

# Association between polymorphisms in TP53 and MDM2 genes and susceptibility to prostate cancer

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Received February 18, 2016; Accepted October 5, 2016

DOI: 10.3892/ol.2017.5739

**Abstract.** Tumor protein 53 (*TP53*), a tumor suppressor gene, is a vital cellular cancer suppressor in multicellular organisms. Murine double minute-2 (*MDM2*) is an oncoprotein that inhibits *TP53* activity. A number of studies have examined the association of *TP53* and *MDM2* polymorphisms with the risk of common forms of cancer, but the findings remain inconclusive. The present study aimed to evaluate the impact of the 40-bp insertion/deletion (I/D) polymorphism (rs3730485) in the *MDM2* promoter region and the 16-bp I/D polymorphism (rs17878362) in *TP53* on the susceptibility of prostate cancer (PCa) in a sample of the Iranian population. This case-control study included 103 patients with pathologically confirmed PCa and 142 patients with benign prostatic hyperplasia. The *MDM2* 40-bp I/D and *TP53* 16-bp I/D polymorphism was determined using polymerase chain reaction analysis. The results demonstrated that the *MDM2* 40-bp I/D polymorphism increased the risk of PCa in a co-dominant inheritance model [odds ratio (OR)=1.88; 95% confidence interval (CI)=1.11-3.19; P=0.023, D/D vs. I/I], while this variant marginally increased the risk of PCa in a dominant model (OR=1.69; 95% CI=1.00-2.83; P=0.051, I/D+D/D vs. I/I). No significant association was observed between the *TP53* 16-bp I/D polymorphism and PCa. In conclusion, the present study demonstrated that the

40-bp I/D polymorphism in the *MDM2* promoter increased the risk of PCa in an Iranian population. Further investigations with diverse ethnicities and larger sample sizes are required to verify these results.

## Introduction

Prostate cancer (PCa) is the most prevalent form of cancer among males in the United States (1). In Iran, the incidence of PCa is ~9.6 cases per 100,000 individuals, with a range of 3.2-16.0 per 100,000 in various geographical settings (2,3). This is comparable with the Asia-Pacific region (9.9 per 100,000), but significantly lower than in the rest of the world (32.8 per 100,000) (4). The median age at diagnosis is ~66 years and the 5-year survival rate of patients with PCa has been estimated to be 98.9% (5).

The molecular mechanisms underlying the progression and carcinogenesis of PCa have not yet been clarified. Additional molecular markers that may be employed to detect PCa and to individualize patient therapy and prognosis are of great clinical importance. A number of large cohort and case-control studies with various populations suggest that family history is a primary risk factor for PCa (6-10).

In humans, the tumor protein 53 (*TP53*) gene is located on the short arm of chromosome 17 (17p13.1) (11). *TP53*, an important tumor suppressor gene, is a crucial regulator of apoptosis and the cell cycle (12-15). When it is mutated, this regulation may be lost, leading to uncontrolled cell proliferation and potentially tumorigenesis (16).

The human *MDM2* gene is mapped on chromosome 12q14.3-15 (17). *MDM2* functions as a key negative regulator of *TP53* (18), inhibiting the transcriptional activity of *TP53* and enhancing proteolytic *P53* degradation (19).

The impact of a 16-bp duplication [insertion (I)/deletion (D)] polymorphism (rs17878362) within intron 3 of *TP53* and a 40-bp I/D variant of *MDM2* on cancer susceptibility has been

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**Key words:** prostate cancer, tumor protein 53, murine double minute-2, insertion/deletion, polymorphism

examined, but studies have reported conflicting results (20-28). Therefore, the current study aimed to determine the possible association of the 16-bp I/D polymorphism within intron 3 of *TP53* and the 40-bp I/D polymorphism in the promoter region of *MDM2* with PCa in a sample of an Iranian population.

## Materials and methods

**Patients.** The present case-control study enrolled 103 histopathologically-confirmed patients with PCa and 142 age-matched men with benign prostatic hyperplasia (BPH), who had been referred to the Department of Urology, Shahid Labbafinejad Medical Center, Shahid Beheshti University of Medical Sciences (Tehran, Iran). The enrollment process and study design were performed as previously described (29). Ethical approval for the study was obtained from the Ethics Committee of Zahedan University of Medical Sciences, and written informed consent was obtained from all participating patients. Blood samples were collected in EDTA-containing tubes from patients and controls, and DNA was extracted using the salting out method as previously described (30).

