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Investigation of the Roles of MTHFR (*C677T* and *A1298C*) and MMP-2 (*–1306C>T*) Variations in Bladder Cancer Development

ABSTRACT

Objective: Bladder cancer is a complex malignancy and has been associated with high morbidity. Since susceptibility to bladder cancer development differs between individuals, determining the roles of MTHFR and MMP-2 gene variations associated with this cancer is important for analyzing differences in individual susceptibility. In this study, we aimed to investigate the role of MTHFR and MMP-2 gene variations in the development of bladder cancer in the Thrace region of Turkey.

Materials and methods: One hundred seventy-nine blood samples were collected, including 98 patients with bladder cancer and 81 healthy controls. DNA extraction was carried out with blood samples. Polymerase chain reaction-restriction fragment length polymorphism was applied to detect MTHFR *C677T* (rs 1801133), MTHFR *A1298C* (rs 1801131), and MMP-2 *(–1306C>T)* (rs 243865) gene variants.

Results: For the MTHFR *A1298C* gene variation, CC genotype was the genetic risk factor ($P = .0001$), while AC genotype was the protective factor ($P < .0001$) in the development of bladder cancer. For the MMP-2 *(–1306C>T)* gene variation, TT genotype ($P < .0001$) and T allele ($P = .0006$) were genetic risk factors, while AC genotype ($P = .0009$) was the protective factor in the development of bladder cancer. For *C677T/A1298C* gene variations, CC–CC combined genotype was the genetic risk factor ($P = .009$), while CT–AC and CC–AC combined genotypes were potential protective biomarkers ($P = .013$ and $P < .001$, respectively).

Conclusion: In our study, TT genotype and T allele were determined as genetic risk factors for MMP-2 *(–1306C>T)* gene variation. For *C677T/A1298C* gene variations, CC–CC combined genotype was detected as the genetic risk factor in the development of bladder cancer.

Keywords: Urinary bladder neoplasms, methylenetetrahydrofolate reductase, polymorphism, matrix metalloproteinases, polymerase chain reaction, restriction fragment length polymorphism

Introduction

Cancer is a multistage process and occurs as a result of interactions between environmental and genetic factors. Investigation of genetic variations associated with cancer susceptibility is extremely important in determining early diagnosis, progression, and therapeutic strategies in cancer.[1] Bladder cancer is characterized by canceration of various cell types in the bladder.[2-5] Interactions between neoplastic cells and microenvironment surrounding these cells have been associated with tumor formation.[5] Bladder cancer is an immunogenic malignancy, and urothelial cancer cells play a role in manipulating the immune system by stimulating the secretion of growth-promoting factors. Tumor-induced excessive cytokine release may lead to inflammatory storm and trigger metastasis through extracellular matrix protein degradation. Effective bladder cancer control has been associated with a strong immune system. There is an important correlation between the microenvironment in tumor tissues and systemic chronic inflammation. Chronic inflammation may be a response to bladder cancer development or a factor that triggers the bladder cancer.[6]

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Folate is an effective coenzyme in DNA synthesis and repair. It provides the methyl group required in deoxynucleoside synthesis.[7-9] Therefore, folate metabolic pathways may contribute to bladder cancer susceptibility.[3] Methylenetetrahydrofolate reductase (MTHFR) is effective in the pathogenesis of various cancer types such as bladder cancer. 5,10-Methylenetetrahydrofolate plays a role in providing the methyl group required for methionine synthesis.[3,10] Various genetic variations have been described in MTHFR gene. The most common variations are MTHFR *C677T* (rs1801133; *Ala222Val*) and MTHFR *A1298C* (rs1801131; *Glu429Ala*) gene variations. These genetic variations affect 30%-50% of the general population and have been associated with various inflammatory diseases such as cancer.[10,11] MTHFR gene variations have also been associated with impaired functions in various metabolic pathways such as neurotransmitter production, immune activation, hormone stimulation, protein function, gene regulation, and DNA repair.[1]

