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Association of *MDM2 T309G* (rs2279744) Polymorphism and Expression Changes With Risk of Prostate Cancer in the Slovak Population

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Abstract. *Background/Aim: The aim of this study was to evaluate the relationship between MDM2 T309G polymorphism and prostate cancer risk in the Slovak population and the association of this polymorphism with MDM2 expression and clinicopathological features. Materials and Methods: The MDM2 T309G polymorphism was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis in 506 prostate cancer patients and 592 controls. Quantitative real-time (RT)-PCR and western blot analysis were applied to examine MDM2 expression in 47 prostate cancer tissues and 43 benign prostatic hyperplasia (BPH) tissues. Results: A decreased risk of prostate cancer in men carrying the GG genotype in comparison with the TT genotype was found. A decrease in the relative MDM2 mRNA and protein levels was found in prostate cancer tissues among patients with the MDM2 GG genotype. Conclusion: There is a potentially protective effect of the MDM2 GG genotype on the risk of prostate cancer in the Slovak male population.*

Prostate cancer is the most commonly diagnosed urological cancer in men; it is the second most common cancer in men worldwide with increasing prevalence (1). The most consistently observed risk factors associated with prostate cancer are age, family history, race and genetic predisposition (2, 3).

MDM2 protein (murine double minute 2, also known as HDM2) negatively regulates the p53 mediated transcriptional transactivation. It is an oncoprotein that escorts p53 from the cell nucleus to the cytoplasm and can mono- or poly-ubiquitinate it depending on the level of MDM2 activity (4, 5). High levels of MDM2 activity promote the polyubiquitination and degradation of p53, whereas low levels of MDM2 activity induce the mono-ubiquitination and nuclear exportation of p53 (6). P53 positively regulates MDM2 expression, thus forming a negative auto-regulatory feedback loop (7).

The human *MDM2* gene is located on chromosome 12q14.3-q15, with a genomic length of 34 kb, and its protein is 491 amino acids in length. MDM2 contains several conserved functional domains, such as an N-terminal p53-binding domain, a bipartite nuclear localization sequence, a nuclear export sequence, an acidic domain, and a C-terminus containing the RING finger domain (8). The *MDM2* promoter region contains several polymorphisms, some of which have been associated with MDM2 expression and cancer risk. Analysis of the sequence of the *MDM2* gene has revealed the presence of a polymorphism located within the second *MDM2* promoter-enhancer region. This polymorphism, a *T* to *G* change at position 309 of intron 1, has been found to create a Sp1 transcription-factor-binding site and to result to increased expression of MDM2 together

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with an attenuation of the p53 pathway in cells stressed with DNA-damaging agents (9, 10).

For the past few years, the relationship between the *MDM2 T309G* polymorphism and prostate cancer risk has been investigated in several studies in multiple populations (11-13); however, this relationship remains unclear. To date, no study has reported the effect of the *MDM2 T309G* polymorphism on MDM2 mRNA and protein levels in prostate cancer. In the current case–control study, we aimed to genotype *MDM2 T309G* and evaluate its association with prostate cancer risk in the Slovak population. We further evaluated the association of *MDM2 T309G* with MDM2 expression and clinicopathological features in prostate cancer patients.

Materials and Methods

Study population. The study population consisted of a total of 1,098 subjects, which included 506 prostate cancer patients and 592 control subjects from the Department of Urology of University Hospital in Martin and the Jessenius Faculty of Medicine in Martin, Comenius University. Prostate cancer patients and controls were registered from May 2005 to May 2019, and all were Caucasians. Prostate cancer patients were diagnosed histologically on the basis of specimens obtained from prostate needle biopsy or transurethral resection of the prostate. Patients older than 50 years without a cancer diagnosis and with PSA values lower than 4 ng/ml were randomly assigned to the control group. Clinical information was abstracted from the medical records of the patients. The clinical characteristics of the subjects are summarized in Table I.

In total, 47 tissue samples from prostate cancer patients and 43 tissue samples from patients with BPH were collected during routine surgery and stored in mRNA stabilizing solution (RNAlater®; Applied Biosystems/Ambion, Foster City, CA, USA) at –80˚C until processed. All tissues were used for protein and total RNA isolation. The study was approved by the Ethical Boards of the Jessenius Faculty of Medicine in Martin, Comenius University, and written informed consent was obtained from all individuals prior to their inclusion in the study.

MDM2 T309G (rs2279744) genotyping. Genomic DNA from all subjects was isolated from whole blood by using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA) and stored at -20˚C until used.

Genotypes of the *MDM2 T309G* (rs2279744) polymorphism were determined by PCR-RFLP assay (14). Briefly, the amplification reactions were carried out in a 25-μl volume consisting of 1 μmol/l of each of the forward and reverse primer (forward 5'-CGCGGGAG TTCAGGGTAAAG-3' and reverse 5'-AGCTGGAGACAAGTCAG GAC-TTAAC-3'), 12.5 μl One*Taq*® Quick-Load® 2X Master Mix with Standard Buffer (New England Biolabs, Ipswich, MA, USA), and 1 μl genomic DNA. The cycling conditions were 94˚C for 4 min, followed by 27 cycles at 94˚C for 30 s, 62˚C for 30 s, and 72˚C for 30 s, with a final cycle at 72˚C for 7 min. A 10 μl aliquot of the appropriate PCR product was digested with 10U of the restriction enzyme *MspA1I* (New England Biolabs) at 37˚C for 16 h and separated on ethidium-bromide-stained 3% agarose gel. The *G* allele produced a 189-bp and 48-bp pattern, whereas the *T* allele was undigested and produced a 237-bp fragment.

