Research Article

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A case–control study on effects of the ATM, RAD51 and TP73 genetic variants on colorectal cancer risk

ATM, RAD51 ve TP73 genetik varyantlarının kolorektal kanser riski üzerine etkileri, bir vaka-kontrol çalışması

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Abstract

Aim: ATM, RAD51 and TP73 are genes that take part in DNA repair pathways. The aim of this prospective case-control study was to determine the genotype and allele distributions of the ATM 5′-UTR G/A, RAD51 135 G/C and TP73 GC/AT polymorphisms and their relationship with clinical parameters in Turkish colorectal cancer (CRC) patients.
Material and methods: One hundred and four CRC patients and 113 healthy individuals were included in this study as control. The polymerase chain reaction-restriction fragment length polymorphism techniques were used.
Results: The ATM 5′-UTR G/A polymorphism GG ($p = 0.001$) and AA ($p = 0.0001$) genotypes were found higher in the patient group, while the GA genotype ($p = 0.0001$) and A allele ($p = 0.001$) were significantly higher in the control group. Moreover, the GG genotype ($p = 0.042$) was higher among patients with advanced-stage cancer and, while GA genotype ($p = 0.047$) was increased in patients without perineural invasion. The RAD51 135 G/C polymorphism GC genotype ($p = 0.0001$) and C allele ($p = 0.0001$) were significantly higher in the patient group, while CC genotype ($p = 0.0001$) was higher in the control group. No statistical significance was observed between the TP73 GC/AT polymorphism genotype and allele distribution and the clinical parameters.
Conclusion: In the Turkish population, the ATM 5′-UTR GG and AA genotypes, and the RAD51 135 G/C GC genotype and the C allele presence may be risk factors for CRC.

Keywords: Colorectal cancer; ATM; RAD51; TP73; PCR-RFLP.

Öz

Amaç: ATM, RAD51 ve TP73 genleri DNA tamir yolağında rol oynayan genlerdir. Bu prospektif vaka-kontrol çalışmasının amacı, Türk kolorektal kanser hastalarında ATM 5′-UTR G/A, RAD51 135 G/C ve TP73 GC/AT polimorfizmlerinin genotip ve allel dağılımlarının ve klinik parametrelerle ilişkisinin belirlenmesidir.
Gereç ve Yöntemler: Bu çalışmaya 104 kolorektal kanser hastası ve 113 sağlıklı birey dahil edildi. Polimorfizmlerin belirlenmesinde, Polimeraz Zincir Reaksiyonu-Restriksiyon Parça Uzunluk Polimorfizmi teknikleri kullanılmıştır.

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Bulgular: ATM 5′-UTR G/A polimorfizmi için GG (p = 0.001) ve AA (p = 0.0001) genotipleri taşıma oranı hasta grubunda kontrol grubuna göre, GA genotipi (p = 0.0001) ve A alleli (p = 0.001) taşıma oranı ise hasta grubuna oranla kontrol grubunda anlamlı düzeyde daha yüksektir. Ayrıca, ileri evre kanser hastalarında GG genotipi (p = 0.042), perinöral invazyonu olmayan hastalarda ise GA genotipi daha yüksek bulunmuştur (p = 0.047). RAD51 135 G/C polimorfizmi için GC genotipi (p = 0.0001) ve C alleli (p = 0.0001) taşıma oranı hasta grubunda kontrol grubuna göre istatistiksel olarak daha yüksek iken, CC genotipi (p = 0.0001) taşıma oranı ise hasta grubuna göre kontrol grubunda anlamlı düzeyde daha yüksektir. TP73 GC/AT polimorfizmi genotip ve allel dağılımları ve klinik parametrelerle ilişkisi açısından incelendiğinde istatistiksel anlamlı bir farklılık görülmemiştir.
Sonuçlar: Türk populasyonunda ATM 5′-UTR varyantı için GG ve AA genotipleri, RAD51 135 G/C varyantı için GC genotipi ve C alleli taşımanın kolorektal kanser için bir risk faktörü olabileceği söylenilebilir.

Anahtar Kelimeler: Kolorektal kanser; ATM; RAD51; TP73; PCR-RFLP.