**Genotyping.** *MDM2* 40-bp I/D polymorphism genotyping was performed using forward (5'-GACCACTATGTTTAAGGAAG-3') and reverse (5'-TGACTCACCTACTTCCAC-3') primers, as described previously (28). Polymerase chain reaction (PCR) was performed using commercially available Prime Taq Premix (Genet Bio, Daejeon, South Korea), according to the manufacturer's recommended protocol. The PCR cycling conditions were as follows: An initial denaturation at 95°C for 5 min, followed by 30 cycles at 95°C for 30 sec, 59°C for 25 sec, 72°C for 30 sec and a final extension step at 72°C for 10 min. The product sizes for the I and D alleles were 287 and 247 bp, respectively. The PCR products were verified using 2.5% agarose gels containing 0.5 µg/ml ethidium bromide, and observed under UV light (Fig. 1).

Genotyping of the 16-bp duplication polymorphism in *TP53* was performed by PCR using forward (5'-CTGAAACAACGTTCTGGTA-3') and reverse (5'-AAGGGGACTGTAGATGGGTG-3') primers (31) as previously described (32). The PCR conditions were as follows: An initial denaturation step at 95°C for 5 min, followed by 30 cycles at 95°C for 30 sec, 60°C for 30 sec and 72°C for 30 sec, with a final extension step at 72°C for 10 min. The *TP53* wild type allele, the D allele (no duplication), resulted in a 119-bp fragment and the variant alleles, the I allele (with 16-bp duplication), resulted in a 135-bp fragment (Fig. 2). Approximately 15% of the samples were randomly selected for confirmation and the results were all consistent.

**Statistical analysis.** Statistical analysis was performed using SPSS v18 (SPSS, Inc., Chicago, IL, USA). Continuous and categorical data were analyzed using the independent sample *t*-test and the  $\chi^2$  test, respectively. Associations of the *MDM2* 40-bp I/D and the *TP53* 16-bp I/D polymorphism with PCa were calculated by computing the odds ratio (OR) and 95% confidence intervals (95% CIs) from logistic regression analyses. The genotype distribution of variants was tested for Hardy-Weinberg equilibrium (HWE) separately for cases and controls.  $P < 0.05$  was considered to indicate a statistically significant difference.

Table I. Clinicopathological characteristics of patients with prostate cancer.

Characteristics	Number of patients
Mean age, years (range)	61.0 (43-72)
PSA level at diagnosis (ng/ml), mean $\pm$ SD	14.1 $\pm$ 13.3
Gleason Score, n (%)	
$\leq 6$	36 (35)
7	46 (44.7)
$> 7$	21 (20.4)
Stage, n (%)	
pT1	8 (7.8)
pT2a	20 (19.4)
pT2b	7 (6.8)
pT2c	39 (37.9)
pT3a	8 (7.8)
pT3b	21 (20.4)
Perineural invasion, n (%)	60 (58.3)
Impotency, n (%)	18 (17.5)
Loss of libido, n (%)	16 (15.5)
Post-void residual, mean $\pm$ SD (ml)	26.7 $\pm$ 27.6
Addiction, n (%)	4 (3.9)
Hypertension, n (%)	8 (7.8)
Diabetes mellitus, n (%)	7 (6.8)
History of smoking, n (%)	12 (11.7)
Alcohol consumption, n (%)	1 (0.97)

PSA, prostate specific antigen; SD, standard deviation. pT1, pT2a, pT2b, pT2c, pT3a and pT3b are clinical stages of prostate cancer.

## Results

**Patient characteristics.** In total, 103 patients with PCa were enrolled with a mean age of 61.03 $\pm$ 6.03 years, and 142 patients with BPH (control group) were enrolled with a mean age of 62.57 $\pm$ 7.85 years. No significant difference in age was observed between the groups ( $P=0.097$ ). The clinicopathological characteristics of the patients with PCa are summarized in Table I.