Quantitative real-time PCR. Total RNA was isolated from prostate cancer/BPH tissue by using the AllPrep DNA/RNA/miRNA Universal Kit (Qiagen GmbH, Hilden, Germany) according the manufacturer's recommendations. The amount of extracted RNA was quantified with a Nanophotometer (Implen GmbH, München, Germany). First-strand cDNAs were synthesized from total RNA (1 μg) by using the RT^2 First Strand Kit (Qiagen GmbH) according to the manufacturer's protocol.

Quantitative PCR was performed on a Viia7 Real Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA) according to the following temperature profile: 50˚C for 2 min, 95˚C for 10 min followed by an amplification composed of 40 cycles of 95˚C for 15 s and 60˚C for 1 min. The primer sequences used in this study were as follows: *β-actin* (housekeeping gene): 5'-GGCGGCACCACCATG TACCCT-3', and 5'-AGGGGCCGGACTCGTCATACT-3'; *MDM2*: 5'-TGTAAGTGAACATTCAGG TG-3', and 5'-TTCCAATAGTCA GCTAAGGA-3' (15). The reaction was performed in a final volume of 20 μL with IQ™ SYBR Green Supemix Master Mix (BioRad Laboratories, Inc.), 0.5 μmol/l of each specific primer, and 9 ng cDNA. Each sample was assayed in triplicate for each primer pair. Results obtained from this experiment were normalized to β-actin by using the $2^{-\Delta\Delta Ct}$ method to determine the fold change in relative gene expression (16).

Western blot analysis. To analyze the correlation between the *MDM2 T309G* polymorphism and protein expression levels, we examined 14 prostate cancer tumor tissues. Total protein samples (30 μg) were run on a 12% SDS-PAGE gel, and following electrophoresis, separated proteins were transferred onto a nitrocellulose membrane by using a semi-dry transfer protocol. Non-specific binding was blocked with 5% non-fat dry milk in TBS-T (50 mM Tris-Cl, pH 7.5, 150 mM NaCl, 0.05% Tween 20) for at least 2 h. They were then incubated with the following primary antibodies at 4˚C overnight: anti-MDM2 (1:500, ab16895) and anti-β-actin (1:2,000, sc-1616). Next day, the membranes were washed with TBS-T solution and then incubated with secondary antibodies conjugated with horseradish peroxidase (1:5,000, Santa Cruz) for 1 h. After extensive washes with TBS-T solution (3 times × 15 min), membranes were incubated in SuperSignal West Pico Chemiluminescent Substrate (ThermoFisher Scientific) solution for 3 min in the dark. After exposure of the membranes to Chemidoc XRS (BioRad Laboratories, Inc.), the intensities of the relevant bands were quantified using Quantity One software (BioRad Laboratories, Inc.). The intensities of the bands of interest were normalized against corresponding intensities of β-actin bands.

Statistical analysis. A genomic association study was performed assuming the general, dominant, recessive, multiplicative, and additive models, with the null hypothesis of independence of genotype, and the case/control status was tested by the chi2 test with the *p*-value being computed by the Monte Carlo method. The Hardy Weinberg equilibrium was tested using the R library Hardy Weinberg. The null hypothesis that the population odds ratio (OR) was equal to 1 was tested by the Fisher exact test. Gene expression was computed by an in-house R code and visualized by a boxplot overlaid with a swarm plot. The null hypothesis that the population median Fold Change (FC) was equal to 1 was tested by the Wilcoxon one sample test. Robust ANOVA was used to test the null hypothesis of the equality of the population median FC among the alleles of a gene. Data analyses were carried out in R ver. 3.5.2 (17).

Table I. *Characteristics of the study groups.*

	Healthy controls	Prostate cancer
Number	592	506
Age (years)		
Mean±SD	65.7±9.0	67.4±7.9
PSA (ng/ml, %)		
PSA <10 ng/ml	592 (100)	225 (44.5)
PSA ≥10 ng/ml	0	281 (55.5)
Mean±SD	3.6±0.2	**38.3±4.2**
Gleason score (%)		
<7	NA	104 (25.8)
≥7	NA	298 (74.2)
Mean±SD	NA	7.3±0.06
Missing	NA	104
Pathological stage (%)		
pT1/pT2	NA	119 (50)
pT3/pT4	NA	119 (50)
Missing	NA	268

NA: Not applicable. Bold characters represent $p<0.05$.

Results of tests with a *p*-value below 0.05 were considered statistically significant.

