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Examination of *RAD51* gene G135C polymorphism in gastric cancer patients in northeastern Anatolia

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Abstract: Polymorphisms of DNA repair and genome integrity genes are associated with DNA repair capacity and elevated cancer risk. To establish an association between the pattern of polymorphism and the incidence of any type of cancer, studies across different populations are required. Polymorphic regions have been identified in the *RAD51* repair gene in various cancer types; however, the influence of specific genetic variants on gastric cancer prevalence has not been empirically demonstrated. We conducted a case-control study with 76 gastric cancer patients and 78 healthy individuals from northeastern Anatolia to examine the association between polymorphism and gastric cancer. We genotyped the previously identified G135C polymorphism of *RAD51* in all individuals and estimated the allele and genotype frequencies in the two groups. Our results indicated that the two groups differed both in allele and genotype frequencies. Additionally, a significant and elevated odd ratio (3.53) of gastric cancer for the C allele of *RAD51* was observed. The genotypes GC and CC had also significant and high odd ratios (>3.75). Our results indicate that G135C polymorphism of the *RAD51* gene was associated with an increased risk of gastric cancer in the examined population.

Keywords: gastric cancer; *RAD51* gene; genetic polymorphism; RFLP; DNA repair

INTRODUCTION

Cancer is a disease that threatens public health worldwide. According to the World Health Organization, 14 million people are diagnosed with cancer each year, and the number of deaths from cancer was around 8.8 million in 2017 [1]. More than 400 genes have been identified to be associated with cancer [2,3]. The genes associated with cancer can be classified as proto-oncogenes, tumor suppressor genes and genes playing a role in DNA repair. The DNA repair machinery is responsible for preventing changes in the DNA sequence during replication and for preventing mutations caused by environmental factors. Mutations in the genes responsible for DNA repair can interrupt the DNA repair machinery and lead to cancer formation [4].

Gastric cancer is the third most common cause of cancer deaths in the world and amounts to about 10% of all cancer deaths [5]. The incidence of gastric cancer is reported to vary among populations and the difference in gastric cancer incidence between various populations can be as high as 20-fold [6]. Therefore, linking polymorphisms residing in the genes involved in the DNA repair mechanism in different populations could help pinpoint the underlying causative polymorphism for gastric cancer.

As a member of the double-strand-break repair protein 51 (RAD51) family, the *RAD51* gene has been reported to be an important DNA repair gene and is crucial for chromosomal stability [2]. Homologous recombination (HR) is reported to be a key pathway for the repair of severe DNA damage in human cells [7]. The HR protein Rad51, a product of the *RAD51* gene, leads the reaction in which a strand transfer between a damaged sequence and its undamaged homolog enables the resynthesis of damaged sites of the genome in all eukaryotes [8].

A large number of polymorphic sites within the *RAD51* gene have been reported previously [9], including the most commonly studied polymorphism that results from the transversion of guanine to cytosine

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at the 135 nucleotide of the 5′-untranslated region (G135C) (rs1801320) [7]. The association between the *RAD51* G135C polymorphic site and colorectal cancer in the Kashmir population [9], in breast and ovarian cancer in Israel [10], colorectal cancer risk in Poland [11-12] and breast cancer [13] have been reported. Nevertheless, the potential role of the *RAD51* gene G135C polymorphism in gastric cancer prevalence has not been studied extensively. Here, we investigated the possible association of *RAD51* G135C polymorphism with gastric cancer in the northeastern Anatolian population.

MATERIALS AND METHODS

Subjects

The approval #B.30.2.ATA.0.01.00/53 and "informed voluntary consent form" were obtained from the Local Ethics Committee of the Atatürk University Medical Faculty. The patients with gastric cancer were usually selected at the second or the third stage of the cancer.

The 76 patients with a clinical diagnosis of gastric cancer who were accepted to the Gastroenterology and Oncology Clinics of the Research Hospitals in northeastern Anatolia within the last six years, were randomly selected and included as the cancer patient group. Of the 76 cancer patients, 22 were females and 54 were males. Twelve of the female patients who were diagnosed with gastric cancer were under the age of 60 and 10 were above that age. Male patients who were diagnosed with gastric cancer were mostly above the age of 60 (30 out of 54), with 24 patients aged 20 to 40 years. We also selected 78 individuals with no gastric cancer who served as the control group, 24 of whom were female and 54 were male. Blood samples (8 mL) were obtained from each cancer and healthy patient and stored in tubes with EDTA.