Introduction

Colorectal cancer (CRC) is one of the most common malign tumors in both men and women, being 3rd in cancer related mortalities. It is estimated that over one million new CRC cases are diagnosed worldwide annually and one of every three CRC patients die due to the disease [1].

Numerous kinds of DNA damage may occur as a result of carcinogens, such as DNA adducts and single or double stranded DNA breaks (DSBs) and elimination of these damages is crucial for the preservation of genetic integrity that later may lead to cancer development [2]. One of the genes that is primarily activated by DSBs is Ataxia-telangiectasia mutated (ATM), positioned on chromosome 11q22-q23 with the full length 150 kb. It contains 66 exons and encodes a 12 kb transcript [3]. As a response to DNA damage due to carcinogens, ionizing radiation and reactive oxygen, ATM is activated rapidly and play roles in many cellular pathways including cell cycle regulation, apoptosis, DNA repair and preserving mitochondrial DNA [3, 4]. ATM also is a significant regulator of the phosphorylation of TP53, a tumor suppressor protein that regulates cell cycle and apoptosis [5]. Lately, it is estimated that the ATM gene has an important part in preserving genomic stability, and a single nucleotide polymorphism (SNP) may give rise to modifications in DNA repair mechanisms and apoptosis, thus, lead to development of various types of cancer [6]. ATM 5′-UTR G/A polymorphism is located at the 5′-UTR of the promoter region of ATM gene. In the different studies, it was clear that ATM 5′-UTR G/A polymorphism in the promoter region of a gene could alter the binding sites of transcription factors that affect gene expression [7]. The SNP in the 5′-UTR of the ATM is thought to have an association with CRC, since the alterations in the 5′-UTR of the ATM gene causes functional modifications [6].

RAD51, a 30 bp long gene located on 15q15.1 and comprised of 10 exons, is the eukaryotic homologous of the bacterial recA protein and take part in the repair of DSBs [8, 9]. During repair, it configures nucleo-protein filaments on single stranded DNA, which promotes homologous pairing and enables the interchange between single and double stranded DNA. That is to say, RAD51 has a crucial role in preserving the genomic stability and reconstruction of DSBs [10, 11]. Therefore, variations within the RAD51 gene may lead to DNA instability and malignancies by altering its DNA repair capacity and reaction against deleterious agents. The location of the RAD51 135G/C polymorphism is related with the transcriptional activity [12]. Studies on the G to C transversion located at the 135th position of the RAD51 5′-UTR revealed that this polymorphism impairs RNA stability or translational efficiency therefore leading to altered polypeptide yield levels, RAD51 protein function and DNA repair capacity [8]. In the past decade, it has been shown that the RAD51 135G/C polymorphism is associated with many types of cancers [9].

The TP53, TP63 and TP73 genes, members of the TP53 family, have separate roles in critical cellular mechanisms, for instance, DNA synthesis and repair, genome stability, apoptosis or senescence and growth arrest [13]. Although they are all notably homologous, p56 and p73 have more significantly similar sequences than p53, with TP73 also having similar functions with TP53, in the way that p73 may trigger TP53-regulated genes and restrain growth or promote apoptosis [14]. In addition, they both respond to DNA damage, yet via different pathways. Polymorphisms that occur in the TP73 dependent apoptosis mechanism have been shown to alter the clinical response to cancer chemotherapy. Two SNPs have been detected, 4G/A and 14C/T, within the AUG region of the second exon of the TP73 gene, which are completely unconnected. Mutations in this region theoretically can fashion the stem loop formation that can affect the initiation of translation and therefore gene expression and bring on functional outcomes [15, 16]. These polymorphisms have also been related with many types of cancers. The AT genotype have been pointed as an increased risk factor for certain cancer

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types by various studies, while others indicated that it plays a preventive role against cancer development [17].

ATM, RAD51 and TP73 are genes that take part in DNA repair pathways and it has been reported that, ATM 5′-UTR G/A (rs189037), RAD51 135 G/C and TP73 GC/AT polymorphisms were significantly associated with susceptibility to various cancer types. However, there is no previous study that has investigated the combined effect of the variations of these DNA repair related genes (synergistic effect) on the pathogenesis of CRC in the Turkish population. This study is the first to investigate the ATM 5′-UTR G/A, RAD51 135 G/C and TP73 GC/AT polymorphisms together in CRC. The aim of this study was to determine the possible influence of these SNPs on the formation and clinical parameters of CRC.