Results

The relevant characteristics of the study subjects are presented in Table I. A significant difference in the mean age was found between the prostate cancer group and the control group ($p<0.05$). The total PSA levels were significantly higher in the patient group than in the control group as expected ($p<0.001$). The majority of the prostate cancer patients had a high Gleason score ≥7 (74.2%), and no difference was noted in the distribution of tumor stages pT1/pT2 and pT3/pT4.

The distributions of the *MDM2 T309G* genotypes in the studied groups are summarized in Table II. The *MDM2 T309G* genotype distribution among our Caucasian control subjects (*TT*: 42.1%, *TG*: 50.5% and *GG*: 7.4%) was similar to that of other studies in identical populations (13, 18), except for the frequency of the variant *G* allele. The genotype distributions in the control group were in Hardy-Weinberg equilibrium ($p>0.05$). The heterozygous mutant *TG* genotype of *MDM2* was associated with no increased risk of prostate cancer (OR=1.19; 95%CI=0.92-1.52, $p>0.05$), whereas the *GG* mutant genotype of *MDM2* showed a protective association (OR=0.69; 95%CI=0.44-1.06, $p>0.05$), in comparison with the wild-type *TT* genotype.

The association of the *MDM2 T309G* polymorphism with clinicopathological features, such as total PSA levels, Gleason score, and pathological T stage in prostate cancer patients was further evaluated (Table III). Patients with the *GG* genotype and PSA levels ≥10 ng/ml tended to have a higher risk of prostate cancer (OR=1.34; 95%CI=0.74-2.44, $p>0.05$) compared with those subjects who had the *TT* genotype and PSA levels <10 ng/ml. No significant association was observed between the Gleason score, pathological T stage, and the polymorphisms in *MDM2*.

Table II. *Distributions of the MDM2 T309G allelic and genotypic frequencies among patients with prostate cancer and healthy controls.*

Genotype	Healthy controls n (%)	Prostate cancer n (%)	OR (95%CI)	*p*-Value
TT	249 (42.1)	223 (44.1)	1.00 (ref.)	
TG	299 (50.5)	226 (44.6)	1.19 (0.92-1.52)	0.20
GG	44 (7.4)	57 (11.3)	0.69 (0.44-1.06)	0.10
TG+GG	343 (58)	283 (55.9)	1.08 (0.85-1.38)	0.54
Allele				
T	797 (67.3)	672 (66.4)	1.00 (ref.)	
G	387 (32.7)	340 (33.6)	0.96 (0.80-1.15)	0.68

Table III. *Association of the MDM2 T309G alleles and genotypes with clinicopathological characteristics of prostate cancer.*

Genotypes	Cases		OR (95%CI)	*p*-Value
	PSA <10 ng/ml n (%)	PSA ≥10 ng/ml n (%)		
TT	106 (47.1)	117 (41.6)	1.00 (ref.)	
TG	96 (42.7)	130 (46.3)	1.23 (0.85-1.78)	0.29
GG	23 (10.2)	34 (12.1)	1.34 (0.74-2.44)	0.37
TG+GG	119 (52.8)	164 (58.4)	1.25 (0.87-1.78)	0.24
Allele T	308 (68.4)	364 (64.8)	1.00 (ref.)	
Allele G	142 (31.6)	198 (35.2)	1.18 (0.91-1.54)	0.23
	Gleason <7 n (%)	Gleason ≥7 n (%)		
TT	47 (45.2)	131 (43.9)	1.00 (ref.)	
TG	45 (43.3)	137 (46.0)	0.92 (0.57-1.47)	0.72
GG	12 (11.5)	30 (10.1)	1.12 (0.51-2.33)	0.85
TG+GG	57 (54.8)	167 (56.0)	0.95 (0.61-1.49)	0.91
Allele T	139 (66.8)	399 (67.0)	1.00 (ref.)	
Allele G	69 (33.2)	197 (33.0)	1.00 (0.72-1.40)	1.00
	pT1/pT2 n (%)	PT3/pT4 n (%)		
TT	52 (43.7)	52 (43.7)	1.00 (ref.)	
TG	51 (42.9)	52 (43.7)	0.98 (0.57-1.70)	1.00
GG	16 (13.4)	15 (12.6)	1.06 (0.47-2.41)	1.00
TG+GG	67 (56.3)	67 (56.3)	1.00 (0.60-1.67)	1.00
Allele T	155 (65.1)	156 (65.5)	1.00 (ref.)	
Allele G	83 (34.9)	82 (34.5)	1.02 (0.70-1.48)	1.00

