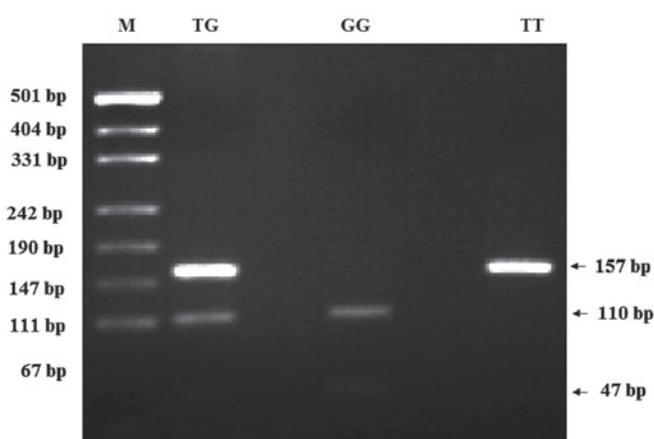


Figure 1. PCR-restriction fragment length polymorphism patterns of polymorphisms of *MDM2 T309G*. M: pUC119/Msp I DNA ladder (501, 489, 404, 331, 242, 190, 147, 111 and 110 bp); *MDM2* PCR product, 157 bp, TG (157, 110 and 47 bp); GG (110 and 47 bp); TT (157 bp). PCR, polymerase chain reaction; *MDM2*, murine double minute-2.

last two decades, it has been confirmed that the *MDM2 T309G* polymorphism is capable of significantly altering the expression levels of *MDM2*, thereby suppressing the p53 signaling pathway. Furthermore, a number of epidemiological studies have investigated the association between the *MDM2 T309G* polymorphism and the risk of various types of cancer (23-26), including renal, hepatocellular, endometrial, bladder and stomach cancers. However, the results of previous studies regarding the association between the *MDM2 T309G* polymorphism and GC risk were different or even contradictory (27,28). Recently, numerous studies demonstrated that significant associations between the *MDM2 T309G* polymorphism and GC risk were identified for the G and T alleles, and the GG and TT genotypes. The G allele was revealed to act as a key factor for increasing the risk of GC, which is consistent with the study by Pan *et al* (29). In addition, in a meta-analysis, Chen *et al* (30) proposed that the G allele of the *MDM2 T309G* polymorphism is a high risk factor for developing GC. In the present study, a statistically significant difference was identified in the GG genotype distribution between GC patients and control subjects in the investigated Turkish population (OR=4.58; 95% CI, 1.18-17.79; P=0.022; Table III). *MDM2 T309G* GG genotype was associated with an increased risk of

gastric carcinoma when compared with the TT genotype or T carriers (P<0.01), and a joint effect between *MDM2 T309G* GG and the *Helicobacter pylori* infection was observed to intensify the GC risk (31). Tian *et al* (32) proposed that the GG genotype is associated with a significantly increased risk of GC (OR=1.43; 95% CI, 1.08-1.91; P=0.013). Consistent with these studies, the current study identified that the overall GC risk for *T309G* GG was significantly increased when it was compared with T carriers (OR=4.58; 95% CI, 1.18-17.79; P=0.022), as presented in Table III. The distribution of *MDM2* genotypes in different populations is provided in Table IV. While the genotype distribution of the control group of the present study was similar to those of the other studies (15,26-29,31,33) the case groups differed.

In conclusion, the present results indicate a significant association between the *MDM2* GG genotype and GC; although, it is not possible to conclude that a single polymorphism determines an individual's susceptibility to GC development. Furthermore, the *MDM2* GG genotype may be defined as an independent marker for GC.

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