Matrix metalloproteinases (MMPs) play an important role in the degradation of extracellular matrix components.[12] MMPs are important in the protection of cellular microenvironment. MMPs also play a role in various stages of cancer development such as immune surveillance, and they may affect tumor cell behavior.[5,12,13] MMPs are responsible for triggering the mechanisms that may support or inhibit tumor growth.[14] Genetic variations occurring in the regulatory regions of MMPs have been associated with alterations in gene expression levels for different cancer types.[15-17] MMP-2 is an important member in the MMP family.[1] The MMP-2 gene is known as gelatinase A and is localized on chromosome 16q13.[1] Functional single-nucleotide gene variations have been identified in the MMP-2 gene, and these genetic variations may lead to increased proteolytic enzyme production associated with an increased risk of carcinogenesis. The Sp1-type promoter site (CCACC box) is required for transcription activation and promoter activity associated with Sp-1 binding in various genes. MMP-2 *(–1306C)* gene variation may cause the elimination of the CCACC box. Thus, lower promoter activity occurs in transcriptional activity of the MMP-2 gene. Molecular epidemiological studies have been performed in different populations in terms of correlation between MMP-2 *(–1306C>T)* gene variation and various malignancies. Therefore, in the Thrace region of Turkey, we aimed to detect the roles of MTHFR *(C677T, A1298C)* and MMP-2 *(–1306C>T)* gene variations in the development of bladder cancer.

MAIN POINTS

- *In our study, in Thrace population, MTHFR C677T gene variation was not determined as the genetic risk factor for the development of bladder cancer.*
- *CC genotype of MTHFR A1298C gene variation and TT genotype and T allele of MMP-2 (–1306C>T) gene variation were identified as genetic risk factors for bladder cancer development.*
- *AC genotype of MTHFR A1298C gene variation and CT genotype of MMP-2 (–1306C>T) were determined as protective markers for bladder cancer.*
- *In addition to this, for C677T/A1298C gene variations, CC–CC combined genotype was detected as the genetic risk factor, while CT–AC and CC–AC combined genotypes were determined as potential protective biomarkers.*
- *Also, CT–AA–CT, CT–AC–CT, and CC–AC–CC combined genotypes of C677T/A1298C/–1306C>T variants were determined as protective markers for bladder cancer.*

Materials and Methods

Patient and Control Subjects

For this study, ethics committee approval was received from Trakya University Faculty of Medicine Non-Invasive Clinical Research Ethics Committee (TÜTF-BAEK 2019/205). Signed informed consent forms were collected from participants. Contrast-enhanced computed tomography and ultrasonography were applied to the patients primarily for diagnosis of bladder cancer. For pathological diagnosis, tumor was removed by Transurethral Resection of Bladder Tumors (TURBT) surgery and was sent to pathology. Men and women younger than 19 years, pregnant women, and those with another malignancy were excluded from the study. A flow diagram is presented in Figure 1. In this diagram, patient and control groups have been reported. According to the diagram, potentially eligible participants were 183 (100 patients and 83 controls). Two patients with any malignancies were excluded from the study. In addition, because of 2 unsuccessful DNA isolation, 2 controls were excluded from our study. Genotyping has been performed in a total of 179 participants (98 patients and 81 controls).

DNA Isolation and Polymerase Chain Reaction-Restriction Fragment Length Polymorphism

DNA isolation was carried out in the participants. For MTHFR and MMP-2 gene variations, polymerase chain reaction (PCR) conditions, primer series, product lengths, and restriction enzymes are presented in Table 1. MTHFR and MMP-2 gene variants were determined using PCR-restriction fragment length polymorphism (PCR-RFLP) methods[18-20] (Figures 2-4). MTHFR *C677T* gene variants are presented in Figure 2. CC genotype (198 bp), CT genotype (198, 175, and 23 bp), and TT genotype (175 and 23 bp) have been observed in Figure 2. A 50 bp marker was used and 23 bp was not observed in the figure. MTHFR *A1298C* gene variation genotypes are presented in Figure 3. CC genotype (84, 31, and 30 bp), AC genotype (84, 56, 31, 30, 28,