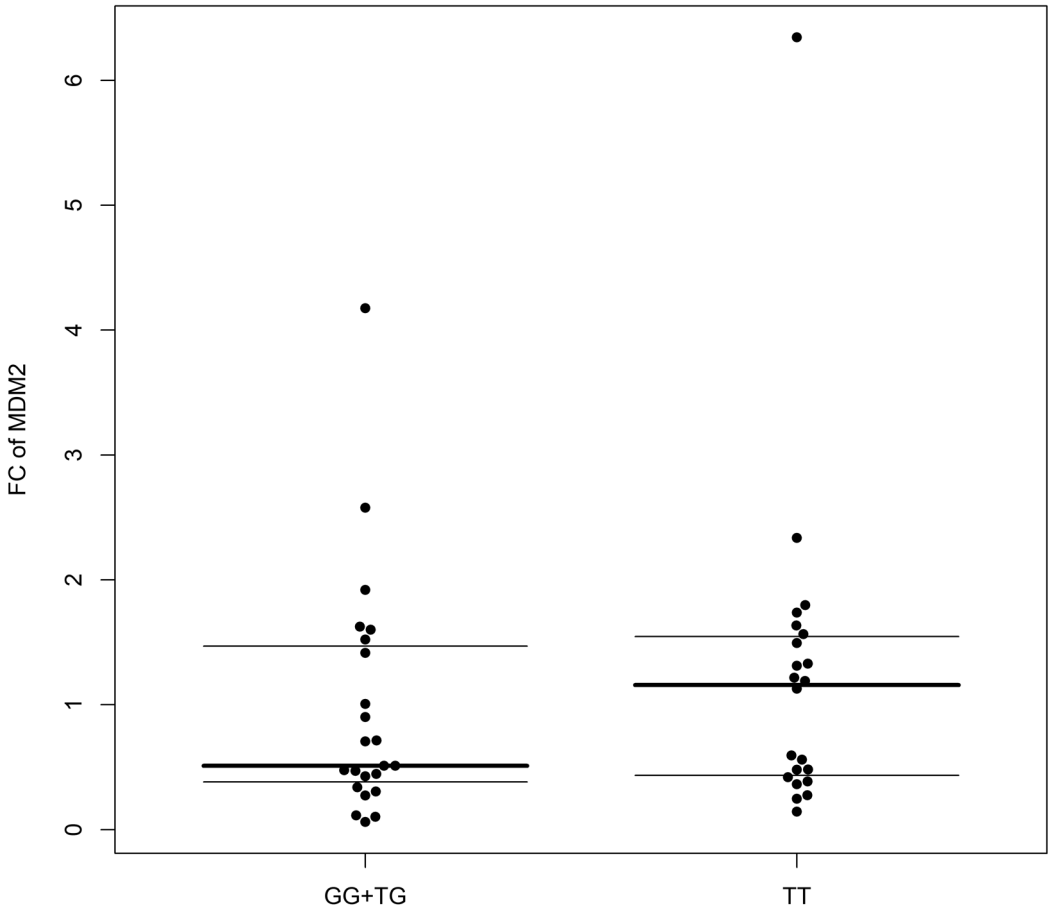

Figure 1. *Fold change (FC) in the relative expression of MDM2 with respect to different MDM2 T309G genotypes among prostate cancer patients.*

Real-time PCR was performed to detect the effect of the *MDM2 T309G* polymorphism on the relative *MDM2* mRNA expression levels in prostate tumor tissues. Because of the low number of individuals who carried the *GG* genotype, we examined the joint effect of the *TG* plus *GG* genotypes *vs*. the *TT* genotype on the relative *MDM2* mRNA expression (Figure 1). The results revealed that the median fold change values were lower in individuals who carried the *TG* plus *GG* genotypes than those with the *TT* genotype (0.51 and 1.16, respectively, $p>0.05$) by 2.3-fold. The effect of selected clinicopathological characteristics on relative *MDM2* mRNA expression levels was also studied (Table IV). Wilcoxon's test between the Gleason score or pathological T stage and mRNA expression showed no statistically significant associations ($p>0.05$).

In order to study whether the *MDM2 T309G* polymorphism had an impact on the MDM2 protein levels, we performed western blot analysis in 14 tumor tissues obtained from the prostate cancer patients. The frequency distribution of the *TT*, *TG*, and *GG* genotypes was 5, 5, and 4, respectively. We found that the MDM2 protein levels were lower in individuals with the *TG* and *GG* genotypes than in those with the *TT* genotype (Figure 2).

Table IV. *Fold chance in the relative MDM2 expression with respect to selected clinicopathological characteristics of prostate cancer patients.*

Variables	Median	Range	*p*-Value
Gleason score			
<7	1.00	0.10-1.32	
≥7	0.48	0.06-6.34	0.10
Pathological stage			
pT1/pT2	0.48	0.10-2.57	
pT3/pT4	0.90	0.11-6.34	0.41

Discussion

The exact mechanism by which *MDM2 T309G* affects cancer susceptibility has not yet been fully elucidated. During the