Materials and methods

Ethical approval

This research has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by the Istanbul Medical Faculty Ethical Committee, Istanbul University (#2014/995).

Study group

All participants' rights were protected and a written informed consents were obtained before the procedures according to the Helsinki Declaration. The sample size to be used in the study was determined with power analysis. According to the results of this analysis, the minimum sample size required to detect a significance difference using this test should be at least 95 individuals in each group (in total 190 individuals), considering type I error (α) of 0.05, power (1-β) of 0.8 and effect size of 0.9. The patient group was consisted of a total of 104 individuals, 46 women and 58 men, that were diagnosed with CRC by the General Surgery Clinic of the Istanbul University Faculty of Medicine, while the control group was consisted of a total of 113 healthy individuals, 47 women and 66 men, who did not have cancer history.

DNA isolation and genotyping procedure

Peripheral blood specimens were collected in tubes containing EDTA, and genomic DNA samples were extracted from whole blood by a salting out procedure. The ATM 5′UTR G/A, RAD51 135 G/C and TP73 GC/AT polymorphisms were analyzed using polymerase chain reaction (PCR) – Restriction fragment length polymorphism (RFLP) methods. For detection of ATM 5′UTR G/A, RAD51 135 G/C and TP73 GC/AT polymorphisms, 50–100 ng genomic DNA was amplified with 10x reaction buffer (10 mM Tris-HCl, 50 mM of KCl, 1.75 mM $MgCl_2$), 2.5 mM of each dNTP, 100 pmol/μL of each primer and 0.3 unit Taq polymerase (Invitrogen) in a 25 μL reaction volume. The PCR reactions for ATM 5′-UTR G/A and RAD51 G/C were set as 94°C for 45 s, 59°C for 45 sec and 72°C for 45 sec for 35 cycles following the 95°C for 5 min initial denaturation and a final elongation step as 72°C for 5 min. For the TP73 GC/AT polymorphism 95°C for 1 min, 55°C for 1 min and 72°C for 1 min for 35 cycles following the 95°C for 10 min initial denaturation and a final elongation step as 72°C for 5 min. The amplified PCR products for ATM 5′UTR G/A and RAD51 135 G/C polymorphisms were cut with MscI and BstNI restriction endonuclease enzymes, respectively. The digested yields were analyzed with agarose gel electrophoresis. The used primers, restriction enzymes and interpretations for determining ATM 5′UTR G/A, RAD51 135 G/C and TP73 GC/AT polymorphisms are shown in Table 1.

Evaluation of the MscI and BstNI restriction enzyme digestion results

The PCR yield of the ATM 5′-UTR G/A polymorphism was 287 bp and the bands obtained following digestion with MscI were 111 and 176 bp, only if the polymorphism was present. Therefore, a single band of 287 was appraised as GG (wild type), 111 and 176 bp as AA (variant type), and 287, 111 and 176 bp as GA (heterozygous type).

In order to evaluate the RAD51 polymorphism BstNI restriction enzyme was utilized. Only the wild type sequences were digested by the enzyme giving 86 and 71 bp long fragments. Variant types were not containing the restriction site giving 157 bp long bands.

TP73 mutation analysis

There are two linked SNPs in the TP73 gene region. Four specific primers were used to determine the variants without using restriction enzymes (Table 1). A yield of 428 bp was evaluated as GC/GC (wild type), bands of 270

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Table 1: PCR–RFLP-based determination of the ATM 5′UTR G/A, RAD51 135 G/C and TP73 GC/AT polymorphisms.