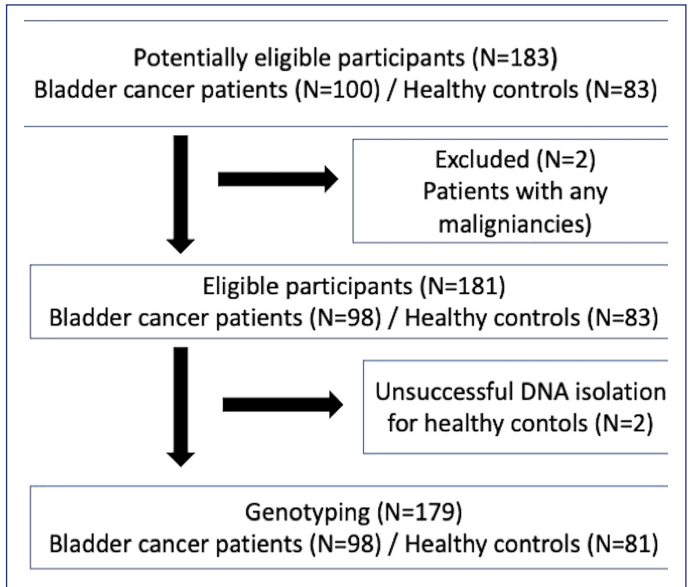

Figure 1. A flow diagram of the bladder cancer patient and control groups.

Table 1. Primer Series, PCR Conditions, Restriction Enzymes, and Product Lengths for MTHFR *(C677T, A1298C)* and MMP-2 *(–1306C>T)* Gene Variations

Gene Variations	Primer Series	PCR Conditions	RE
MTHFR *(C677T)*	FP: 5′-TGAAGGAGAAGGTGTCTGCGG GA-3′ RP: 5′-AGGACGGTGCGGTGAGAG TG-3′	5 minutes at 94^{0}C 30 seconds at 94^{0}C 30 seconds at 62^{0}C 30 seconds at 72^{0}C 5 minutes at 72^{0}C	*HinfI* 30cycle
MTHFR *(A1298C)*	FP: 5′-CTTTGGGGAGCTGAAGGACTA CTAC-3′ RP: 5′CACTTTGTGACCATTCCGGTTTG-3′	5 minutes at 94^{0}C 30 seconds at 94^{0}C 30 seconds at 62^{0}C 30 seconds at 72^{0}C 10 minutes at 72^{0}C	*MboII* 35cycle
MMP-2 *(–1306C>T)*	FP: 5′-CTTCCTAGGCTGGTCCTTACTGA-3′ RP: 5′-CTGAGACCTGAAGACCTAAAGAGCT-3′	5 minutes at 94^{0}C 1 minute at 95^{0}C 1 minute at 62^{0}C 1 minute at 72^{0}C 7 minutes at 72^{0}C	*BfaI (XspI)* 35cycle
Gene Variation	PCR Product Length	RFLP Product Lengths	
MTHFR *C677T*	198 bp	CC ⟶ 198bp CT ⟶ 198bp, 175bp, 23bp TT ⟶ 175bp, 23bp (23bp is not observed; 50bp marker	
MTHFR *A1298C*	163 bp	CC ⟶ 84bp, 31bp, 30bp AC ⟶ 84bp, 56bp, 31bp, 30bp, 28bp, 18bp AA ⟶ 56bp, 31bp, 30bp, 28bp, 18bp (30bp, 28bp and 18bp are not observed; 50bp marker	
MMP-2 *(–1306C>T)*	188 bp	CC ⟶ 188bp, 5bp CT ⟶ 188bp, 162bp, 26bp, 5bp TT ⟶ 162bp, 26bp, 5bp (26bp and 5 bp are not observed; 100bp marker	

FP, forward primer; PCR, polymerase chain reaction; RE, restriction enzyme; RP, reverse primer.