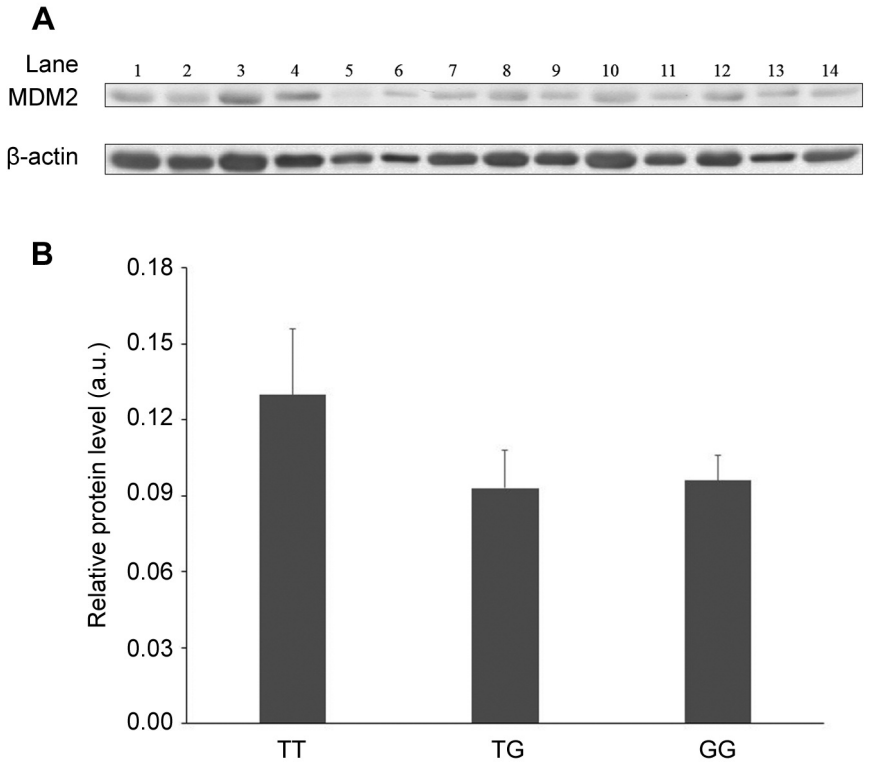

Figure 2. *Association between the MDM2 T309G polymorphism and MDM2 protein levels. (A) Western blot analysis of MDM2 protein levels in prostate cancer tissues from individuals with different MDM2 T309G genotypes. Individual genotype designation: lanes 1 to 5, TT genotype (n=5); lanes 5 to 10, TG genotype (n=5); lanes 11 to 14, GG genotype (n=4); (B) Relative protein expression levels were calculated densitometrically in reference to the β-actin expression level. Values are given as means±SEM.*

past few years, several studies have focused on the effects of the *MDM2 T309G* polymorphism on prostate cancer risk within various ethnic populations (11, 18, 19). Results from these studies are inconsistent mainly for Europeans, whereas the data from individuals of Asian ancestry indicate that this polymorphism increases cancer risk (20). Therefore, we have conducted a case-control study consisting of 506 cases and 592 controls, all of European ancestry, within Slovakia to validate these results. Our study has shown that the mutant *GG* genotype of *MDM2 T309G* has a protective association with prostate cancer risk and decreases the relative MDM2 mRNA and protein levels in prostate cancer tissues.

Both the *T* and *G* alleles have previously been shown to be associated with a high risk of malignancy (9, 21). Kibel *et al*. suggest that the presence of at least one copy of the *T* allele of *MDM2* is associated with an increased risk of advanced prostate cancer for the European-American population (18). On the contrary, a study on a Chinese population has shown that the *T* alleles of *MDM2* are associated with a decreased prostate cancer risk (22). One hospital-based case control study from northern India has found significant association of the *GG* variant with reduced risk of prostate cancer (12). Studies carried out in Germany (13), Norway (23), Japan (24), and China (25) have reported no correlation between a *MDM2 T309G* polymorphism and the development of prostate cancer. A meta-analysis of Yang *et al*. has shown that the *MDM2 309G* variant is significantly associated with a decreased prostate cancer risk in Caucasians but not in Asians (11). The same results of a decreased prostate cancer risk in the European population and in a hospital-based population with the *MDM2 T309G* polymorphism have been obtained from the meta-analysis of Chen *et al*. (26). A subsequent meta-analysis reported that the *G* allele was not associated with an increased risk of prostate cancer in either the total population or after grouping by ethnicity (27, 28).

A number of possible explanations can be proposed for the different results observed in the present study as compared with other previous studies: 1) the different frequencies of the *MDM2 T* and *G* alleles across ethnicities; 2) the different study designs and sample sizes; 3) the genotyping method employed in the study; 4) the distinct SNP in the *MDM2* P2 promoter, *e.g*., *MDM2 SNP285* is located 24 bp upstream from *MDM2 T309G*, which overlaps with the SP1 site (29); and 5) gene-gene (*e.g*., *p53-MDM2*) and gene-environment interactions.