SNPs	Primers	Restriction enzymes	Interpretation (bp)
ATM 5′UTR G/A	F: 5′-GCTGCTTGGCGTTGCTTC-3′ R: 5′-CATGAGATTGGCGGTCTGGT-3′	MscI	GG: 287 GA: 287 + 176 + 111 AA: 176 + 111
RAD51 135 G/C	F: 5′-TGGGAACTGCAACTCATCTGG-3′ R: 5′-CTCTGGCACTCGGTGACAT-3′	BstNI	GG: 86 + 71 GC: 157 + 86 + 71 CC: 157
TP73 GC/AT	F1: 5′-CCACGGATGGGTTCTGATCC-3′ R1: 5′-GGCCTCCAAGGGCAGCTT-3′ F1: 5′-CCTTCCTTCCTGCAGAGCG-3′ R1: 5′-TTAGCCCAGCGAAGGTGG-3′	–	GC/GC: 428 GC/AT: 428 + 270 + 193 AT/AT: 270 + 193

F, Forward primer; R, reverse primer.

and 193 bp as (variant type), and 428, 270 and 193 bp as GC/AT (heterozygous type).

Statistical analysis

Results were evaluated using the SPSS 20.0 package program and values of $p < 0.05$ were considered significant. Obtained results were presented as median and interquartile range (IQR) or mean standard deviation. Allele frequencies were determined according to the gene counting method, the genotype and allele frequency differences between groups using chi-square and Fisher's tests, and the effects of the alleles and genotypes on activity were appraised using the Student's t-test and One-way ANOVA.

Determination of Hardy-Weinberg equilibrium

Each of the patient and control groups was checked for all polymorphisms by the Hardy-Weinberg equilibrium. According to Hardy-Weinberg equilibrium, frequency of ATM 5′-UTR ($p = 0.001$) and RAD51 ($p = 0.001$) genotypes in the control subjects and frequency of TP73 genotype in patients ($p = 0.02$) were not in the Hardy-Weinberg equilibrium.

Results

The mean age of the patient group, which consisted of 46 women and 58 men, was 58.56 ± 12.05, while the control group, 47 women and 66 men, was 55.09 ± 10.51. Demographic and clinical parameters of the patient group are given in Table 2.

Table 2: Demographic details of the study groups.

Demographic and clinical parameters	Patient n (%)
Stage	
Early (T1–T2)	38 (36.5%)
Advanced (T3–T4)	66 (63.5%)
Node	
Early (N1–N2)	99 (95.2%)
Advanced (N3–N4)	5 (4.8%)
Metastasis	
Positive	29 (27.9%)
Negative	75 (72.1%)
Perineural invasion	
Positive	36 (34.6%)
Negative	68 (65.4%)
Angiolymphatic invasion	
Positive	38 (36.5%)
Negative	66 (63.5%)
Tumor localization	
Rectum	50 (48.1%)
Sigmoid	31 (29.8%)
Cecum	6 (5.8%)
Left colon	8 (7.7%)
Right colon	1 (1%)
Transverse colon	8 (7.7%)

n, Number of individuals.

The ATM 5′-UTR G/A polymorphism genotype and allele distribution among patient and control groups

The allele and genotype distribution analyses showed that the GG genotype was significantly higher in the patient group than the control ($p = 0.001$, X^2: 10.173, OR: 2.58, 95% CI: 1.43–4.65). The GA genotype ($p = 0.0001$, X^2: 28.755, OR: 0.208, 95% CI: 0.11–0.37) and A allele ($p = 0.001$, X^2: 10.173, OR: 0.38, 95% CI: 0.215–0.699) were significantly higher in the control group. Also, we did not

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Table 3: The ATM 5′-UTR G/A genotype/allele distributions in the patient and control groups.

	Control (n: 113)	Patient (n: 104)	p-Value
Genotype			
GG	25 (22.1%)	44 (42.3%)[a]	0.001
GA	88 (77.9%)[a]	44 (42.3%)	0.0001
AA	0 (0%)	16 (15.4%)[a]	0.0001
Allele			
G	138 (61.06%)	132 (63.46%)	>0.05
A	88 (38.93%)[a]	76 (36.53%)	0.001

n, Number of individuals.
[a]Statistically significant at p-Value < 0.05.

observed any AA genotype in control group (p = 0.0001) (Table 3). According to these results, it can be concluded that the ATM 5′-UTR GG and AA genotypes may be a risk factor for CRC.