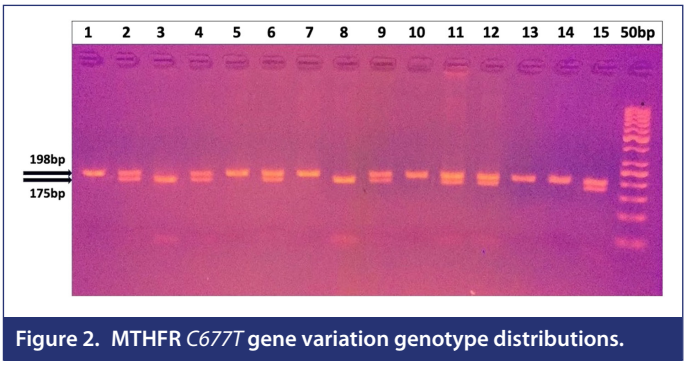

Figure 2. MTHFR *C677T* gene variation genotype distributions.

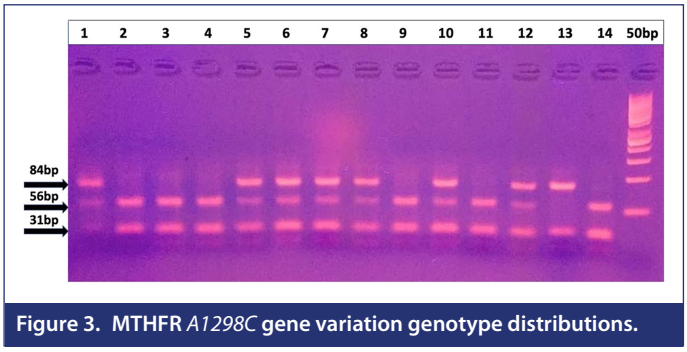

Figure 3. MTHFR *A1298C* gene variation genotype distributions.

and 18 bp), and AA genotype (56, 31, 30, 28, and 18 bp) have been observed in Figure 3. A 50-bp marker was used, and 30, 28, and 18 bp were not observed in the figure. MMP-2 *(–1306T>C)* gene variation genotype distributions are presented in Figure 4. CC genotype (188 and 5 bp), CT genotype (188, 162, 26, and 5 bp), and TT genotype (188, 162, and 5 bp) have been observed in Figure 4. A 100-bp marker was used, and 26 and 5 bp were not observed in the figure.

Statistical Analysis

The statistical analysis was carried out using the Statistics Package of Social Science (SPSS) v.20.0 program (IBM SPSS Corp.; Armonk, NY, USA). The chi-square test was used to compare clinical and

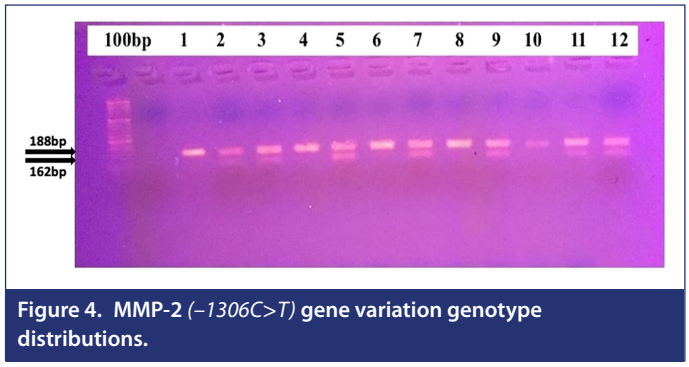

Figure 4. MMP-2 *(–1306C>T)* gene variation genotype distributions.

