INTRODUCTION

Bladder cancer (BC) is a major problem and, 11th most common cancer in the world(1). BC is higher in men than women, and its incidence increases with age for both gender, peaking at the seventh decade(2). Genetic and potential environmental factors play role in the etiology of the disease and, familial cancer history increasing risk of BC. In addition, there is important heterogeneity in terms of its clinical and genetic backgrounds of urothelial carcinomas and, the heterogeneity partly originates from different changes in different genes that affect various mechanisms associated with cell proliferation and cancer(3-4). A genome-wide association studies (GWAS) have informed that new locus on chromosome (chr) 3q may be correlated with BC risk. In addition, it has been reported that the locus on chromosome 3q is included in MYNN gene and TERC gene and, these genes are strong candidates for the association with bladder cancer(5).

Myoneurin (MYNN) gene locates on 3q26.2 and encodes a member of the BTB/POZ and zinc finger (ZF) domain-containing protein family that is involved in the control of gene expression(5). Certain polymorphic regions were discovered on the MYNN gene. The rs10936599 polymorphism is one of these regions. The function of rs10936599 polymorphism on the MYNN gene is not known. Some research concludes that this synonymous variation is associated with both longer telomeres and the colorectal cancer or patients with adenomas(6-8). Additionally, it has been suggested that the MYNN polymorphism may be associated with ovary and bladder cancers(6-9). Furthermore, the variation which is close to Telomerase RNA component (TERC) gene participates at least partly in tumorigenesis at early stages(7,10).

Telomerase is a specialized ribonucleoprotein polymerase that adds TTAGGG repeats to telomere ends in soma human cells, including stem cells. The holoenzyme consists of a protein component with reverse transcriptase (TERT) activity, and a RNA component (TERC). The TERT utilizes the TERC as a template to add repeats to the existing telomeres(11). The TERC is a 451 base pairs (bp) long gene, located on chromosome 3q26.2. TERC is a template for telomeric DNA.
MYNN and TERC gene polymorphisms in bladder cancer-Polat et al.

Table 1. Demographic characteristics of bladder cancer patients and controls

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Patients n = 70 (%)</th>
<th>Controls n = 150 (%)</th>
<th>p value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Age year ± SD (range)</td>
<td>61.95 ± 10.63 (25-81)</td>
<td>59.41 ± 12.92 (22-94)</td>
<td>.15</td>
<td></td>
</tr>
<tr>
<td>*Sex</td>
<td></td>
<td></td>
<td>.09</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>61 (87.1)</td>
<td>118 (78.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>9 (12.9)</td>
<td>32 (21.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>*Smoking status</td>
<td></td>
<td></td>
<td>.002*</td>
<td>2.55 (1.42-4.58)</td>
</tr>
<tr>
<td>Smoker</td>
<td>36 (51.4)</td>
<td>44 (29.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-smoker</td>
<td>34 (48.6)</td>
<td>106 (70.7)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: ±SD, Standard derivation; *p < .05; significantly different from control group; Continuous variables were compared by independent samples t-test; Categorical variables including sex and smoking status were compared by chi square test.

MATERIALS AND METHODS

Study Population: The patients and controls were selected among the ones from urology clinic of Luleburgaz and Niğde State Hospital, Turkey. We investigated the MYNN rs10936599 and TERC rs2293607 SNPs (Single Nucleotide Polymorphisms) in 70 bladder cancer patients and 150 healthy controls. Protocol of the present study was reviewed and approved by the institutional review board of the Local Human Ethics Committee (Decision number: KAEK 2014/144). Informed consent was submitted by all subjects when they were enrolled.

Inclusion and exclusion criteria: Patient group was generated with individuals who have been diagnosed bladder cancer by histopathological examination. Bladder cancer type of all patients is transitional cell carcinoma. Patients, who have received any chemotherapy or radiotherapy, were not accepted in the study. The control group matched with age and gender distributions of patients was selected from healthy volunteers without bladder cancer history.