MDM2 overexpression has been demonstrated to be significantly associated with a large number of human tumors including prostate cancer (30, 31). In the present study, we have evaluated whether MDM2 mRNA and protein expression in prostate tumors is influenced by the *MDM2 T309G* polymorphism. To the best of our knowledge, we report the first analysis of this association in prostate cancer. We have not observed any significant effect of the *MDM2 G* allele on relative *MDM2* mRNA expression levels. Our results are in agreement with those from previous studies in various types of malignancies (32, 33). On the other hand, several studies have demonstrated a correlation between the *MDM2 T309G* polymorphism and mRNA levels (34, 35). Woelfelschneider *et al*. have analyzed whether genetic variation in *MDM2 T309G* contributes to changes in the mRNA expression, as measured in the peripheral blood lymphocytes in prostate cancer patients (36). They found a higher median *MDM2* mRNA level in homozygous *GG* individuals as compared with *T* allele carriers. The direct impact of this polymorphism in tumorigenesis has been determined in two genetically engineered mouse models carrying either the *MDM2 309G* or *MDM2 309T* alleles. They have shown that mice carrying two *MDM2 309G* alleles have increased levels of MDM2 mRNA and protein and exhibit accelerated tumor formation (37). More interestingly, our data have demonstrated decreased MDM2 protein levels in individuals with *TG* and *GG* genotypes in comparison with individuals with the *TT* genotype. We hypothesize that a positive association between the *MDM2 309G* allele and MDM2 mRNA and protein levels might have affected tumorigenesis through regulation of the p53 proteasomal degradation. Moreover, it is possible that in a particular environment or genetic background, the *MDM2* variant may protect, while in another, the same variant may predispose cells to malignant transformation (18, 38).

Finally, the *MDM2 T309G* genotype and MDM2 expression profiles have been correlated with selected clinicopathological features of prostate cancer, including total PSA levels, pathological stage, and Gleason score. In most studies, the authors have not discussed the association of this polymorphism with the clinical behavior of the prostate tumor, whereas this aspect is addressed in our present study. We observed no significant associations between the genotypes and MDM2 mRNA and protein expression with the clinical outcomes of prostate cancer. Similarly, other authors have found no association of the *MDM2 T309G* polymorphism with tumour risk, age at tumour onset, histopathological characteristics of the tumours, or prognosis (13, 19, 39). By contrast, a large hospital-based study in a Caucasian population, performed by Sun *et al*. has revealed that the *MDM2 309T* allele is associated with earlier onset prostate cancer, higher Gleason scores, and higher tumor stages in men undergoing radical prostatectomy (40). A later study by Yang *et al*. has shown that the *MDM2 309G* variant is significantly associated with both a lower malignant degree and a slower clinical progression in Caucasians but not in Asians (11).

In conclusion, our results have shown a protective association between the *MDM2 GG* genotype and prostate cancer risk. We have further demonstrated that the *MDM2 T309G* polymorphism is associated with decreased expression of MDM2 in prostate cancer tissues. Neither the *MDM2 T309G* polymorphism nor MDM2 expression exhibit significant correlations with clinicopathological features. The molecular mechanisms underlying this association remain to be determined, and larger population studies are required to verify this conclusion.

Conflicts of Interest

The Authors declare no conflicts of interest regarding this study.

Authors' Contributions

MKS and JK conceived the idea for this study and wrote the paper. JJ, MH, AT, and HD contributed to the design of the research. RD and DE were involved in the data collection. PK and MG analyzed the data. All Authors edited and approved the final version of the manuscript.

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References

1 Siegel RL, Miller KD and Jemal A: Cancer Statistics, 2017. CA Cancer J Clin *67*: 7-30, 2017. PMID: 28055103. DOI: 10.3322/caac.21387

2 Rawla P: Epidemiology of prostate cancer. World J Oncol *10*: 63-89, 2019. PMID: 31068988. DOI: 10.14740/wjon1191

3 Pernar CH, Ebot EM, Wilson KM and Mucci LA: The epidemiology of prostate cancer. Cold Spring Harb Perspect Med *8*: a030361, 2018. PMID: 29311132. DOI: 10.1101/cshperspect.a030361

4 Mendoza M, Mandani G and Momand J: The MDM2 gene family. Biomol Concepts *5*: 9-19, 2014. PMID: 25372739. DOI: 10.1515/bmc-2013-0027

5 Wang X and Jiang X: Mdm2 and MdmX partner to regulate p53. FEBS Lett *586*: 1390-1396, 2012. PMID: 22673503. DOI: 10.1016/j.febslet.2012.02.049

6 Li M, Brooks CL, Wu-Baer F, Chen D, Baer R and Gu W: Mono- *versus* polyubiquitination: differential control of p53 fate by Mdm2. Science *302*: 1972-1975, 2003. PMID: 14671306. DOI: 10.1126/science.1091362

7 Bond GL, Hu W and Levine AJ: MDM2 is a central node in the p53 pathway: 12 years and counting. Curr Cancer Drug Targets *5*: 3-8, 2005. PMID: 15720184. DOI: 10.2174/1568009053332627

8 Zhao Y, Yu Hand and Hu W: The regulation of MDM2 oncogene and its impact on human cancers. Acta Biochim Biophys Sin (Shanghai) *46*: 180-189, 2014. PMID: 24389645. DOI: 10.1093/abbs/gmt147

9 Bond GL, Hu W, Bond EE, Robins H, Lutzker SG, Arva NC, Bargonetti J, Bartel F, Taubert H, Wuerl P, Onel K, Yip L, Hwang SJ, Strong LC, Lozano G and Levine AJ: A single nucleotide polymorphism in the MDM2 promoter attenuates the p53 tumor suppressor pathway and accelerates tumor formation in humans. Cell *119*: 591-602, 2004. PMID: 15550242. DOI: 10.1016/j.cell.2004.11.022