Genotyping

DNA isolation was performed according to the salting-out method from all blood samples taken from cancer patients and healthy controls following the procedures described in [14-15]. Isolated DNA samples were quantified using a Nanodrop Spectrophotometer (Thermo ND1000) and stored at -80°C until use. The previously identified [15] forward primer (5′-TGG-GAACTGCAACTCATCTGG-3′) and the reverse primer (5′-GCGCTCCTCTCTCCAGCA-3′) from the *RAD51* gene were used to amplify the part of the gene that contains the polymorphic region. The PCR reactions were conducted as follows: each reaction consisted of a total of 25 µL and included 2.5 µL PCR buffer, 1 µL $MgCl_2$ (2mM), 2 µL dNTP mix (0.2 mM), 2.5 µL of forward and 2.5 µL reverse primers, 1 µL genomic DNA (100ng/ml), 0.2 µL 10X Taq polymerase (5 u/mL), and 13.3 µL Nanopure water. PCR conditions were set to an initial denaturation for 3 min at 95°C and then 35 cycles at 94°C for 30 s, at 57°C for 30 s, at 72°C for 45 s, with an extension for 1 cycle of 72°C for 7 min.

To detect the *RAD51* polymorphism, we applied a digestion step to the process. The 157 bp PCR amplified products were digested with 5 U *Micrococcus varians (MvaI)* restriction endonuclease (New England Biolabs, INC,UK). The post-digestion PCR products were visualized by agarose gel electrophoresis (at 90V, 300A for 1.5 h) on 2% agarose gels containing ethidium bromide, and the fluorescent intensity of each band was evaluated with a UV Transilluminator (Gel Logic Pro 2000, Canada) [16]. After digestion with the restriction endonuclease, the 157-bp fragment produced 86-bp and 71-bp fragments (Supplementary Fig. S1). These digested fragments corresponded to the G135 allele, whereas the 157-bp fragment represented the C135 variant allele [10].

Statistical analysis

The estimation of expected genotype frequencies and allele frequencies was based on the Hardy-Weinberg equilibrium. Deviations of allele and genotype frequencies from the expected were estimated using the chi-square test. The relative importance of variant allele/genotype in comparison to the wild-type allele/genotype was assessed via estimation of the odd ratio. A significance value of $p<0.05$ was used in all the statistical analyses.

RESULTS

Our study consisted of a population of 76 gastric cancer patients (22 females and 54 males), and 78 healthy in-

Table 1. Genotype and allele frequencies of the G135C site of *RAD51* polymorphism in gastric cancer patients and controls.

Genotype	Cancer cases, [n=76, n (%)]	Controls [n=78, n (%)]	OR (95% CI); P-value	χ2; P-value
GG-wild	44 (58)	66 (85)	1	13.58; 0.0011
GC-heterozygous	25 (32)	10 (13)	3.75 (1.64-8.57); 0.002	
CC-variant	7 (9)	2 (3)	5.25(1.02-26.45); 0.044	
GC+CC	32 (42)	12 (15)	4.00 (1.86-8.60); <0.001	
G allele	0.74	0.91	1	15.04; <0.001
C allele	0.26	0.09	3.53 (1.82-6.81); <0.001	

CI – confidence interval; OR – odds ratio; χ^2 – chi-square test statistics.

Table 2. Association between *RAD51* polymorphism and age group among gastric cancer cases.

	Cases (n=76)				
Variables	Total (n (%))	GG (n =44)	GC (n=25)	CC (n =7)	χ^2 (P-value)
Age Group					
≤60	40 (52.6)	22	14	4	0.29; 0.86
>60	36 (47.4)	22	11	3	

χ^2 – chi-square test statistics.

dividuals (24 females and 54 males). We first evaluated the departures from the Hardy-Weinberg equilibrium for the whole sample as well as for each group using the chi-square test. The results indicated that there were no departures from the Hardy-Weinberg equilibrium for the G135C polymorphic site of the *RAD51* gene (data not shown).