Table 2. Comparison of Clinical and Demographic Parameters Between Patient and Healthy Control Groups

Clinical and Demographic Parameters	Patient Group (n = 98)	Control Group (n = 81)	*P*
Gender (male/female)	88 (63.8%)/10 (24.4%)	50 (36.2%)/31 (75.6%)	***<.001***[a*]
Hypertension (+)/(–)	38 (76.0%)/60 (46.5%)	12 (24.0%)/69 (53.5%)	***<.001***[a*]
Diabetes mellitus (+)/(–)	19 (70.4%)/79 (52.0%)	8 (29.6%)/73 (48.0%)	.119[a]
Cholesterol (+)/(–)	6 (37.5%)/92 (56.4%)	10 (62.5%)/71 (43.6%)	.234[a]
Heart disease (+)/(–)	23 (95.8%)/75 (48.4%)	1 (4.2%)/80 (51.6%)	***<.001***[a*]
Alcohol (+)/(–)	36 (57.1%)/62 (53.4%)	27 (42.9%)/54 (46.6%)	.635[a]
Smoking (+)/(–)	38 (66.7%)/60 (49.2%)	19 (33.3%)/62 (50.8%)	***.029***[a]

(+)/(–), existent/absent.
[a]Chi-square test.
*Significance ($P < .05$).

demographic parameters between patients and controls. These variations in genotype distributions were compared between these groups using the chi-square test. In addition, the Hardy–Weinberg distribution was used to compare allele frequencies of MTHFR and MMP-2 gene variations in these groups. Combined genotype analyses were carried out and allele frequencies and odds ratio values were calculated for MTHFR and MMP-2 gene variations in these groups.

Results

In our study, clinical and demographic parameters have been presented in Table 2. According to the results, a significant difference was detected in terms of gender ($P < .001$), hypertension ($P < .001$), heart disease ($P < .001$), and smoking ($P = .029$) parameters between patient and control groups. However, the significant difference was not determined in terms of diabetes mellitus ($P = .119$), cholesterol ($P = .234$), and alcohol ($P = .635$) parameters between these groups. The significant difference was not detected for the MTHFR *C677T* gene variation between patient and control groups ($P > .05$). For the MTHFR *A1298C* gene variation, CC genotype was determined as a genetic risk factor (odds ratio: 7.033; 95% CI: 2.587-19.118; $P = .0001$), while AC genotype was determined as a protective factor (odds ratio: 0.209; 95% CI: 0.110-0.400; $P < .0001$) in the development of bladder cancer. For the MMP-2 *(–1306C>T)* gene variation, TT genotype (odds ratio: 8.826; 95% CI: 3.267-23.841; $P < .0001$) and T allele (odds ratio: 2.111; 95% CI: 1.374-3.243; $P = .0006$) were determined as a genetic risk factor, while CT genotype (odds ratio: 0.353; 95% CI: 0.191-0.652; $P = .0009$) was determined as a protective factor in the development of bladder cancer (Table 3). The allele frequencies of these gene variations were detected significantly different according to the Hardy–Weinberg distribution in these groups ($P < .05$) (Table 4).

For *C677T/A1298C* gene variations, CC–CC combined genotype was detected as genetic risk factor (odds ratio: 3.897; 95% CI: 1.392-10.913; $P = .009$), while CT–AC and CC–AC combined genotypes were determined as potential protective biomarkers (odds ratio: 0.374; 95% CI: 0.172-0.814; $P = .013$; odds ratio: 0.240; 95% CI: 0.104-0.554; $P < .001$, respectively). CT–AA–CT, CT–AC–CT, and CC–AC–CC combined genotypes of *C677T/A1298C/–1306C>T* variants were determined as protective markers for bladder cancer (odds ratio: 0.309; 95% CI: 0.104-0.918; $P = .035$; odds ratio: 0.120; 95% CI: 0.026-0.552; $P = .007$; odds ratio: 0.139; 95% CI: 0.039-0.499; $P = .002$, respectively) (Table 5).

