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Lack of an Association between a Functional Polymorphism in the *MDM2* Promoter and Breast Cancer in Women in Northeast Iran

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Abstract

Background: Breast cancer is one of the most common cancers among women worldwide. Tumor protein 53 (TP53) and its regulator, the mouse double murine 2 (MDM2) protein homologue, influence tumorigenesis through their key roles in cell division and response to DNA damage. The *MDM2* SNP309T>G (rs2279744) polymorphism in the promoter region of the MDM2 can cause dysfunction and inactivation of TP53, which promotes tumor progression. The aim of this study was to investigate the possible association between this polymorphism and breast cancer in a northeastern Iranian population.

Methods: A case-control study with 128 female breast cancer patients and 143 healthy women was conducted. PCR-ARMS was performed to assess the *MDM2* SNP309T>G (rs2279744) polymorphism.

Results: No significant association was found between the GG genotype or G allele polymorphisms and breast cancer in patients or controls (p = 0.116, OR [95% CI]: 1.267 [0.616, 2.603] and p= 0.143, OR [95% CI]: 1.326 [0.908, 1.935], respectively). For the G allele polymorphism, a significant difference of 8 years in the average cancer diagnosis age was observed between TT and TG carriers (40.57 vs. 48.15 years, respectively, p=0.029).

Conclusions: The SNP309T>G polymorphism in *MDM2* may not be associated with breast cancer in this Iranian population.

Keywords: Breast cancer, Case-control study, Mouse double murine 2 (MDM2), Polymorphism.

Introduction

Breast cancer is one of the most common cancers among women worldwide, with Caucasian women affected at a median age of 61 years (1, 2). Malignancy incidence and mortality rates of 23.1 and 3.52 per 100,000, respectively, make breast cancer the fifth-most-common cause of death in Iranian women (3-5).

Although the exact cause is not yet known, breast cancer is a heterogeneous disease influenced by both environmental and genetic factors. Mutation and inactivation of tumor protein p53 (TP53) have been frequently reported in numerous cancers, including breast cancer (6).

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TP53 has pivotal role in the cell cycle and DNA damage mechanisms (7, 8). TP53 transcription and activity of its corresponding protein in humans is regulated by the mouse double murine 2 (MDM2) protein homologue, an ubiquitin protein ligase that negatively regulates TP53 by targeting it for proteasomal degradation. MDM2 is a nuclear phosphoprotein that also inhibits the transcriptional activity of TP53 by directly binding to it (9, 10). In 2004, a functional T to G substitution polymorphism at nucleotide 309 (SNP309, rs2279744) in the promoter region of *MDM2*, was identified (11).

The GG genotype of this polymorphism leads to increased affinity of the *MDM2* promoter for the transcription factor SP1, resulting in *MDM2* overexpression (11). *MDM2* overexpression can cause dysfunction and inactivation of TP53, which promotes tumor progression (10, 12). Results of studies performed to investigate the possible role of this polymorphism in the pathogenesis of cancers, including breast cancer, have been inconsistent (10,13-18). The aim of this study was to investigate the allele and genotype frequency of the *MDM2* SNP309T>G (rs2279744) polymorphism in northeast Iranian breast cancer patients.

Materials and Methods

Subjects

One hundred and twenty-eight women with clinically and pathologically-confirmed breast cancer and 143 age-matched healthy women enrolled in this study**.** Before enrollment, all participants completed the consent form, which was approved by the Institutional Ethical Committee and Research Advisory Committee of Mashhad University of Medical Sciences. All procedures performed in studies involving human participants were in accordance with the ethical standards of the committee. Demographic variables including age, family history of breast cancer, menopause status, and marriage status were obtained from all participants.

Genotyping

Peripheral blood was obtained from each participant and genomic DNA was extracted using a commercially-available kit (Biogene Company, IRAN) according to the manufacturer's protocol. The genotype of the *MDM2* SNP309T>G polymorphism was determined by amplification refractory mutation system polymerase chain reaction (ARMS-PCR), modified from a technique described by Nunobiki et al. (19). The *MDM2* SNP309T>G region was amplified by PCR using two pairs of specific primers for the two alleles, as shown in Table 1.

Two independent PCR assays were performed for each allele in 20 μL final volumes, using 100 ng of genomic DNA, 2 μL of buffer, 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 0.5 pmol of each primer, and 0.5 unit of Taq DNA polymerase. The amplification conditions for PCR were initial denaturation at 95 °C for 5 min followed by 35 cycles of denaturation at 95 °C for 45 seconds, annealing at 67 °C for 45 seconds, DNA extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min. All amplifications were performed in a Biometra Thermal cycler (Biometra Ltd., Kent, UK)**.** The PCR products were electrophoresed on 2% agarose gels containing ethidium bromide and visualized with ultraviolet illumination. The fragment lengths of the T and G allele amplification products are shown in Table 1. All assays were conducted blindly with no knowledge of case or control status. For quality controls, 10% of the samples were randomly repeated, and the results were 100% concordant.