DNA Isolation and Genotyping: Genomic DNA was extracted from the whole blood treated with EDTA using the QIAamp DNA Blood Mini Kit (Maryland, USA), according to the manufacturer’s guidelines. PCR amplifications of both the polymorphic regions in MYNN and TERC genes were using specific primer sets (for MYNN rs10936599 polymorphism F:5'- TCAGGTAAAAATTCCCATCTG-3' and R:5'- TCCACAGAGAAAAACCTGCTTCC-3'; for TERC rs2293607 polymorphism F:5'-AGTTCGCTTTCTGGTGTTG-3' and R:5'- ATTCATTGTGGCCGACCT-3'). The PCR was performed in a final reaction volume of 20 µl containing 10 ng of genomic DNA, 10 pmol of each primer, 5x FIREPol Master Mix (Solis BioDyne). PCR reaction was made on these conditions: after initial denaturation at 95°C for 5 minutes, then followed by 38 cycles including of denaturation at 95°C for 40 seconds for denaturation, 58°C in the rs10936599 polymorphism, 59°C in the rs2293607 polymorphism for 30 seconds for annealing, and 72°C for 30 seconds for extension. The reaction was completed by a final extension of 5 min at 72°C.

Evaluation: SNP rs10936599 is a point mutation occurring with C → T substitution at nucleotide 18 in MYNN gene and, this mutation causes coding of a synonymous variant (His6His). SNP rs2293607 is a point mutation occurring with A → G substitution at nucleotide 514 in TERC gene (15). PCR products of MYNN and TERC polymorphic regions were digested with HpyCH4III at 37°C for over-night and BsrDI at 65°C for 30 min, respectively. PCR products were separated by electrophoresis on 2% agarose gels, and visualized under ultraviolet (UV) illumination after nucleic acid staining solution (ECO Safe). The PCR product size for MYNN rs10936599 SNP was 104 base pair (bp) and the wild-type allele (C) contains two fragments of 58 and 46 bp. The polymorphic variant (T) was seen a fragment of 104 bp. PCR product size for TERC rs2293607 SNP, was 159 bp. After enzymatic digestion of these products, the fragment sizes were 94 bp and 65 bp for the wild type (A). A fragment of 159 bp was seen for the variant allele (G).

Statistical analysis: The genotype and allele frequencies of two SNPs were tested for Hardy-Weinberg Equilibrium using a chi-square (χ²) test. Deviations from Hardy-Weinberg equilibrium (HWE) were analyzed by using Michael H. Court’s (2005-2008) online calculator. On the result of power analysis which was performed for detecting an association between BC and the studied polymorphisms, sample sizes were found to
**MYNN and TERC gene polymorphisms in bladder cancer - Polat et al.**

**Table 2.** The Genotypes and allele frequencies of MYNN (rs10936599) C/T and TERC (rs2293607) A/G genes SNPs in bladder cancer patients and control individuals in Turkish population

<table>
<thead>
<tr>
<th>Gene/Genotypes</th>
<th>Patients n = 70 (%)</th>
<th>Controls n = 150 (%)</th>
<th><strong>Crude values</strong></th>
<th><strong>Adjusted values</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MYNN (rs10936599)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>46 (66)</td>
<td>63 (42)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>CT</td>
<td>19 (27)</td>
<td>78 (52)</td>
<td>0.001*</td>
<td>0.33 (0.17-0.62)</td>
</tr>
<tr>
<td>TT</td>
<td>5 (7)</td>
<td>9 (6)</td>
<td>0.013*</td>
<td>0.076 (0.23-2.42)</td>
</tr>
<tr>
<td>CT+TT</td>
<td>24 (34)</td>
<td>87 (58)</td>
<td>0.001*</td>
<td>0.37 (0.20-0.68)</td>
</tr>
<tr>
<td><strong>Alleles</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>111 (79)</td>
<td>204 (68)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>T</td>
<td>29 (21)</td>
<td>96 (32)</td>
<td>0.01*</td>
<td>0.55 (0.34-0.89)</td>
</tr>
<tr>
<td><strong>TERC (rs2293607)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>27 (39)</td>
<td>68 (45)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>AG</td>
<td>39 (55)</td>
<td>77 (51)</td>
<td>0.001*</td>
<td>1.27 (0.70-2.29)</td>
</tr>
<tr>
<td>GG</td>
<td>4 (6)</td>
<td>5 (4)</td>
<td>0.001*</td>
<td>2.91 (1.30-6.51)</td>
</tr>
<tr>
<td>AG+GG</td>
<td>43 (60)</td>
<td>82 (55)</td>
<td>0.001*</td>
<td>1.32 (0.74-2.35)</td>
</tr>
<tr>
<td><strong>Alleles</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>93 (66)</td>
<td>213 (71)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>G</td>
<td>47 (34)</td>
<td>87 (29)</td>
<td>0.37</td>
<td>1.23 (0.80-1.90)</td>
</tr>
</tbody>
</table>