Relationship of the ATM 5′-UTR G/A polymorphism genotype and allele distribution with clinical parameters

The relationship between the ATM 5′-UTR G/A variant and clinical parameters is given in Table 4. The GG genotype was significantly higher in patients with T3 and T4 (Advanced) tumors than with T1 and T2 tumors (early) (p = 0.042, X^2: 4.118, OR: 0.43, 95% CI: 0.192–0.979). The GA genotype was higher in patients without perineural invasion (p = 0.047, X^2: 3.959, OR: 0.436, 95% CI: 0.191–0.995). Furthermore, A allele presence was higher among patients without metastasis (p = 0.036, X^2: 4.384, OR: 2.519, 95% CI: 1.048–6.051).

The RAD51135 G/C polymorphism genotype and allele distribution among patient and control groups

CC genotype frequency was found significantly higher in the control group (p = 0.0001, X^2: 13.22, OR: 0.36, 95% CI: 0.20–0.63), while GC genotype (p = 0.0001, X^2: 14.74, OR: 3.01, 95% CI: 1.701–5.328) and C allele (p = 0.0001, X^2: 13.22, OR: 2.75, 95% CI: 1.58–4.77) were significantly higher in the patient group. Therefore, it can be said that in the Turkish population, the RAD51 GC genotype and the C allele presence may be a risk factor for CRC (Table 5).

Relationship between the RAD51 135 G/C polymorphism genotype and allele distributions and clinical parameters

The association between the RAD51 135 G/C variant genotype and allele distribution and clinical parameters are given in Table 6. No statistical significance was observed.

Table 4: Relationship between the ATM 5′-UTR G/A variant and clinical parameters.

Clinical parameters	Genotype			Allele	
	GG n (%)	GA n (%)	AA n (%)	G n (%)	A n (%)
Metastasis					
Positive	17 (38.6%)	10 (22.7%)	2 (12.5%)	27 (30.7%)	12 (20%)
Negative	27 (61.4%)	34 (77.3%)	14 (87.5%)	61 (69.3%)	48 (80%)[a]
Node					
Early (N1–N2)	42 (95.5%)	42 (95.5%)	15 (93.8%)	84 (95.5%)	57 (95%)
Advanced (N3–N4)	2 (4.5%)	2 (4.5%)	1 (6.3%)	4 (4.5%)	3 (5%)
Stage					
Early (T1–T2)	21 (47.7%)	12 (27.3%)	5 (31.3%)	33 (37.5%)	18 (30%)
Advanced (T3–T4)	23 (52.3%)[a]	32 (72.7%)	11 (68.8%)	55 (62.5%)	42 (70%)
Perineural invasion					
Positive	14 (31.8%)	20 (45.5%)	2 (12.5%)	34 (38.6%)	22 (36.7%)
Negative	30 (68.2%)	24 (54.5%)[a]	14 (87.5%)	54 (61.4%)	38 (63.3%)
Angiolymphatic invasion					
Positive	12 (27.3%)	20 (45.5%)	6 (37.5%)	32 (36.4%)	26 (43.3%)
Negative	32 (72.7%)	24 (54.5%)	10 (62.5%)	56 (63.6%)	34 (56.7%)

n, Number of individuals.
[a]Statistically significant at p-Value < 0.05.

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Table 5: The RAD51 135 G/C genotype/allele distributions in the patient and control groups.

	Control (n: 113)	Patient (n: 104)	p-Value
Genotype			
GG	67 (59.3%)	36 (34.6%)	>0.05
GC	29 (25.7%)	53 (51%)[a]	0.0001
CC	17 (15%)[a]	15 (14.4%)	0.0001
Allele			
G	163 (72.13%)	125 (60.09%)	>0.05
C	63 (27.87%)	83 (39.9%)[a]	0.0001

n, Number of individuals.
[a]Statistically significant at p-Value < 0.05.

The TP73 GC/AT polymorphism genotype and allele distributions among patient and control groups

The TP73 GC/AT polymorphism genotype and allele distributions among patient and control groups are given in Table 7. No statistical significance was observed. Homozygote variant, the AT/AT genotype was only encountered in 1 patient and control, therefore, no difference was observed.

Relationship between the TP73 GC/AT polymorphism genotype and allele distributions and clinical parameters

Relationship between the TP73 GC/AT polymorphism genotype and allele distributions and clinical parameters are given in Table 8. No significant difference was observed.

