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Association between the DNA Repair Gene Polymorphisms and Lung Cancer in Turkish Population

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Abstract

Introduction: DNA repair enzymes continuously monitor DNA to correct damaged nucleotide residues generated by exposure to environmental mutagenic and cytotoxic compounds or carcinogens. Our objective was to investigate the association among XRCC1 (Arg399Gln and Arg194Trp), XRCC3 (Thr241Met), XPD-ERCC2 (Lys751Gln), APE1 (Asp241Glu), PARP-ADPRT (Val762Ala) DNA repair gene polymorphisms and lung cancer in Turkish population. **Materials and Methods:** Our patient group consists of 90 patients with lung cancer and the control group had 100 healthy individuals all of those smoking. DNA was extracted using the whole blood samples. PCR-RFLP technique was used to investigate the polymorphisms on target genes. **Results:** There was no significant difference in the genotype distributions of XPD Lys751Gln, XRCC1 Arg194Trp, XRCC3 Thr241Met, APE1 Asp241Glu between lung cancer patients and controls for each polymorphism ($p > 0.05$). However, there was a significant difference between the genotype distributions of XRCC1 Arg399Gln, and PARP Val762Ala in patients and the control group ($p > 0.05$). **Discussion:** Only the polymorphisms of XRCC1 codon 399 and PARP Val762Ala alleles are associated with the risk of lung cancer. Other genotypes were not related to lung cancer.

Keywords

Lung Cancer, Polymorphism, DNA Repair Genes, Turkish Population, PCR-RFLP

1. Introduction

Defined as the loss of control in a cellular cycle, cancer is one of the most im-

portant health problems, for which huge amounts of funds and research endeavors are allocated to understand, prevent and cure this important public health problem. Investigation of the biochemical processes of cancer at the molecular level has a great potential for a better understanding of the multifactorial etiology of this disease [1].

According to WHO, causing the death of 1.8 million people every year, lung cancer is the most common type of cancer among males that causes death, and it is the third most common type of cancer among females right after breast cancer and colorectal cancer [1]. As a multifactorial disease, 70% of lung cancer cases are associated with cigarette smoking. However, there is a long list of questions that are still unanswered about the cigarette-lung cancer relationship, such as "Why do not all cigarette smokers develop lung cancer? Is it the molecular-level differences among people that cause overcoming or failing to sufficiently neutralize the dangerous effects of cigarettes on the organism?" Since cancer stems from errors in molecular-level basic regulatory mechanisms, it is a disease that needs to be examined particularly at the molecular level [2].

It is a well-known fact that the toxic chemicals in cigarettes cause a host of damages to the genomic DNA, one-chain and double-chain fractures being the leading major effects. The damages are repaired by DNA repair genes which play an important role in transmitting genetic information to the new offspring. DNA damage can be repaired through direct repair (DR), base excision repair (BER), and nucleotide excision repair (NER) pathways in the DNA repair system. Inadequate repairability can increase the risk of lung cancer. Maintaining these repairing mechanisms as intact, functional, and healthy has a significant place in protecting the organism from diseases [2] [4].

Sometimes, structural changes might occur to the genes in charge of repairing DNA damages. Some of these changes comprise various polymorphic structures such as single nucleotide polymorphism (SNP) and tandem repeating double nucleotide polymorphism (DNP). Because they change the aminoacid sequences in synthesized proteins and enzymes, the polymorphic structures within the nucleotide sequences of repair genes might happen to be functional polymorphic structures. Therefore, polymorphic changes with these genes might lead to functional changes in DNA repairing capacity and eventually it might influence the individuals' sensitivity (proneness or resistance) against different types of cancer diseases [5].

In this study, we have aimed to compare the polymorphic structures that are known to be important in certain DNA repair genes among patients who have lung cancer and among healthy individuals who neither themselves nor their family members have a history of lung cancer.

2. Materials and Methods

Subjects

In this study, we have included 90 cigarette smoking patients diagnosed with

lung cancer and 100 healthy individuals who also smoke cigarettes but neither themselves nor their families have a history of lung cancer. We have divided the sample into three groups based on the average number of cigarettes: light smokers (less than 10 cigarettes/per day), medium smokers (10 to 1 pack of cigarettes/per day), and heavy smokers (more than 1 pack/per day). The patient group is classified into two groups according to the clinical examination: Primary Tumor and Nodular Metastasis. The study was initiated upon the Ethical Board Approval of the hospital on 18/06/2014; Approval number: 9. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

DNA Extraction and Isolation

In this study, genomic DNA was extracted from ethylenediaminetetraacetic acid anticoagulant whole-blood samples using the commercial DNA extraction kit according to the method recommended by the manufacturer (AXAP-MN-BL-GDN AxyPrep Blood Genomic DNA Miniprep Kit). DNAs were stored at −20˚C until the polymerase chain reaction (PCR) analysis.

Analysis of Gene Regions Using PCR Technique

We used restriction fragment length polymorphism (RFLP) technique in order to extract targeted gene polymorphisms.