**Table 2**. Distinct distributions of genotypes in groups were compared by chi square test. dCrude values of odds ratios were calculated by Fisher exact test; eldependent variables were compared by logistic regression. Adjusted with smoking habit and gender Adjusted values of odds ratios were calculated by using the statistic method. *P < .05 indicates statistically significant.

RESULTS

The demographic characteristics of patients and controls were demonstrated on Table 1. When frequencies of mean age and gender were compared in both groups, the control group was found to be compatible with patients (for mean age P = .051, for gender P = .09). Smoker count in patient group (51.4%) compared with those in controls was found significantly higher (P = .002).

Frequencies of genotype and allele for two polymorphisms in patients and control groups were shown in Table 2. Genotype distribution of MYNN rs10936599 SNP among both groups was different and, the value was statistically significant (P = .001). In addition, patients with CT genotype and CT+TT genotype combination versus CC genotype and T allele versus C allele of the MYNN polymorphism compared those with controls have a decreased odds ratio for BC. Similar odds ratio was also obtained when the heterozygous genotype together with other risk factors such as gender and smoking habit were evaluated. All these data are illustrated in Table 2. Interestingly, the frequency of CC genotype (wild type for rs10936599) in patients was higher than one in controls. When CC genotype was compared to other genotypes of the MYNN polymorphism among case-control groups, approximately 2 times increased odds ratio between BC development and this genotype was found [P = .001, OR = 2.58 (1.46-4.77)].

Frequencies of genotypes (AA, AG, GG) and alleles (A and G) for TERC rs2293607 polymorphism were observed as 38.6, 55.7, 5.7% and 66.4, 33.6% in patients, respectively and 45.3, 51.3, 3.3% and 71, 29% in controls, respectively. For the TERC SNP, we detected that GG genotype versus AA genotype resulted in odds ratio of two fold, but the ratio was not statistically significant (P = .44).

In addition, gene-gene interaction and haplotype analysis among cases and controls were made in the study. It was observed in gene-gene interaction analysis that genotype combination of GG+AG/CC (TERC gene/ MYNN gene) versus wild type genotypes of two polymorphisms (AA/CC) revealed stronger correlation (Table 3). Besides, four possible haplotypes of MYNN (rs10936599) and TERC (rs2293607) SNPs were identified in our study. C-A haplotype was accepted as a reference haplotype because it was more common in both groups. For the two variants, a linkage was found in both patients and controls (c2 = 24.09, P = .0001 for patients; c2 = 178.77, P = .0001 for controls). We obtained statistically significant relationship between patients and controls for the C-G haplotypes (P = .0001) (Table 3).

There is no data about histological types and stage of patients with bladder cancer, so these parameters were not evaluated in the study.