Statistical analysis

Descriptive statistics, logistic regression test, independent sample *t*-test, one-way ANOVA, Pearson Chi-square, and Fisher's exact test were analyzed using SPSS software version 17.0. P values less than 0.05 were considered statistically significant.

Table 1. Primer sequences and specification of amplified fragments for *MDM2* SNP309 genotype.

Primers	Primer sequence	Fragment lengths	Genotype
F1	5'- GGA TTT CGG ACG GCT CTC-3'	121 bp	Wild-type of T allele
R1	5'-TCC GGA CCT CCC GCG CCG A-3'		
F2	5'- GTT TTG TTG GAC TGG GGC TA-3'	168 bp	Mutant-type of G allele
R2	5'- ATC CGG ACC TCC CGC GCC GC-3'		

Results

This study included 128 breast cancer patients with a mean age of 46.1 ± 10.2 years and 143 healthy control subjects with a mean age of 43.8 ± 11.8 years (p = 0.07). No significant association was found between the genotype and allele frequencies of the *MDM2* SNP309T>G polymorphisms in either patients or controls and breast cancer risk (p = 0.116, Table 2). The T and G allele frequencies in patients were 28 and 72%, and in controls, 34 and 66%, respectively. These differences were not significant (p = 0.143, Table 2).

Also, no associations were found between the co-dominant, dominant, or recessive inheritance models of the *MDM2* SNP309T>G polymorphism and breast cancer. However, a slight increase in disease was seen in the overdominant inheritance model (p=0.048, OR [95% for CI]:1.712 [1.00-2.92]) (Table 2). The genotype distributions were in Hardy-Weinberg equilibrium in controls (X^2: 3.45, X^2 p=0.06), but not in patients (X^2: 16.29, X^2 p < 0.05).

To determine the relationship between average breast cancer diagnosis age and the *MDM2* SNP309T>G polymorphism, the patients were divided into two groups based on their ages at diagnosis: those diagnosed at ≤ 35 years (n = 24) and those diagnosed at > 35 years (n=104). The genotype frequencies between age-classified patients were not significantly different (p = 0.075, Table 3); however, a significant difference was found in average cancer diagnosis age based on genotype. TT genotype patients were diagnosed at a significantly younger age than those with the TG genotype (40.57 vs. 48.15 years, p = 0.029, Table 4).

Breast cancer types in the study patients are shown in Table 5. Invasive ductal carcinoma was the most common type of breast cancer among patients (82%). No significant association was found between the *MDM2* SNP309T>G genotypes and breast cancer type in our study patients (p =0.627).

Finally, no significant associations were found in patients between the *MDM2* SNP309T>G genotypes and menopause status (p = 0.307) or family history of breast cancer (p=0.112, data not shown).

Table 2. Distribution of genotypes and allele frequencies and their association with breast cancer.

MDM2 SNP309T>G		Patients n= 128 (%)	Controls n= 143 (%)	P value	Odds ratio (95% CI)
Codominant					
	TT	19 (14.8)	19 (15.1)		1.0 (reference)
	TG	33 (25.8)	47 (37.3)	0.116	0.702 (0.323, 1.526)
	GG	76 (59.4)	60 (47.6)		1.267 (0.616, 2.603)
Dominant					
	TT	19 (14.8)	19 (15.1)	0.958	1.0 (reference)
	TG-GG	109 (85.2)	107 (84.9)		1.018 (0 .511, 2.030)
Recessive					
	TT-TG	52 (40.6)	66 (52.4)	0.0603	1.0 (reference)
	GG	76 (59.4)	60 (47.6)		1.607 (0.978, 2.641)
Overdominant					
	TG	33 (25.8)	47 (37.3)	0.048	1.0 (reference)
	TT-GG	95 (74.2)	79 (62.7)		1.712 (1.002, 2.927)
Alleles					
	T	71 (28)	85 (34)	0.143	1.0 (reference)
	G	185 (72)	167 (66)		1.326 (0.908, 1.935)

Table 3. MDM2 SNP309T>G genotype frequencies among patients based on age at breast cancer diagnosis.

		Age at breast cancer diagnosis			
		Below 35 years (%)	Above 35 years (%)	Total	P value
MDM2 SNP309 T>G	T/T	7 (29.2)	12 (11.5)	19 (14.8)	0.075
	T/G	4 (16.7)	29 (27.9)	33 (25.8)	
	G/G	13 (54.2)	63 (60.6)	76 (59.4)	
Total		24 (100)	104 (100)	128 (100)	

Table 4. Average age of cancer diagnosis in breast cancer patients based on MDM2 SNP309T>G genotypes.