For the ERCC2 Lys751Gln polymorphism, we employed 5'-CCTCTGTTCTCTGCAGGAGGA-3' and 5'-CCTGCGATTAAAGGCTGTGGA-3' primers with the PCR product of the 235 base pairs.

For the XRCC1 Arg399Gln polymorphism, we employed 5'-TTGTGCTTTCTCTGTGTCCA-3' and 5'-TCCTCCAGCCTTTTCTGATA-3' primers with the PCR product of the 615 base pairs.

For the XRCC1 Arg194Trp polymorphism, we employed 5'-GCCAGGGCCCCTCCTTCAA-3' and 5'-TACCCTCAGACCCACGAGT-3' primers with the PCR product of the 485 base pairs.

For the XRCC3 Thr241Met polymorphism, we employed 5'-GGTCGAGTGACAGTCCAAAC-3' and 5'-TGCAACGGCTGAGGGTCTT-3' primers with the PCR product of the 456 base pairs.

For the APE1 Asp148Glu polymorphism, we employed 5'-CTGTTTCATTTCTATAGGCTA-3' and 5'-AGGAACTTGCGAAAGGCTTC-3' primers with the PCR product of the 164 base pairs.

For the PARP-1 Val763Ala polymorphism, we employed 5'-TTTGCTCCTCCAGGCCAACAG-3' and 5'-TGGAAGTTTGGGACCGCTGC-3' primers with the PCR product of the 210 base pairs.

For all gene regions, we prepared the following reaction mixture with the stated ratios: 1.8 µl 10 pmol each of dNTPs (ThermoFisher), 2 µl 10 × PCR tampon (ThermoFisher), 1 µl $MgCl_2$ (25 mM) (ThermoFisher), 1.2 µl 10 pmol primer mix (Genplaza), 0.2 µl DNA Taq polymerase (ThermoFisher), 11.6 µl dH_2O and 2 µl genomic DNA.

Amplification was carried out on GeneAmp PCR System 2700 Model. The PCR cycling conditions consisted of an initial denaturation step at 94˚C for 3 min, 10 cycles of 94˚C for 0.15 min, Tm1 for 0.15 min, and 72˚C for 0.15 min, 25 cycles of 94˚C for 0.15 min, Tm2 for 0.15 min, and 72˚C for 0.15 min and final extension step at 72˚C for 3 min.

Double-Checking PCR Products, Enzyme Cuts and Electrophoresis

After PCR, we controlled whether the projected products are obtained and whether the product meets the expected quality by running electrophoresis (Axygen Scientific HGB20) under UV illimunator (BTLabSystems-BT501) on 2% agarose gel (AgaPure HR), of which was prepared with 5 µl TAE (Tris Acit, Asetic Acit, and EDTA) tampon (ThermoFisher). The gene products are put into tubes and kept at +4˚C for enzyme cutting. If the expected PCR product was not obtained, all procedures were repeated for unsuccessful samples.

Later, we have added 5 µl restriction enzyme mixtures to the remaining 15 µl PCR products (PstI (BIORON) for ERCC2 Lys751Gln gene region, PvuII (New England BioLabs-NEB) for XRCC1 Arg194Trp region, MspI (NEB) for XRCC1 Arg399Gln region, Hsp92 (NIaIII) for XRCC3 Thr241Met region, FspBI-MaeI (ThermoFisher) for APE1 Asp148Glu region, and BstUI (NEB) for PARP-1 Val763Ala region) and incubated them at 37˚C for 12 hours.

At the end of the 12-hour incubation period, all PCR products were separated by 2.0% or 6.0% (short length of the PCR outcomes of the restriction enzyme products) agarose gel electrophoresis that was stained with ethidium bromide (ThermoFisher), visualized and photographed using a UV transilluminator (BT LabSystems-BT501). We used 100 pair-base ladder markers (Invitrogen) to make sure that the produced stribe are the expected ones.

Determining Allele Distributions in Polymorphisms

Digestions of PCR products by restriction enzymes yield for XRCC1 Arg399Gln polymorphism, 615 bp bands for AA (Gln/Gln) homozygote genotype, 615, 376 and 239 bp bands for AG (Arg/Gln) heterozygote genotype, 376 and 239 bp bands for GG (Arg/Arg) homozygote genotype; for XRCC1 Arg194Trp polymorphism 485 bp bands for CC homozygote genotype, 485, 396 and 89 bp bands for CT heterozygote genotype, 396, 89 bp bands for TT homozygote genotype for XRCC3 Thr241Met polymorphism 456 bp bands for Thr/Tr homozygote genotype, 456, 315 and 141 bp bands for Thr/Met heterozygote genotype, and 315, 141 bp bands for Met/Met homozygote genotype; for XPD Lys751Gln polymorphism, 235 bp bands for AA (Lys/Lys) homozygote, 235, 171 and 64 bp bands for AC (Lys/Gln) heterozygote, and 171, 64 bp bands for CC (Gln/Gln) homozygote genotypes; for APE1 Asp148Glu polymorphism, 164 bp bands for Asp/Asp homozygote genotype, 164, 144 and 20 bp bands for Asp/Glu heterozygote genotype, 144 and bp bands for Glu/Glu homozygote genotype. We have employed 6% electrophoresis gel in analyzing the PARP-1 Val762Ala polymorphism because of the short length of the PCR outcomes of the restriction enzyme products. At the end of the electrophoresis, we monitored 210 bp bands for TT

(Val/Val) genotype, 210, 190 and 20 bp bands for TC (Val/Ala) genotype, 190 and 20 bp bands as CC (Ala/Ala) genotype.

