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XRCC1 Gene Polymorphisms and Risk of Lung Cancer in Turkish Patients

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ABSTRACT Polymorphisms in the X-ray repair cross complementing 1 (*XRCC1*) gene have been found to be associated with susceptibility to various types of cancers. We investigated the association between the *XRCC1* gene Arg399Gln polymorphism and the susceptibility to lung cancer in Turkish patients. To determine the association of this polymorphism with the risk of lung cancer in Turkish patients, a hospital-based case-control study was designed, involving 67 patients with lung cancer and 60 control subjects with no cancer history who were matched for age and gender. *XRCC1* genotypes (Arg/Arg, Arg/Gln, and Gln/Gln) were determined using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) analysis on genomic DNA. No statistically significant relationship was determined between the lung cancer and control groups ($p>0.05$). Among the patients, 61% were Arg/Arg, 28% were Arg/Gln, and 11% were Gln/Gln. Among the controls, 50% were Arg/Arg, 38% were Arg/Gln, and 12% were Gln/Gln. There was no difference in the distribution of *XRCC1* genotypes or the frequencies of the Arg (75% versus 69%) and Gln (25% versus 31%) alleles between the lung cancer patients and controls. Our results suggest that the *XRCC1* gene Arg399Gln polymorphism is not associated with an increased risk for the development of lung cancer in Turkish patients.

INTRODUCTION

Currently, lung cancer is the most common cancer worldwide. Histologically, 80% are non-small-cell cancers, and 20% are small-cell lung cancers. The inhalation of tobacco smoke and other environmental carcinogens is considered a major etiologic factor (Hu et al. 2002; Wang et al. 2009). Reactive oxygen species present in cigarette smoke cause DNA damage and single strand breaks that are repaired through the base excision repair (BER) pathway (David-Beabes and London 2001; Wilson and Thompson 1997). The X-ray repair cross complementing 1 (*XRCC1*) gene plays a role in base excision repair and in repairing DNA strand breaks (Thompson and West 2000).

Shen et al (1998) identified three coding polymorphisms in the *XRCC1* gene in codons 194 (Arg to Trp), 280 (Arg to His), and 399 (Arg to Gln). These polymorphisms encode non-conserved amino acid changes that could alter *XRCC1* function. In particular, the 399Gln polymorphism resulting from a guanine to adenine mutation occurs in the poly(ADP-ribose) polymerase binding domain and may affect complex assembly or repair efficiency (Park et al. 2002; Shen et al. 1998). Lunn et al (1999) reported that the 399Gln allele was significantly associated with higher levels of aflatoxin B1-DNA adducts and glycophorin. These studies propose that individuals with the 399 Gln allele are less able to repair DNA damage.

In this study, we investigated the relationship between the development of cancer and general polymorphisms that play a role in DNA repair. Specifically, the *XRCC1* gene Arg 399 Gln polymorphism was studied in Turkish patients with lung cancer.

MATERIALS AND METHODS

1. Study Subjects

This prospective study was conducted in the Department of Medical Oncology at the Medi-

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cal School of Uludag University. In this study, the patient group consisted of 67 cases diagnosed with lung cancer, and the age-matched control group consisted of 60 cases with no cancer history. Both groups visited our clinic during the same period. Members of both the patient and control groups signed an informed consent form.

2. DNA Isolation and Genotyping of *XRCC1* (Arg399Gln)

Blood samples obtained from patient and control individuals were collected in EDTA tubes. Genomic DNA was extracted from whole blood using a DNA isolation kit (Dr. Zeydanlý, Turkey) according to the manufacturer's instructions.

Genotypic analysis of the *XRCC1* gene Arg 399 Gln polymorphism was performed using a modification of a previously described PCR-restriction fragment length polymorphism (RFLP) assay (Canalle et al. 2006). Briefly, PCR primers for codon 399 were used to generate a 615-bp product containing the polymorphic site. The 25-μl amplification reaction contained 200 ng genomic DNA, 150 ng each of codon 399 primers F (52 -TTGTGCTTTCTCTGTGTC CA-32) and R (52 -TCCTCCAGCCTTTTCT GATA-32), 300 μM dNTPs, 2.5 μl 10x PCR buffer, 2 mM MgCl2, and 1 U Taq DNA polymerase (Bioron, Germany). After an initial denaturation at 94 °C for 5 min, 35 cycles of 30 s at 94 °C, 45 s at 65 °C, and 45 s at 72 °C were performed with a final extension step of 10 min at 72 °C. The PCR products were digested with Msp I (Bioron, Germany) at 37 °C overnight and analyzed on a 2% agarose gel. Arg/Arg individuals had 374- and 221-bp fragments, Arg/Gln individuals had 615-, 374-, and 221-bp fragments, and Gln/Gln individuals had only a 615-bp fragment (Fig. 1).