We evaluated the relative changes in allele and genotype frequencies of the evaluated site. Results indicated that both allele and genotype frequencies were significantly different between the gastric cancer group and the control (Table 1). The G allele frequency was estimated to be 0.74 among cancer patients, whereas the frequency of the same allele was about 0.91 in healthy individuals. Conversely, the C allele had a much higher frequency among cancer patients, with a frequency of 0.26. The frequency of the C allele among the controls was as low as 0.09. The odd ratio of the C allele for gastric cancer was 3.53 and was highly significant (Table 1). The chi-square statistics for the differences between the allele frequencies was 15.04 and was highly significant. The genotype frequency of heterozygous GC was 0.32, that of homozygous CC was 0.09, and the frequency for homozygous GG was 0.58 among the gastric cancer patients. The genotype frequency of heterozygous GC was 0.13, that of homozygous CC was 0.03, and the frequency for homozygous GG was 0.66 among the controls. Since the G allele is considered as a wild allele and had a higher frequency, we also estimated the cumulative frequency of the GC and CC genotypes. The cumulative genotype frequency (GC+CC) was 0.42 for cancer patients, whereas the frequency was 0.15 and much lower for the control group. The odd ratio of each genotype in comparison to the wild genotype of GG was from 3.75 to 5.25, indicating elevated risk as the number of C alleles increased (Table 1).

We also analyzed the correlation of *RAD51* polymorphism with the age of the cancer patients. We found that age was not a significant (p>0.05) parameter for cancer prevalence (Table 2).

DISCUSSION

The correct repair of DNA damage is crucial for maintaining genome stability. The genomic machinery is composed of several genes that are employed to achieve such an integral role. Thr241Met and Arg188His polymorphisms in the DNA repair gene *XRCC3* were proposed for use as cancer markers for gastric, colorectal, cervical and soft-tissue sarcomas [14]. Polymorphisms in a number of other DNA repair genes were also demonstrated to be associated with lung cancer [17-19]. The associations between polymorphic sites in *RAD51* and various cancer types were previously investigated and significant associations were reported [20-21]. Sliwinski et al. [20] reported that *RAD51* genetic variants are associated with increased risk for breast cancers in the Polish population. In a study conducted by Zhang et al. [22], *RAD51* polymorphism was thought to influence

different cancer types among white and Asian populations. Genetic variation within the *RAD51* gene was also linked to esophageal cancer [23-25]. A relatively large study targeting a *RAD51* (rs1801320) variant in DNA samples of 548 breast cancer patients and 360 healthy women found no statistically significant association between the variant and breast cancer in a Turkish population [26]. Here, we used the G135C polymorphic site to test the association of *RAD51* gene polymorphism with gastric cancer in the northeastern Anatolian population.

The results reported here indicated that the G allele was the common allele (wild) with a frequency of 0.73. Similar results were reported in another study [9]. Our results also showed that the 135G>C polymorphism in *RAD51* might be associated with tumor formation in the digestive system, more specifically in the stomach. The results indicated that the C allele in the polymorphic site increased the risk for the gastric cancer. The presence of at least one C allele in the genotype increased the risk of cancer 4-fold. Results indicated that the 135G>C polymorphism of the *RAD51* may increase colorectal cancer occurrence [9]. Zhou et al. [17] proposed that gastric cancer patients carrying the *RAD51* G172T genetic variant have a significantly higher mean survival rate than the other patients.

A meta-analysis based on 39 case-control studies was performed to investigate the association between cancer susceptibility and *RAD51* 135G>C. The meta-analysis suggested that the *RAD51* variant 135C homozygote is associated with elevated breast cancer risk among *BRCA2* mutation carriers [27]. Since the presence of the C variant at the site is linked with various cancer types, it can be expected that it is a mutation that prevents the accurate repair of DNA. Although there is a variation among the populations in terms of the relative risk of cancer, it would be interesting to conduct another meta-study to regress the C allele frequency increase with the prevalence of different cancer types in relation to the population of interest. Such a meta-study would also provide insight into the global pattern of the association between the cancer type and the polymorphism in *RAD51*. Since there is a number of other polymorphic sites in the gene of interest, considering all polymorphic sites and conducting a candidate gene-based association mapping study would be invaluable in pinpointing the true causative polymorphism within the *RAD51* gene.

The reported association needs to be further investigated in various parts of the world to discern differences among different populations. Furthermore, because of the polygenic nature of gastric cancer along with environmental effects, other candidate genes known to be involved in the DNA repair machinery should also be investigated to deduce a more comprehensive picture of the genetic causes of gastric cancer.

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Supplementary Data

Supplementary Fig. S1.
Available at: http://serbiosoc.org.rs/NewUploads/Uploads/Gok%20and%20Ozden_3793_Supplementary%20Fig.%20S1.pdf