***TP53* 16-bp I/D and *MDM2* 40-bp I/D polymorphism variant and PCa risk.** The *TP53* 16-bp I/D variant was not associated with PCa in any tested inheritance models (co-dominant, dominant and recessive; Table II). The genotype and allele frequencies of the *MDM2* 40-bp I/D polymorphism in patients with PCa and the controls are presented in Table III. A significant difference was observed between patients with PCa and controls regarding the *MDM2* 40-bp I/D polymorphism ( $\chi^2=7.06$ ;  $P=0.029$ ). The *MDM2* 40-bp I/D polymorphism increased the risk of PCa in a co-dominant inheritance model (OR=1.88; 95% CI=1.11-3.19;  $P=0.023$ , I/D vs. I/I), while the variant marginally increased the risk of PCa in a dominant model (OR=1.69; 95% CI=1.00-2.83;  $P=0.051$ , I/D+D/D vs. I/I). The D allele was not associated

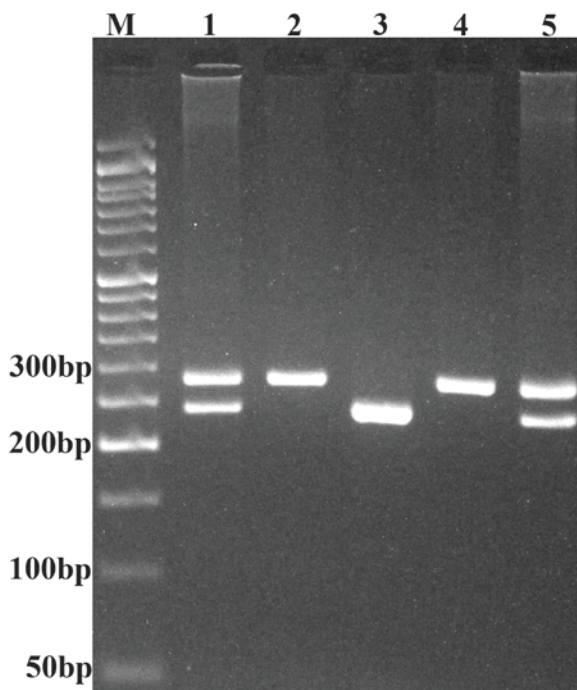


Figure 1. Electrophoresis pattern of the polymerase chain reaction product of the 40-bp I/D polymorphism of murine double minute-2 resolved by 2.5% agarose gel electrophoresis. M, 50 bp DNA marker; lanes 1 and 5, I/D; lanes 2 and 4, I/I; lane 3, D/D. I, insertion; D, deletion.

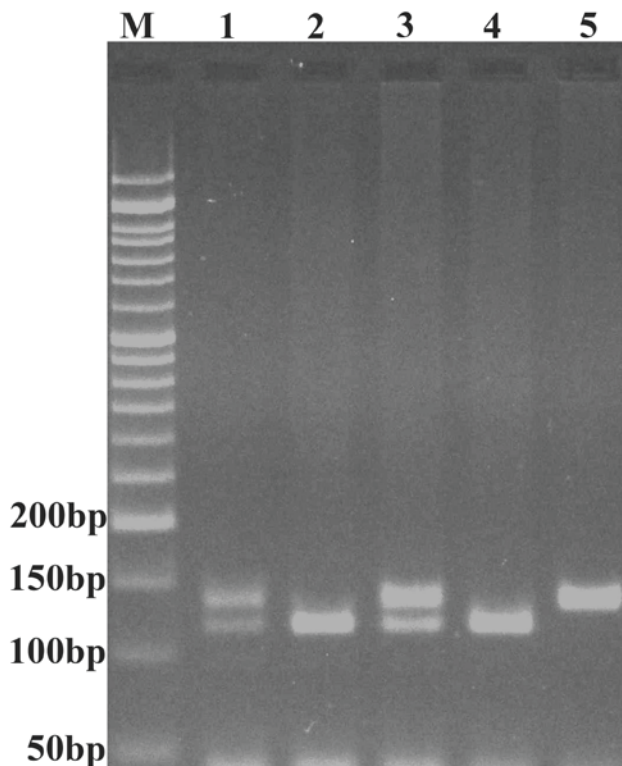


Figure 2. Representative polymerase chain reaction products resolved by agarose gel electrophoresis to detect the presence or absence of the 16 bp I/D polymorphism of the tumor protein 53 gene. M, 50 bp DNA marker; lanes 1 and 3, I/D; lanes 2 and 4, D/D; lane 5, I/I. I, insertion; D, deletion.

The *MDM2* I/D and *TP53* 16-bp variant genotype frequencies were individually tested for patients and controls using the HWE. The *TP53* 16-bp I/D polymorphism in patients and controls were observed to be in equilibrium ( $\chi^2=0.002$ ,  $P=0.958$ ;  $\chi^2=0.52$ ,  $P=0.473$ , respectively). For the *MDM2* 40-bp I/D, the genotype was in equilibrium in controls ( $\chi^2=0.51$ ;  $P=0.820$ ), but not in patients ( $\chi^2=10.36$ ,  $P=0.001$ ).