10 Basu S and Murphy ME: Genetic Modifiers of the p53 Pathway. Cold Spring Harb Perspect Med *6*: a026302, 2016. PMID: 27037420. DOI: 10.1101/cshperspect.a026302

11 Yang J, Gao W, Song NH, Wang W, Zhang JX, Lu P, Hua LX and Gu M: The risks, degree of malignancy and clinical progression of prostate cancer associated with the MDM2 T309G polymorphism: a meta-analysis. Asian J Androl *14*: 726-731, 2012. PMID: 22902907. DOI: 10.1038/aja.2012.65

12 Mandal RK and Mittal RD: Are cell cycle and apoptosis genes associated with prostate cancer risk in North Indian population? Urol Oncol *30*: 555-561, 2012. PMID: 20822933. DOI: 10.1016/j.urolonc.2010.05.006

13 Stoehr R, Hitzenbichler F, Kneitz B, Hammerschmied CG, Burger M, Tannapfel A and Hartmann A: Mdm2-SNP309 polymorphism in prostate cancer: no evidence for association with increased risk or histopathological tumour characteristics. Br J Cancer *99*: 78-82, 2008. PMID: 18577987. DOI: 10.1038/sj.bjc.6604441

14 Ohmiya N, Taguchi A, Mabuchi N, Itoh A, Hirooka Y, Niwa Y and Goto H: MDM2 promoter polymorphism is associated with both an increased susceptibility to gastric carcinoma and poor prognosis. J Clin Oncol *24*: 4434-4440, 2006. PMID: 16983111. DOI: 10.1200/JCO.2005.04.1459

15 Xiao M, Zhang L, Zhu X, Huang J, Jiang H, Hu S and Liu Y: Genetic polymorphisms of MDM2 and TP53 genes are associated with risk of nasopharyngeal carcinoma in a Chinese population. BMC Cancer *10*: 147, 2010. PMID: 20398418. DOI: 10.1186/1471-2407-10-147

16 Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods *25*: 402-408, 2001. PMID: 11846609. DOI: 10.1006/meth.2001.1262

17 R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: https://www.R-project.org/ [Last accessed September 2, 2020]

18 Kibel AS, Jin CH, Klim A, Luly J, A Roehl K, Wu WS and Suarez BK: Association between polymorphisms in cell cycle genes and advanced prostate carcinoma. Prostate *68*: 1179-1186, 2008. PMID: 18459109. DOI: 10.1002/pros.20784

19 Wang G, Firoz EF, Rose A, Blochin E, Christos P, Pollens D, Mazumdar M, Gerald W, Oddoux C, Lee P and Osman I: MDM2 expression and regulation in prostate cancer racial disparity. Int J Clin Exp Pathol *2*: 353-360, 2009. PMID: 19158992.

20 Hu Z, Jin G, Wang L, Chen F, Wang X and Shen H: MDM2 promoter polymorphism SNP309 contributes to tumor susceptibility: evidence from 21 case□control studies. Cancer Epidemiol Biomarkers Prev *16*: 2717-2723, 2007. PMID: 18086778. DOI: 10.1158/1055-9965.EPI-07-0634

21 Economopoulos KP and Sergentanis TN: Differential effects of MDM2 SNP309 polymorphism on breast cancer risk along with race: a meta-analysis. Breast Cancer Res Treat *120*: 211-216, 2010. PMID: 19590949. DOI: 10.1007/s10549-009-0467-1

22 Xue L, Han X, Liu R, Wang Z, Li H, Chen Q, Zhang P, Wang Z and Chong T: MDM2 and P53 polymorphisms contribute together to the risk and survival of prostate cancer. Oncotarget *7*: 31825-31831, 2016. PMID: 26025918. DOI: 10.18632/oncotarget.3923

23 Gansmo LB, Knappskog S, Romundstad P, Hveem K, Vatten L and Lønning PE: Influence of MDM2 SNP309 and SNP285 status on the risk of cancer in the breast, prostate, lung and colon. Int J Cancer *137*: 96-103, 2015. PMID: 28158999. DOI: 10.1002/ijc.29358

24 Hirata H, Hinoda Y, Kikuno N, Suehiro Y, Shahryari V, Ahmad AE, Tabatabai ZL, Igawa M and Dahiya R: Bcl2 -938C/A polymorphism carries increased risk of biochemical recurrence after radical prostatectomy. J Urol *181*: 1907-1912, 2009. PMID: 19237173.

25 Xu B, Xu Z, Cheng G, Min ZC, Mi Y, Zhang ZZ, Tao J, Li PC, Wang ML, Tang JL, Zhang ZD, Zhang W, Wu HF, Feng NH and Hua LX: Association between polymorphisms of TP53 and MDM2 and prostate cancer risk in southern Chinese. Cancer Genet Cytogenet *202*: 76-81, 2010. PMID: 20875869. DOI: 10.1016/j.cancergencyto.2010.02.014

26 Chen T, Yi SH, Liu XY and Liu ZG: Meta-analysis of associations between the MDM2-T309G polymorphism and prostate cancer risk. Asian Pac J Cancer Prev *13*: 4327-4330, 2012. PMID: 23167337. DOI: 10.7314/apjcp.2012.13.9.4327

27 Chen Y, Zhong H, Gao JG, Tang JE and Wang R: A systematic review and meta-analysis of three gene variants association with risk of prostate cancer: An update. Urol J *12*: 2138-2147, 2015. PMID: 26135929.