Discussion

Systemic inflammation is an important susceptibility factor and is effective in the pathogenesis of various diseases and different types of cancer. Various genetic variations have been associated with carcinogenesis processes.[1] High folate supplements are associated with a significant reduction in bladder cancer risk. Therefore, it is thought that functional genetic variations in genes associated with folate metabolism may be effective in bladder carcinogenesis.[21] As a result

Table 3. Comparison of MTHFR (*C677T* and *A1298C*) and MMP-2 *(–1306C>T)* Gene Variations, Genotype Distributions, and Allele Frequencies Between Patient and Healthy Control Groups

Genotype Distributions		Patient Group (n = 98)	Control Group (n = 81)	Odds Ratio (95% CI)	*P*
MTHFR *C677T*	CC	43 (55.8%)	34 (44.2%)	Reference	Reference
	CT	37 (50.0%)	37 (50.0%)	0.721 (0.397-1.312)	.285
	TT	18 (64.3%)	10 (35.7%)	1.598 (0.692-3.687)	.272
	C	123 (62.8%)	105 (64.8%)	Reference	Reference
	T	73 (37.2%)	57 (35.2%)	1.093 (0.709-1.686)	.687
MTHFR *A1298C*	AA	45 (60.8%)	29 (39.2%)	Reference	Reference
	AC	22 (31.9%)	47 (68.1%)	0.209 (0.110-0.400)	***<.0001***[*]
	CC	31 (86.1%)	5 (13.9%)	7.033 (2.587-19.118)	***.0001***[*]
	A	112 (57.1%)	105 (64.8%)	Reference	Reference
	C	84 (42.9%)	57 (35.2%)	1.382 (0.900-2-122)	.140
MMP-2 *(–1306C>T)*	CC	32 (50.8%)	31 (49.2%)	Reference	Reference
	CT	30 (40.0%)	45 (60.0%)	0.353 (0.191-0.652)	***.0009***[*]
	TT	36 (87.8%)	5 (12.2%)	8.826 (3.267-23.841)	***<.0001***[*]
	C	94 (48.0%)	107 (66.1%)	Reference	Reference
	T	102 (52.0%)	55 (33.9%)	2.111 (1.374-3.243)	***.0006***[*]

*Significance ($P < .05$).
AA, adenine–adenine; AC, adenine–cytosine; CC, cytosine–cytosine; CT, cytosine–thymine; TT, thymine–thymine.

Table 4. Comparison of MTHFR (*C677T* and *A1298C*) and MMP-2 *(−1306C>T)* Gene Variations Allele Frequencies Between Patient and Healthy Control Groups with Hardy–Weinberg Equilibrium Test

Gene Variations	Patient Group (n = 98)	Control Group (n = 81)
MTHFR *C677T*	Hardy–Weinberg equilibrium test: Pearson chi^2 = 3.625 Pr = 0.0569[a]	Hardy–Weinberg equilibrium test: Pearson chi^2 = 0.000 Pr = 0.9892[a]
MTHFR *A1298C*	Hardy–Weinberg equilibrium test: Pearson chi^2 = 28.753 Pr = ***0.0000***[a*]	Hardy–Weinberg equilibrium test: Pearson chi^2 = 6.001 Pr = ***0.0143***[a*]
MMP-2 *(−1306C>T)*	Hardy–Weinberg equilibrium test: Pearson chi^2 = 14.657 Pr = ***0.0001***[a*]	Hardy-Weinberg Equilibrium Test: Pearson chi^2 = 4.617 Pr = ***0.0317***[a*]

[a]Hardy–Weinberg Equilibrium test.Pr: Pearson's chi-squared test.
*Significance ($P < .05$).