Discussion

Impairment in DNA repair is one of the main reasons for genetic alterations to occur and consequently lead to cancer development. ATM is thought to be one of the main controllers in cell cycle regulation pathways necessary for cellular response to DNA damage and genomic stability. It is activated if DNA is damaged due to ionized radiation or reactive oxygen and termination of cell cycle, apoptosis, DNA repair and transactivation of various proteins that induce centrosome duplication [18]. Polymorphisms within the promoter region can alter the transcription binding sites, therefore, will affect gene expression. The GA polimorphism (rs189037) is located on the 5′-UTR of the ATM gene. No significant relationship was shown between the ATM 5′-UTR G/A polymorphism and leukemia [19], nasopharyngeal carcinoma [20] and papillar thyroid carcinoma [21, 22]. In a meta-analysis, heterozygotous ATM mutation carriers were found to have increased risk of breast cancer development [23] and in another the ATM 5′-UTR G/A polymorphism AA genotype was associated with head-neck and lung cancer susceptibility [7]. In a meta-analysis study conducted in 2019, it has been shown that the A allele of ATM 5′-UTR G/A polymorphism increased the risk of lung cancer, breast cancer, and oral cancer in East Asian and Latino, but not associated with Caucasian [3]. While Hsia et al. reported no relationship between lung cancer and the ATM 5′-UTR G/A polymorphism, Liu et al. indicated a higher risk of lung cancer among people carrying the AA genotype or A allele [24]. In two studies conducted in 2010, the A allele was also

Table 6: Relationship between the RAD51 135 G/C variant and clinical parameters.

Clinical parameters	Genotype			Allele	
	GG n (%)	GC n (%)	CC n (%)	G n (%)	C n (%)
Metastasis					
Positive	8 (22.2%)	15 (28.3%)	6 (40%)	23 (25.8%)	21 (30.9%)
Negative	28 (77.8%)	38 (71.7%)	9 (60%)	66 (74.2%)	47 (69.1%)
Node					
Early (N1–N2)	34 (94.4%)	50 (94.3%)	15 (100%)	84 (94.4%)	65 (95.6%)
Advanced (N3–N4)	2 (5.6%)	3 (5.7%)	0 (0%)	5 (5.6%)	3 (4.4%)
Stage					
Early (T1–T2)	10 (27.8%)	21 (39.6%)	7 (46.7%)	31 (34.8%)	28 (41.2%)
Advanced (T3–T4)	26 (72.2%)	32 (60.4%)	8 (53.3%)	58 (65.2%)	40 (58.8%)
Perineural invasion					
Positive	14 (38.9%)	14 (26.4%)	8 (53.3%)	28 (31.5%)	22 (32.4%)
Negative	22 (61.1%)	39 (73.6%)	7 (46.7%)	61 (68.5%)	46 (67.6%)
Angiolymphatic invasion					
Positive	16 (44.4%)	17 (32.1%)	5 (33.3%)	33 (37.1%)	33 (32.4%)
Negative	20 (55.6%)	36 (67.9%)	10 (66.7%)	56 (62.9%)	46 (67.6%)

n, Number of individuals.

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Table 7: The TP73 GC/AT genotype/allele distributions in the patient and control groups.

	Control (n: 113)	Patient (n: 104)	p-Value
Genotype			
GC/GC	74 (65.5%)	60 (57.7%)	>0.05
GC/AT	38 (33.6%)	43 (41.3%)	>0.05
AT/AT	1 (1%)	1 (1%)	>0.05
Allele			
GC	186 (82.3%)	163 (78.36%)	>0.05
AT	40 (17.69%)	45 (21.63%)	>0.05

n, Number of individuals.

shown as a risk factor for oral and breast cancers, and suggested to be utilized in early diagnosis [25, 26]. This polymorphism is very variable in terms of ethnicity and it is not found significant in Caucasian race. On the contrary, our results indicates that both GG and AA genotypes may be a risk factor for CRC in the Turkish population. Therefore, our results are valuable in terms of revealing the relationship between ATM 5′-UTR G/A polymorphism and CRC development in Turkish population.