28 Ding H, Dai Y, Ning Z, Fan N, Wang Z, Li P, Zhang L, Tao Y and Wang H: Murine double minute 2 SNP T309G polymorphism and urinary tract cancer risk: A meta-analysis. Medicine (Baltimore) *95*: e2941, 2016. PMID: 27015167. DOI: 10.1097/MD.0000000000002941

29 Knappskog S: Re: Murine double minute 2 promoter SNP309 polymorphism and prostate cancer risk: a meta-analysis. Int J Urol *19*: 966, 2012. PMID: 22846160. DOI: 10.1111/j.1442-2042.2012.03108.x

30 Leite KR, Franco MF, Srougi M, Nesrallah A, Bevilacqua RG, Darini E, Carvalho CM, Meirelles MI, Santana I and Camara-Lopes LH: Abnormal expression of MDM2 in prostate carcinoma. Mod Pathol *5*: 428-436, 2001. PMID: 11353053. DOI: 10.1038/modpathol.3880330

31 Khor LY, Desilvio M, Al-Saleem T, Hammond ME, Grignon DJ, Sause W, Pilepich M, Okunieff P, Sandler H and Pollack A: MDM2 as a predictor of prostate carcinoma outcome: an analysis of Radiation Therapy Oncology Group Protocol 8610. Cancer *104*: 962-967, 2005. PMID: 16007688. DOI: 10.1002/cncr.21261

32 Rihani A, Van Maerken T, De Wilde B, Zeka F, Laureys G, Norga K, Tonini GP, Coco S, Versteeg R, Noguera R, Schulte

JH, Eggert A, Stallings RL, Speleman F and Vandesompele J: Lack of association between MDM2 promoter SNP309 and clinical outcome in patients with neuroblastoma. Pediatr Blood Cancer *61*: 1867-1870, 2014. PMID: 24391119. DOI: 10.1002/pbc.24927

33 McEvoy J, Ulyanov A, Brennan R, Wu G, Pounds S, Zhang J and Dyer MA: Analysis of MDM2 and MDM4 single nucleotide polymorphisms, mRNA splicing and protein expression in retinoblastoma. PLoS One *7*: e42739, 2012. PMID: 22916154. DOI: 10.1371/journal.pone.0042739

34 Dong HJ, Fang C, Fan L, Zhu DX, Wang DM, Zhu HY, Zhuang Y, Miao KR, Liu P, Xu W and Li JY: MDM2 promoter SNP309 is associated with an increased susceptibility to chronic lymphocytic leukemia and correlates with MDM2 mRNA expression in Chinese patients with CLL. Int J Cancer *130*: 2054-2061, 2012. PMID: 21647873. DOI: 10.1002/ijc.26222

35 Chien WP, Wong RH, Cheng YW, Chen CY and Lee H: Associations of MDM2 SNP309, transcriptional activity, mRNA expression, and survival in stage I non-small-cell lung cancer patients with wild-type p53 tumors. Ann Surg Oncol *17*: 1194-1202, 2010. PMID: 19941079. DOI: 10.1245/s10434-009-0853-2

36 Woelfelschneider A, Popanda O, Lilla C, Linseisen J, Mayer C, Celebi O, Debus J, Bartsch H, Chang-Claude J and Schmezer P: A distinct ERCC1 haplotype is associated with mRNA expression levels in prostate cancer patients. Carcinogenesis *29*: 1758-1764, 2008. PMID: 18332046. DOI: 10.1093/carcin/bgn067

37 Post SM, Quintás-Cardama A, Pant V, Iwakuma T, Hamir A, Jackson JG, Maccio DR, Bond GL, Johnson DG, Levine AJ and Lozano G: A high-frequency regulatory polymorphism in the p53 pathway accelerates tumor development. Cancer Cell *18*: 220-230, 2010. PMID: 20832750. DOI: 10.1016/j.ccr.2010.07.010

38 Onat OE, Tez M, Ozçelik T and Törüner GA: MDM2 T309G polymorphism is associated with bladder cancer. Anticancer Res *26*: 3473-3475, 2006. PMID: 17094469.

39 Jaboin JJ, Hwang M, Perez CA, Cooper C, Chen H, Ye C, Cai Q, Wills ML and Lu B: No evidence for association of the MDM2-309 T/G promoter polymorphism with prostate cancer outcomes. Urol Oncol *29*: 319-323, 2011. PMID: 19523862. DOI: 10.1016/j.urolonc.2009.03.031

40 Sun T, Lee GS, Oh WK, Pomerantz M, Yang M, Xie W, Freedman ML and Kantoff PW: Single-nucleotide polymorphisms in p53 pathway and aggressiveness of prostate cancer in a Caucasian population. Clin Cancer Res *16*: 5244-5251, 2010. PMID: 20855462. DOI: 10.1158/1078-0432.CCR-10-1261

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