MDM2 SNP309T>G	Mean diagnosis age ± SD	95% confidence interval	P value
T/T	40.57 ± 8.15	36.64, 44.5	0.029
T/G	48.15 ± 11.25	44.09, 52.21	
G/G	46.69 ± 9.98	44.41, 48.97	

Table 5. Association between MDM2 SNP309T>G genotype frequencies and breast cancer type

Diagnosis (%)	MDM2 SNP309T>G				
	T/T	T/G	G/G	Total	P value
Invasive ductal carcinoma (%)	15 (14.28)	29.53 (31)	59 (56.19)	105 (100)	0.627
Invasive lobular carcinoma (%)	0 (0)	2 (25)	75 (6)	100 (8)	
Comedo carcinoma (%)	0 (0)	0 (0)	100 (5)	100 (5)	
Medullary Carcinoma (%)	2 (50)	0 (0)	50 (2)	100 (4)	
Mixed ductal and lobular carcinoma (%)	2 (66.67)	0 (0)	1 (33.33)	100 (3)	
Mucinous carcinoma (%)	0 (0)	0 (0)	2 (100)	2 (100)	
Paget's disease (%)	0 (0)	0 (0)	1 (100)	1 (100)	
Total (%)	19 (14.84)	33 (25.78)	76 (59.37)	128 (100)	

Discussion

The nuclear phosphoprotein MDM2 homolog is the main regulator of *TP53* expression and activation, and plays an essential role in cell cycle events and DNA damage mechanisms (7-10). In the present study, the association of the *MDM2* SNP309T>G polymorphism and breast cancer were investigated in a series of 128 breast cancer patients and 143 healthy controls in a sample of Iranian women.

No significant association was found between genotype or allele frequencies of this polymorphism and breast cancer in our study population. These findings are consistent with the other studies that reported no significant association between the *MDM2* SNP309T>G polymorphism and increased breast cancer risk in other populations (10,15, 20-24). Campbell et al. observed no association between the *MDM2* GG genotype and risk of breast cancer in 351 English women (OR 1.04, 95% CI: 0.67–1.600) (15). Similarly, Ma et al. found no association between the *MDM2* SNP309T>G polymorphism and breast cancer risk in Chinese women (OR, 1.03; 95% CI, 0.74–1.42) (25).

In a meta-analysis of 21 case-controls studies of various cancers, including eight breast cancer studies (3284 patient samples and 3853 controls), Hu et al. found no association between GG genotype and cancers, including breast cancer (GG versus TT OR: 1.00, 95% CI: 0.89-1.12) (26). Moreover, in agreement with this meta-analysis report, Wilkening et al., in another meta-analysis of 11 case and control studies, reported that the *MDM2* SNP309T>G G allele has no effect on breast cancer risk (OR:0.97, 95% CI:0.87– 1.08) (10).

However, in a study of 236 breast cancer patients and 203 healthy controls in southeast Iran, Hashemi et al. reported that this polymorphism is associated with increased breast cancer risk. In this study, the *MDM2* SNP309 GG genotype frequencies in

patients and controls were 16.1 and 9.4%, respectively (p= 0.018, OR: 2.09, 95% CI: 1.14-3.85) (27). The difference between the results of this study and ours may be due to ethnicity differences in northeastern and southeastern Iranian populations.

Despite the role of MDM2 in tumor progression, the null association between the G allele of the *MDM2* SNP309T>G polymorphism and breast cancer may be due to estrogen receptor (ER) expression variation on cancerous cells (26, 28). Studies showed that ER-positive breast cancer cells express more MDM2 than ER-negative cells, which leads to breast cancer progression (26, 29-31). In the presence of the G allele, the estrogen signaling pathway can induce *MDM2* expression in estrogen-positive cells (32). Therefore, the G allele of the *MDM2* SNP309T>G polymorphism, accompanied by estrogen receptor expression, is associated with breast cancer and lower onset age (22, 31).

Another finding of our study was the high G allele frequency in our study population, which is similar to reports from Asian populations (13, 21).

Results from studies of a possible correlation between breast cancer onset age and the *MDM2* G allele have been controversial. Some reports indicated a direct correlation (11, 31), while others found no correlation (15, 20, 21, 24). In agreement with the San Lum et al. report (13), in our study the TT genotype was also associated with a younger breast cancer onset age.

These different results may be due to the type of familial or non-familial/sporadic breast cancer patients studied. Studies that reported correlation between the G allele and breast cancer onset age were conducted on familiar cancer cases, while the studies that found no such association were conducted on non-familiar/sporadic breast cancer cases. Based on these findings, it appears that some variables, such as the *MDM2* SNP309T>G polymorphism, affect carcinogenesis differently in familiar vs. non-familiar/sporadic breast cancers (13).

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