RAD51 plays a critical role in preserving genomic integrity, since it initiates the repair of double strand DNA breaks in the homologous recombination process. An SNP within the RAD51 gene can alter the DNA repair capacity and response to hazardous agents. In a meta-analysis, it has been reported that the RAD51 135 G/C polymorphism increases the risk of cancer development [27]. Furthermore, in a meta-analysis have been investigated the association between the RAD51 135 G/C variant and squamous cell carcinoma of the head and neck (SCCHN), CRC, ovarian cancer and acute leukemia. As a result, the GG genotype of RAD51 135 G/C was only found associated with increased risk of SCCHN [28]. Recently, an association was found between CRC risk and RAD51 135 G/C [29]. In the Polish population, while Mucha et al. found no significant relationship between CRC development and progression and the RAD51 variant [30], Krupa et al. reported that the CC genotype has a preventive effect [31]. On the other hand, in another study conducted on the Polish population the CC genotype was associated with CRC, however, not with node metastasis, tumor size and tumor stage [32]. In a study conducted in the Iranian population in 2018, this polymorphism have been reported not to be associated with CRC [12]. Our results are compatible with the findings of Nissar et al. [33] and Cetinkunar et al. [34] with heterozygote GC frequency being higher in the patient group. According to our results, it can be concluded that the RAD51 GC genotype and C allele carriage may be a risk factor for CRC.

Repair of DNA damage is indeed crucial for the organism, but if the damage is beyond repair, cells would not be able to pass the cell cycle check points and will be directed to apoptotic pathways. One of the apoptosis inducing genes, TP73, aside being a tumor suppressor, it also plays important roles in embryonic development and differentiation [35]. The p73 GC/AT polymorphism have been linked with many types of cancers. For instance, in a meta-analysis, the GC/AT + AT/AT genotypes were indicated

Table 8: Relationship between the TP73 GC/AT variant and clinical parameters.

Clinical parameters	Genotype			Allele	
	GC/GC n (%)	GC/AT n (%)	AT/AT n (%)	GC n (%)	AT n (%)
Metastasis					
Positive	13 (30.2%)	13 (30.2%)	1 (100%)	28 (27.2%)	14 (31.8%)
Negative	30 (69.8%)	30 (69.8%)	0 (0%)	75 (72.8%)	30 (68.2%)
Node					
Early (N1–N2)	41 (95.3%)	41 (95.3%)	0 (0%)	99 (96.1%)	41 (93.2%)
Advanced (N3–N4)	2 (4.2%)	2 (4.2%)	1 (100%)	4 (3.9%)	3 (6.8%)
Stage					
Early (T1–T2)	16 (37.2%)	16 (37.2%)	0 (0%)	38 (36.9%)	16 (36.4%)
Advanced (T3–T4)	27 (62.8%)	27 (62.8%)	1 (100%)	65 (63.1%)	28 (63.6%)
Perineural invasion					
Positive	14 (32.6%)	14 (32.6%)	1 (100%)	35 (34%)	15 (34.1%)
Negative	29 (67.4%)	29 (67.4%)	0 (0%)	68 (66%)	29 (65.9%)
Angiolymphatic invasion					
Positive	15 (34.9%)	15 (34.9%)	1 (100%)	37 (35.9%)	16 (36.4%)
Negative	28 (65.1%)	28 (65.1%)	0 (0%)	66 (64.1%)	28 (63.6%)

n, Number of individuals.

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to increase the risk of lung cancer, SCCHN cancer, oral cancer and CRC [36]. In the Swedish population, it was shown that the AT/AT genotype create higher risk of CRC development, and AT carrier CRC patients had better prognosis [37], in addition, in the Tunisian population the AT/AT genotype was significantly associated with poor prognosis in CRC [38]. In the Korean population, the GC/AT and AT/AT genotypes were associated with CRC risk and the GC/GC with reduced survival [39]. On the other hand, rectal cancer patients carrying the GC/GC genotype was reported to show better survival [40]. Consequently, the TP73 GC/AT polymorphism shows various effects depending on the cancer type and ethnicity. In this study, no significant difference was observed between the patient and control groups in terms of the TP73 GC/AT polymorphism genotype and allele distributions.

To date, there was no study present that evaluated the cell cycle and DNA repair related ATM 5′-UTR G/A, RAD51 135 G/C and TP73 GC/AT polymorphisms together. Thus our study is the first to focus on the mentioned polymorphisms in such a group and we believe that it might be a data source for the further studies.

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