3. Statistical Analysis

Age was represented as the mean and standard deviation. Polymorphisms, histologic types

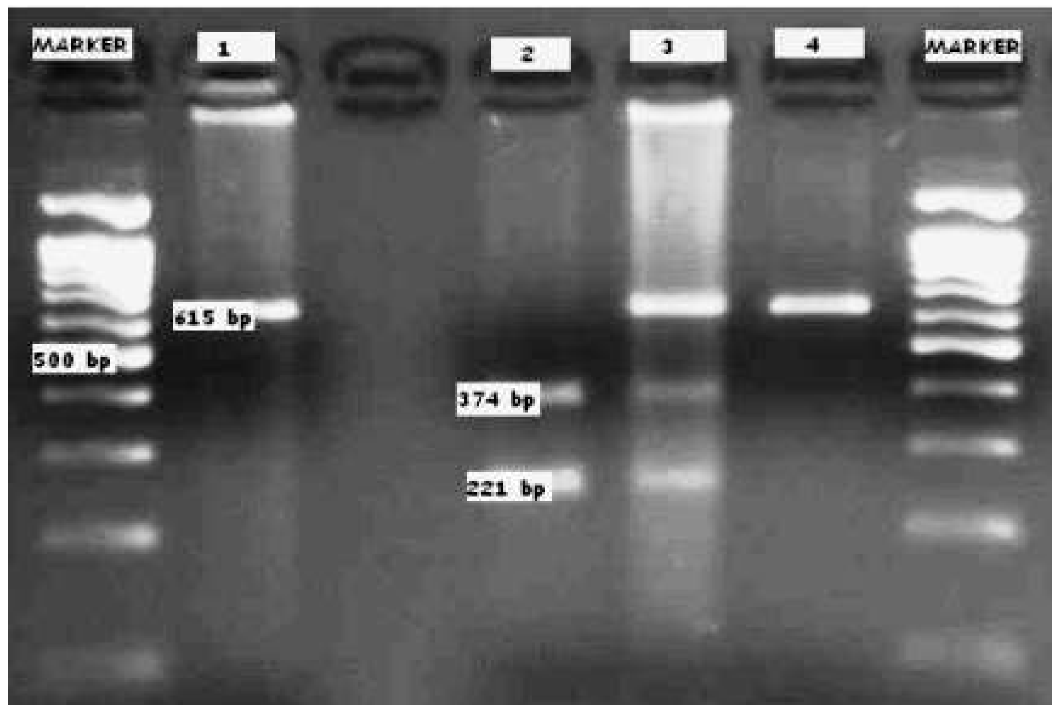

Fig. 1. Photograph of the XRCC1 gene PCR products after Msp I enzyme digestion that were run on a 2 % agarose gel. Lane MARKER shows the 100-bp DNA ladder, lane 1 shows the uncut PCR product, lane 2 shows the Arg/Arg genotype (374-bp and 221 bp products), lane 3 shows the Arg/Gln genotype (615-bp, 374-bp, and 221-bp products) and lane 4 shows the Gln/Gln genotype (615-bp product)

and allele types were expressed by frequency and associated percentages. The Student's t test was performed to compare age between the patient and control groups. Gender, genotypes and allele frequencies were compared between the control and patient groups using the chi-square test. Statistical significance was set at $p<0.05$. SPSS 13.0 (Chicago, IL) software was used to perform the statistical analyses.

RESULTS

The distribution of demographic variables, such as age, gender, and histologic type, is shown in Table 1. The mean age of the 67 cases in the patient group (62 males and 5 females) was 60.94±10.46 years and that of the 60 cases in the control group (53 males and 7 females) was 58.35±13.72 years. There was no significant difference in age or gender between the two groups. Among the patients, 18% had a diagnosis of small-cell cancer, 31% of adenocarcinoma, 37% of squamous-cell cancer, and the remaining 14% of other types of lung cancer.

Table 1: Characteristics of lung cancer patients and controls

Variables	*Patients n=67*	*Control n=60*	*p-value*
*Age (years)**	60.94±10.46	58.35±13.72	0.231
Gender			
Female	5	7	0.614
Male	62	53	
Histologic Types			
Squamous-cell cancer	25(37%)	-	
Adenocarcinoma	21(31%)	-	
Small-cell cancer	12(18%)	-	
Other types	9(14%)	-	

* Age value was shown as the Mean± Standard Deviation.