DISCUSSION

MYNN rs10936599 and TERC rs2293607 SNPs in 70 bladder cancer patients and 150 healthy controls were analyzed in this current study. In our study, genotype distributions in controls for two polymorphisms were not compatible with the principle of HWE. The cause of the drift from HWE is selection from hospital-based individuals of control group in this study. In addition, we evaluated as reference allele, so C allele for the MYNN gene polymorphism is wild type in the current study.
cells from patients with bladder cancer than in control
that telomere length was significantly shorter in buccal
omere length. However, Broberg et al., (2005) reported
the polymorphism may be affected by alteration of tel
omeration studies (GWAS), Wang et al., (2014) reported
the SNP of the MYNN gene was associated with adenoma
risk in colorectal cancers
been found in some studies by Houlston et al.,
been identified that rs10936599 could change the regu
bladder cancer cell lines
suggested that
another study, it has been reported that this susceptibil
the smoking risk factor in the Chinese population
predict the risk of bladder cancer in combination with
bladder cancer risk. These loci showed the potential to
change the transcript’s secondary mRNA structure.
Furthermore, Jones et al. stated that data from the EN
ocode Project had indicated H3K4Me1 and H3K4Me3
histones in the immediate vicinity of rs2293607. Ad-
itionally, the same region is considerably sensitive to
DNAse1 and estimated binding site of multiple transcrip-
tion factors as like NFKB, PU.1, POU3F2 and MYC
DNaseI and estimated binding site of multiple transcrip-
gene polymorphisms in bladder cancer-Polat et al.

### Table 3. Analysis of gene- gene interaction and haplotype for MYNN (rs10936599) and TERC(rs2293607) polymorphisms

<table>
<thead>
<tr>
<th>Gene Genotypes</th>
<th>Patients n (%)</th>
<th>Controls n (%)</th>
<th>P value</th>
<th>OR CI (95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MYNN gene (C/T)/TERC gene (A/G) Haplotypes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C A</td>
<td>45</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C G</td>
<td>11</td>
<td>20</td>
<td>.0001*</td>
<td>12.22 (2.60-57.42)</td>
</tr>
<tr>
<td>T A</td>
<td>2</td>
<td>6</td>
<td>1</td>
<td>0.74 (0.14-3.81)</td>
</tr>
<tr>
<td>T G</td>
<td>12</td>
<td>42</td>
<td>.29</td>
<td>0.63 (0.30-1.32)</td>
</tr>
</tbody>
</table>

*P<.05 indicates statistically significant.