**Association of *MDM2* I/D and *TP53* I/D with clinicopathological characteristics.** As presented in Table IV, the *MDM2* I/D variant was not associated with clinicopathological characteristics, including age, stage, prostate specific antigen level, grade (Gleason score), perineural invasion or surgical margin. However, there was an association between the *TP53* I/D polymorphism and surgical margin ( $P=0.035$ ).

## Discussion

It has been suggested that genetic and environmental factors each contribute to the pathogenesis of human PCa (33-35). The present study investigated the impact of the 16-bp I/D polymorphism within intron 3 of *TP53* and the 40-bp I/D polymorphism in the promoter region of *MDM2* on the risk of PCa in a sample of the Iranian population. The results demonstrated that the *TP53* 16-bp I/D polymorphism was not associated with the risk of PCa. However, a significant association was observed between the *MDM2* I/D variant and the risk of PCa, and the I/D genotype increased the risk of PCa, compared with I/I.

The *TP53* gene is a key tumor suppressor that encodes a 53-kDa protein, which is essential in DNA repair, cell cycle arrest and apoptosis in response to DNA damage (12-15,26,36). *TP53* mutations are the most frequent gene mutations in the majority of types of human cancer, and they permit cells to proliferate and survive (37). Rapid phosphorylation of *TP53* by ataxia telangiectasia mutated (ATM) following DNA damage leads to increased *TP53* stability and activity (38,39). *TP53*-induced gene expression leads to cell cycle arrest and apoptosis (40). When *TP53* is mutated, this effect may be lost, resulting in uncontrolled cell proliferation that may result in tumorigenesis (16). It has been proposed that genetic polymorphisms in *TP53* may affect a number of its functions (41).

*MDM2*, a key negative regulator of *TP53*, is able to bind directly to *TP53* and inhibit its transcriptional activity (18). *MDM2* also promotes the ubiquitination and subsequent degradation of *TP53* (19,42). Promoter polymorphisms in the *MDM2* gene may affect *MDM2* cellular protein levels (43), and *MDM2* overexpression has been reported in several forms of human cancer (44-46).

In agreement with the results of the present study, Mittal *et al* (47) reported no significant association between the *TP53* 16-bp I/D polymorphism and PCa in an Indian population. Certain studies have demonstrated that the 16-bp I/D variant increased the risk of various types of cancer, including breast (20,21,48-50), colorectal (22), lung (23,24,51), esophageal and gastric (52) cancer. Conversely, a number of studies observed no association between the *TP53* 16-bp I/D variant and certain forms of cancer, including breast (53) and pancreatic (54) cancer. The 16-bp I/D polymorphism in *TP53* has been demonstrated to be associated with lymph node metastasis in breast cancer (55).

with PCa (OR=1.23; 95% CI=0.84-1.82;  $P=0.320$ ), compared with the I allele.



Table II. Genotypic and allelic frequencies of the *TP53* 16-bp I/D polymorphism in patients with PCa, and in controls.

TP53 16-bp I/D	PCa, n (%)	Control, n (%)	OR (95% CI)	P-value
Codominant				
D/D	36 (35.0)	57 (40.1)	1.00	-
D/I	50 (48.5)	69 (48.6)	1.15 (0.66-1.99)	0.673
I/I	17 (16.5)	16 (11.3)	1.68 (0.76-3.76)	0.223
Dominant				
D/D	36 (35.0)	57 (40.1)	1.00	-
D/I+I/I	67 (65.0)	84 (59.9)	1.26 (0.75-2.14)	0.425
Recessive				
DD+D/I	86 (83.5)	126 (88.7)	1.00	-
I/I	17 (16.5)	16 (11.3)	1.56 (0.75-3.25)	0.259
Allele				
D	122 (59.2)	183 (64.4)	-	-
I	84 (40.8)	101 (35.6)	1.25 (0.86-1.81)	0.258

TP53, tumor protein 53; I, Insertion; D, deletion; PCa, prostate cancer; OR, odds ratio; CI, confidence interval.

Table III. Genotypic and allelic frequencies of the *MDM2* 40-bp I/D polymorphism in patients with PCa and controls.