Table 5. Combined Genotype Analysis, Frequencies, and Odds Ratio Values for MTHFR *(C677T and A1298C)* and MMP-2 *(−1306C>T)* Gene Variations in Patients and Healthy Controls

Combined Genotypes	Patients (N = 98)	Frequency (%)	Controls (N = 81)	Frequency (%)	Odds Ratio (P/C)	95% CI	*P*
C677T/A1298C							
CT–AA	17	***17.3***	15	***18.5***	0.923	0.429-1.988	.839
CT–AC	12	***12.2***	22	***27.2***	0.374	0.172-0.814	***.013****
CC–AC	9	***9.2***	24	***29.6***	0.240	0.104-0.554	***<.001****
CC–CC	20	***20.4***	5	***6.2***	3.897	1.392-10.913	***.009****
CC–AA	14	***14.3***	5	***6.2***	2.533	0.871-7.365	.088
TT–AA	14	***14.3***	9	***11.1***	1.333	0.545-3.262	.529
TT–AC	1	***1.0***	1	***1.2***	0.825	0.051-13.395	.892
C677T/A1298C/(−1306C>T)							
CT–AA–CC	7	***7.1***	2	***2.5***	3.038	0.613-15.051	.173
CT–AC–TT	4	***4.1***	2	***2.5***	1.681	0.300-9.420	.555
CT–AA–CT	5	***5.1***	12	***15.0***	0.309	0.104-0.918	***.035****
CT–AC–CC	5	***5.1***	7	***8.6***	0.568	0.173-1.864	.351
CT–AA–TT	6	***6.1***	1	***1.2***	5.217	0.615-44.261	.130
CT–AC–CT	2	***2.0***	12	***14.8***	0.120	0.026-0.552	***.007****
CC–AC–CC	3	***3.1***	15	***18.5***	0.139	0.039-0.499	***.002****
CC–AC–CT	3	***3.1***	9	***11.1***	2.629	0.687-10.057	.158
CC–CC–CC	6	***6.1***	2	***2.5***	2.576	0.506-13.126	.255
CC–AA–CT	6	***6.1***	3	***3.7***	1.696	0.411-7.004	.466
CC–AA–CC	5	***5.1***	2	***2.5***	2.124	0.401-11.248	.376
CC–CC–CT	6	***6.1***	3	***3.7***	1.696	0.411-7.004	.466
CC–AC–TT	2	***2.0***	1	***1.2***	1.667	0.148-18.719	.679
TT–AA–TT	4	***4.1***	1	***1.2***	3.404	0.373-31.079	.278
TT–AA–CT	5	***5.1***	5	***6.1***	0.817	0.228-2.928	.757
TT–AA–CC	5	***5.1***	3	***3.7***	1.398	0.324-6.035	.898

AA, adenine–adenine; AC, adenine–cytosine; CC, cytosine–cytosine; CT, cytosine–thymine; P/C, patients/controls; TT, thymine–thymine.
*Significance ($P < .05$).

of folate deficiency, DNA methylation decreases and decreased DNA methylation may cause genomic instability and activation of oncogenes; thus, it may lead to carcinogenesis.[3]

In recent studies, the C allele of the MTHFR *A1298C* gene variation has been associated with alterations in the MTHFR gene expression.[21] In Asian population, CC and CA genotypes of this gene variation have been associated with increased bladder cancer risk. In the European population, the CC genotype of the MTHFR *A1298C* gene variation plays a protective role in bladder cancer development[3]. In the Iranian population, a significant correlation was detected between AC and CC genotypes of MTHFR *A1298C* variation and increased risk of bladder cancer. Also, in this study, *677CT/1298AC* and *677CC/1298CC* combined genotypes of MTHFR gene variations have been associated with increased bladder cancer susceptibility.[7]

The extracellular matrix of tumor is known as a basic regulator of carcinogenesis and has a different organization. In a previous study, in a murine model, *MMP* inhibitor suppresses bladder tumor growth and angiogenesis. Increased tissue inhibitor levels of *MMPs* have been associated with invasive diseases. *MMPs* may play a protective role when the balance between enzyme and inhibitor is achieved. Increased expression of MMP-2 has been associated with accelerated tumor progression in various types of cancer.[1-5]

In some previous studies, the CC genotype of MMP-2 gene variation has a higher promoter activity than the TT genotype and is associated with excessive MMP-2 expression.[12] In the Indian population, a significant association was detected between MMP-2 gene variation and reduced cancer risk.[1]