The investigation of the *XRCC1* gene Arg 399 Gln polymorphism revealed that in the lung cancer patient group, 41 (61%) had the Arg/Arg genotype, 19 (28%) had the Arg/Gln genotype and 7 (11%) had the Gln/Gln genotype. In the control group, 30 (50%) had the Arg/Arg genotype, 23 (38%) had the Arg/Gln genotype and 7 (12%) had the Gln/Gln genotype. With respect to genotype distribution, no significant difference between the lung cancer patients and the controls without lung cancer was observed (p=0.426) (Table 2).

The frequency of the Gln allele was 25% in the patient group and 31% in the control group.

Table 2: Genotype frequencies of XRCC1 polymorphisms in patients and controls

Variables	*Cancer patients (%) (N=67)*	*Controls (%) (N=60)*	*p-value*
Genotypes			
Arg/Arg	41(61%)	30(50%)	
Arg /Gln	19(28%)	23(38%)	0.426
Gln /Gln	7(11%)	7(12%)	
Frequency of Allele			
Arg (%)	75	69	
Gln (%)	25	31	0.345

Although the frequency of the Gln allele was high in the control group, there was no significant difference between the two groups with respect to allele distribution (p=0.345) (Table 2). The Gln allele frequency of the control group in this study is summarized in Table 3.

Table 3: Gln allele frequency in control groups from studies conducted with Turkish patients

Study	*The compared disease group*	*Number of control cases*	*Gln allele frequency (%)*
Tumer et al.	Childhood acute lymphoblastic leukemia	190	31
Engin et al.	Gastric and colorectal cancer	108	31
Karahalil et al.	Bladder cancer	100	38
Güven et al.	Primary open angle glaucoma	121	40
Unal et al.	Cataract	194	40
Deligezer et al.	Breast cancer	133	37
Batar et al.	Childhood acute lymphoblastic leukemia.	75	43
Kocabaþ et al.	Healthy subjects	166	40
Vural et al.	Pre-eclampsia	107	34
Erdal et al.	Healthy subjects	75	35
This study	Lung cancer	60	31

DISCUSSION

In addition to exposure to environmental carcinogenic agents, such as polycyclic aromatic hydrocarbons, that might cause DNA damage, genetic susceptibility can lead to the development of lung cancer. Such genetic susceptibility may arise from polymorphisms in the genes involved in carcinogen metabolism and DNA damage repair (Neumann et al. 2005; Shields and Harris 2000; Wang et al. 2009).

Many studies have investigated the relationship between lung cancer and the *XRCC1* gene Arg399Gln polymorphism, which is a gene involved in DNA repair. There, however, appear

to be inconsistencies among these epidemiological studies. Thus, several meta-analyses of these studies were performed recently. Kiyohara et al. found that while an increase in the development of lung cancer has been identified in Asians with the Gln/Gln genotype, no increase in this risk has been observed in Caucasians (Kiyohara et al. 2006). In contrast, both Hu et al. and Hung et al. found no relationship between lung cancer and the *XRCC1* gene Arg399Gln polymorphism (Hu et al. 2005; Hung et al. 2005). In a meta-analysis of 30 studies comprising 10214 cases and 12599 controls, Wang et al. found a protective effect of the *XRCC1* codon 399 Gln/Gln and Arg/Gln or Gln/Gln polymorphisms for lung cancer on the basis of population control (OR = 0.73, 95% CI: 0.58–0.92; OR = 0.86, 95% CI: 0.77–0.97, respectively) (Wang et al. 2009). Here, we report in the first prospective population-based study of the Turkish population that there was no association between the *XRCC1* gene Arg399Gln polymorphism and the risk of lung cancer ($p>0.05$). Our study is of importance because it was conducted on Turkish patients suffering from lung cancer. In the present study, 60 healthy controls from the Turkish population were included, and the frequency of the *XRCC1* 399Gln allele was determined to be 31% in this group. This frequency was found to be similar to that found in other studies with Turkish control populations (Engin et al. 2011; Tumer et al. 2010). Although the frequency of the Gln allele varies, the results are close (Table 3). In general, the Gln allele frequency ranges from 34 to 43 % (Batar et al. 2009; Deligezer and Dalay 2004; Erdal et al. 2004; Guven et al. 2007; Karahalil et al. 2006; Kocabas and Karahalil 2006; Unal et al. 2007; Vural et al. 2009). These differences in allele frequency may be due to different ethnic and geographic backgrounds. In summary, our study is the first study to investigate the relationship of lung cancer with the *XRCC1* gene Arg399Gln polymorphism in Turkish patients. Although no significant relationship was identified between lung cancer and the *XRCC1* gene Arg399Gln polymorphism, when compared with the studies conducted in other populations, the number of cases in our study is restricted.

Thus, the conclusion from this study would need to be supported with additional studies conducted with a higher number of Turkish lung cancer patients. In addition, studies that investigate the relationship between lung cancer and other polymorphisms in the *XRCC1* gene should be conducted.

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