MDM2 40-bp I/D	PCa, n (%)	Control, n (%)	OR (95% CI)	P-value
Codominant				
I/I	39 (37.9)	72 (50.7)	1.00	-
I/D	60 (58.2)	59 (41.6)	1.88 (1.11-3.19)	0.023
D/D	4 (3.9)	11 (7.7)	0.67 (0.20-2.25)	0.578
Dominant				
I/I	39 (37.9)	72 (50.7)	1.00	-
I/D+D/D	64 (62.1)	70 (49.3)	1.69 (1.00-2.83)	0.051
Recessive				
I/I+I/D	99 (96.1)	131 (92.3)	1.00	-
D/D	4 (3.9)	11 (7.7)	0.45 (0.15-1.56)	0.284
Alleles				
I	138 (67.0)	203 (71.5)	1.00	-
D	68 (33.0)	81 (28.5)	1.23 (0.84-1.82)	0.320

MDM2, murine double minute-2; I, insertion; D, deletion; PCa, prostate cancer; OR, odds ratio; CI, confidence interval.

The intron sequences in *TP53* are involved in regulating gene expression and in DNA-protein interactions (56,57). It has been proposed that the *TP53* intron 3 16-bp I variant is associated with lower levels of *TP53* transcripts, which suggests that this duplication polymorphism causes an alteration in mRNA processing and may be a risk factor for developing cancer (57). In addition, it has been suggested that the *TP53* codon 72 variant may be a low-penetrant risk factor for developing PCa in Caucasians, but not in Asians (58), and variants within the *TP53* binding sites may be valuable biomarkers for the prognosis of patients with PCa (59).

The current study observed that the 40-bp I/D polymorphism in the *MDM2* promoter increased the risk of PCa. A recent study reported that the *MDM2* 40-bp I/D variant

increased the risk of breast cancer in an Iranian population (28). Furthermore, a significant association between *MDM2* I/D and lung cancer was detected in the Chinese population (26). In addition, the *MDM2* 40-bp I/D variant has been demonstrated to be a risk factor for hepatocellular carcinoma in the Chinese population (25). By contrast, no association was identified between the *MDM2* 40-bp I/D polymorphism and breast cancer in the Chinese population (27).

The limitations of the present study are as follows: Firstly, relatively small sample sizes were used, therefore repetition with larger samples is required; secondly, gene-environment interactions were not determined. It has been proposed that environmental and genetic factors each serve a role in PCa development. In conclusion, the results of the present study

Table IV. Association between *MDM2* 40-bp I/D and *TP53* 16-bp I/D polymorphisms with clinicopathological parameters in patients with prostate cancer.

Factors	MDM2 40-bp I/D			P-value	TP53 16-bp I/D			P-value
	II	ID	DD		DD	DI	II	
Age at diagnosis (years), n				0.976				0.529
≤65	30	45	3		25	40	13	
>65	9	15	1		11	10	4	
Stage				0.152				0.241
pT1	3	5	0		4	3	1	
pT2a	5	12	3		7	6	7	
pT2b	3	4	0		2	4	1	
pT2c	18	21	0		16	18	5	
pT3a	1	6	1		2	4	2	
pT3b	9	12	0		5	15	1	
PSA level at diagnosis (ng/ml), n				0.647				0.538
≤4	1	0	0		1	0	0	
4-10	18	29	1		19	21	8	
>10	20	31	3		16	29	9	
Gleason score, n				0.475				0.674
≤7	32	46	4		30	38	14	
>7	7	14	0		6	12	3	
Perineural invasion, n				0.886				0.878
Positive	22	36	2		21	30	9	
Negative	17	24	2		15	20	8	
Surgical margin, n				0.925				0.035
Positive	16	24	2		13	26	3	
Negative	23	36	2		23	24	14	

MDM2, murine double minute-2; TP53, tumor protein 53; I, insertion; D, deletion; PSA, prostate specific antigen. pT1, pT2a, pT2b, pT2c, pT3a and pT3b are clinical stages of prostate cancer.

indicate that the 40-bp I/D polymorphism in the *MDM2* gene promoter increases the risk of PCa in a sample of the Iranian population. Larger sample sizes with differing ethnicities are required to further investigate these findings.

### Acknowledgements

The current study was supported by Zahedan University of Medical Sciences, Zahedan, Iran (grant no. 6728), a University of Manitoba Start-Up grant (grant no. 315992) and a Manitoba Medical Services Foundation grant (grant no. 8-2015-11). The authors would like to thank all individuals who willingly participated in the study. In addition, all authors acknowledge Dr Judi Smith (Research Facilitator, Health Sciences, University of Manitoba, Canada) for editing the original manuscript.

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