Race and ethnicity are extremely important in determining the role of genetic variations in susceptibility to diseases and in individual genetic variability. Our study was carried out with the Thrace population. This population is thought to be similar to the European population in terms of gene variations and allelic distributions. Demographic characteristics may be preserved in this region. There are some limitations in this study. First of all, the number of samples was limited in our study. Detailed results may be obtained if this study is performed with a larger population. The second limitation is the lack of detailed selection criteria for bladder cancer patients and healthy controls. More detailed results may be obtained by specifying detailed selection criteria for these groups. Thirdly, this study did not elaborate on bladder cancer staging, tumor type, and tumor grading. More detailed results can be obtained if the genotypes of these genetic variations are compared according to bladder cancer clinical stages, tumor type, and tumor grades. Finally, genotyping analyses of gene variations determined according to bladder cancer risk factors and clinical subtypes will also enable us to obtain more detailed results in our study. The relationship between bladder cancer risk and these genetic variations should also be investigated in larger and different populations.

Many epidemiological studies have reported the association between bladder cancer risk and smoking. In addition, bladder cancer is more common in men than women, and the incidence of bladder cancer increases with age.[22-24] In our study, in line with the information, the significant difference was detected in terms of gender, hypertension, heart disease, and smoking parameters between patient and control groups. Individual differences in bladder cancer occurrence have been associated with polymorphic variants related to bioactivation and detoxification of carcinogens.[22] In our study, the significant difference was not detected for MTHFR *C677T* gene variation between patient and control groups ($P > .05$). For the MTHFR *A1298C* gene variation, CC genotype was a genetic risk factor ($P = .0001$), while AC genotype was a protective factor ($P < .0001$) in the development of bladder cancer. For the MMP-2 *(–1306C>T)* gene variation, TT genotype ($P < .0001$) and T allele ($P = .0006$) were genetic risk factors, while CT genotype ($P = .0009$) was a protective factor for bladder cancer. The allele frequencies of these gene variations were detected significantly different according to the Hardy–Weinberg distribution in these groups ($P < .05$). For *C677T/A1298C* gene variations, CC–CC combined genotype was a genetic risk factor ($P = .009$), while CT–AC and CC–AC combined genotypes were potential protective biomarkers ($P = .013$; $P < .001$, respectively). CT–AA–CT, CT–AC–CT, and CC–AC–CC combined genotypes of *C677T/A1298C/–1306C>T* variants were determined as protective markers for bladder cancer ($P = .035$; $P = .007$; $P = .002$, respectively). Determining the roles of MTHFR and MMP-2 gene variations that may be effective in various cancer types associated with immune system imbalance may provide new therapeutic targets for inflammatory diseases such as bladder cancer.

In our study, in the Thrace population, the CC genotype of MTHFR *A1298C* gene variation and TT genotype and T allele of MMP-2 *(–1306C>T)* gene variation were identified as genetic risk factors for bladder cancer development. On the other hand, the AC genotype of MTHFR *A1298C* gene variation and CT genotype of MMP-2 *(–1306C>T)* were determined as protective markers for bladder cancer. In addition to this, for *C677T/A1298C* gene variations, CC–CC combined genotype was detected as a genetic risk factor, while CT–AC and CC–AC combined genotypes were determined as potential protective biomarkers. Furthermore, CT–AA–CT, CT–AC–CT, and CC–AC–CC combined genotypes of *C677T/A1298C/–1306C>T* variants were determined as protective markers for bladder cancer. Identifying these genetic variations as important biomarkers, which are effective in the pathogenesis of bladder cancer, will provide us with very important information in terms of early diagnosis, prognosis, progression, and treatment strategies of the disease.

Ethics Committee Approval: *Ethical committee approval was received from Trakya University Faculty of Medicine Non-Invasive Clinical Research Ethics Committee (TÜTF-BAEK 2019/205).*

Informed Consent: *Written informed consent was obtained from all participants who participated in this study.*

Peer-review: *Externally peer-reviewed.*

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