However, for rs10936599 SNP, more of the individu-
als with C allele were obtained in patients compared
to controls in our study. Therefore, all genotypes with-
out pointing out a reference allele were analyzed again.
We obtained significant odds ratio for CC genotype
for bladder cancer among patients (these data were not
shown on table). We suggest that T allele may have a
protective effect in spite of C allele for bladder cancer.
As consisted with our data, in a genome wide associ-
ation studies (GWAS), Wang et al., (2014) reported
that C allele of MYNN rs10936599 polymorphism may
entail a risk for bladder cancer and the SNP together
with other polymorphic side may be used, collective-
ly, to effectively measure inherited risk for bladder
cancer (16). Besides, it has been reported that T allele
for rs10936599 polymorphism at 3q26.2 shows a pro-	ection effect on bladder cancer in another GWAS by
Figueroa et al., (2013) (ORadj per T allele = 0.85, 95%
CI 0.81-0.90 and p = 4.53x10-9)99.
When the effect of the polymorphism in other cancer
species has been investigated, similar results were de-
tected. Carvajal-Carmona et al. (2013) examined some
SNPs in colorectal cancers. They found that rs10936599
SNP of the MYNN gene was associated with adenoma
risk99. At the same time, in a GWAS studies conducted
by Houlston et al., (2010), Lubbe et al., (2012), and Real
et al., (2014), rs10936599 polymorphism was found
risk in colorectal cancers89,99,100. Furthermore, Speedy
et al., (2014) identified new susceptibility loci mapping
to 3q26.2 (rs10936599) for chronic lymphocytic leu-
kemia (CLL) in a genome-wide association study100. It
has been reported in some studies by Houlston et al.,
(2010), Lubbe et al., (2012), and Kantor et al., (2014)
that, C allele for rs10936599 SNP was major and risk
Furthermore, as similar to our finding, T allele was
shown as effective allele when the association of
rs10936599 SNP and telomere length for coronary heart
disease (CHD) was investigated in Han Chinese popu-
lation21. In addition, it has been found that the T allele
may have a protective effect in the study OR = 5.97
(0.825-0.995). Telomere length was not analyzed in
current study but we thought that bladder cancer risk
of the polymorphism may be affected by alteration of tel-
omere length. However, Broberg et al., (2005) reported
that telomere length was significantly shorter in buccal
cells from patients with bladder cancer than in control
subjects. Telomere shortening increases the cancer risk
rather than preventing it. It has been identified as the
reason of the discrepancy in the study that short tel-
omeres may increase the risk of developing cancer,
particularly epithelial cancers via non-reciprocal trans-
locations22. The reason of this discrepancy may be the
effects of other genes and risk factors. Wang et al., 2014
found that seven significant variants including MYNN
rs10936599 had a strong cumulative association with
bladder cancer risk. These loci showed the potential to
predict the risk of bladder cancer in combination with
the smoking risk factor in the Chinese population100.
In another study, it has been reported that this susceptibil-
ity locus rs10936599 at 3q26.2 is in linkage disequi-
librium with SNPs in TERC. In addition, it has been
suggested that TERC gene suppresses cell growth in
bladder cancer cell lines23. On the other hand, it has
been indicated that rs10936599 could change the regu-
larity elements of MYNN or nearby genes to discuss the
bladder cancer risk100.
Moreover, with regard to the results from a study
by Jones et al. on MYNN and TERC, rs10936599 al-
leles were associated with both longer telomeres and
colorectal cancer risk. They reported that this variation
close to TERC probably acts at an early stage in tum-
origenesis. TERC rs2293607 is estimated by RNA fold
to change the transcript’s secondary mRNA structure.
Furthermore, Jones et al. stated that data from the EN-
code Project had indicated H3K4Me1 and H3K4Me3
histones in the immediate vicinity of rs2293607. Ad-
ditionally, the same region is considerably sensitive to
DNAse1 and estimated binding site of multiple transcrip-
tion factors as like NFKB, PU.1, POUS2F2 and MYC107.
Figueroa et al. (2014) found significantly higher TERC
mRNA expression in muscle-invasive bladder tumors
than adjacent normal bladder tissues107. According to
their report, TERC gene may have functional relevance
for predisposition to bladder cancer but the possible
functional effect of the MYNN gene in the associated
LD block cannot be excluded as a molecular cause of
this association. From some GWAS studies, some re-
searchers found that rs10936599 SNP is in strong or
moderate LD with SNP within the region 3q26.2 that
includes the TERC gene107. In our study, we also inves-
tigated whether there is an association between TERC
rs2293607 (A/G) polymorphism and bladder cancer
risk. No significant association was found for the poly-

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morphism. We did not find any data related to the TERC polymorphisms and bladder cancer risk in literature. So, our findings associated with TERC (rs2293607) SNP were not compared. When the data related to localization of MYNN rs10936599 which is close to TERC genes was considered, together with these MYNN and TERC SNPs were analyzed for bladder cancer risk in the present study. After gene-gene interaction analysis, a stronger correlation was obtained between GG+AG genotype combination for TERC (rs2293607) and CC genotype for MYNN (rs10936599) polymorphisms. It has been predicted that GG+AG combination of the TERC gene may cause telomere shortening. The remarkable finding was found consistent with those from Broberg et al. (2005). In addition, it has been shown in our study that C and G haplotypes (for MYNN and TERC SNPs, respectively) had odds ratio value of approximately 12 fold in development of bladder cancer.

CONCLUSIONS
We think that MYNN and TERC genes together may be associated with development of bladder cancer in the current study. In addition, establishing larger numbers of study groups, increasing the number of SNPs in the studied genes, and measuring the telomere lengths and evaluating them together will conclude more effective results.

ACKNOWLEDGEMENT
The authors confirm that all data underlying the findings are fully available without restriction. All relevant data are contained within the paper. The laboratory facilities of Medicine Faculty both Kocaeli and Sivas were used. A part of the manuscript has been presented in 5th International Molecular Biology and Biotechnology Congress in Tetova, Macedonia (orally-) at 25-29 August 2016. We would like to thank to Scientific Research Project Unit of Kocaeli University (Project No: 2014-045)

CONFLICT OF INTEREST STATEMENT
All authors have no potential conflicts of interest to disclose.

REFERENCES