Statistical Analyses

We have statistically examined the allele distributions for each polymorphism type alone and compared the variations of these polymorphisms across the patient and control groups. We used SPSS 10.0 in analyzing data.

We have summarized the data with descriptive statistics and presented percentages, mean scores and standard deviations for variables. We have employed the Chi-Square test in analyzing the relationship between categorical variables. Furthermore, we have also provided the Odds Ratios (OR) and 95% Confidence Intervals as well. For all analyses, we have accepted $p = 0.05$ as the threshold value.

3. Results

We have conducted this study on a sample of 90 patients who were diagnosed with lung cancer and 100 healthy individuals. The neither themselves nor the families of the individuals in the control group have a history of lung cancer.

All subjects in the study are cigarette smokers. 4.4% of the patient group and 13.0% of the control group are light users, 18.9% of the patient group and 26.0% of the control group are medium users, 76.7% of the patient group and 61.0% of the control group are heavy smokers.

The pathology results of the patient group revealed that 31.1% of the patients had small cell lung carcinoma, 44.4% of them had squamous cancer, and 24.4% of them had adeno cancer types. 10 patients (11.1%) were at Stage 2, 8 patients (8.9%) were at Stage 3a, 6 patients (6.7%) at were Stage 3b, 35 patients (38.9%) were at Stage 4, 21 patient (23.3%) were at Advanced Stage, 2 patients (2.2%) were at Stage 1b, 5 patients (5.6%) were at Stage 2b, 2 patients (2.2%) were at Stage 1b, 5 patients (5.6%) were at Stage 2b, 2 patients (2.2%) were at Stage 1a, and 1 patient (1.1%) were at Stage 2a according to pathological analyses (Table 1).

Statistical Analyses of XPD Lys751Gln Polymorphism

Our analysis of XPD (Lys751Gln) genotype distribution showed that there are three types of genotypes: Lys/Lys genotype (41.0% in the control group and 36.7% in the patient group), Lys/Gln genotype (45.0% in the control group & 52.2% in the patient group), Gln/Gln genotype (14.0% in the control group and 11.1% in the patient group). There is not a statistically significant difference between these genotypes across the patient and the control groups ($\chi^2 = 1.05$, $p = 0.59$). Even after we repeated the same analysis by combining the Lys/Gln heterozygote and Gln/Gln homozygous genotypes and comparing its distribution with the wild type Lys/Lys homozygote genotype across groups did not change the non-significant relationship result ($\chi^2 = 0.37$, $p = 0.54$).

As we see in Table 2, when we analyzed the allele frequency distribution across the control and patient groups, the Lys allele distributions were 63.5% and

Table 1. General characteristics of the samples.

	Control Group n = 100	Patient Group n = 90
Cigarette Consumption		
Light user	13 (13.0)	4 (4.4)
Medium user	26 (26.0)	17 (18.9)
Heavy user	61 (61.0)	69 (76.7)
Pathology		
Small cell carcinoma	-	28 (31.1)
Squamoz cell carcinoma	-	40 (44.4)
Adenocarcinoma	-	22 (24.4)
TNM Staging		
Stage 2	-	10 (11.1)
Stage 3a	-	8 (8.9)
Stage 3b	-	6 (6.7)
Stage 4	-	35 (38.9)
Advanced stage	-	21 (23.3)
Stage 1b	-	2 (2.2)
Stage 2b	-	5 (5.6)
Stage 1a	-	2 (2.2)
Stage 2a	-	1 (1.1)

Data are given as n (%).

Table 2. Distribution of XPD (Lys751Gln) polymorphisms across groups.

	XPD (Lys751Gln) Genotypes			Allele Frequency (%)	
	Lys/Lys	**Lys/Gln**	**Gln/Gln**	**Lys**	**Gln**
Control Group (n = 100)	41 (41.0)	45 (45.0)	14 (14.0)	63.5	36.5
Patient Group (n = 90)	33 (36.7)	47 (52.2)	10 (11.1)	62.8	37.2
	$\chi^2 = 1.05$, $p = 0.59$			$\chi^2 = 0.02$, $p = 0.88$	
	Merged Genotypes				
	Lys/Lys		**Lys/Gln + Gln/Gln**		
Control Group (n = 100)	41 (41.0)		59 (42.0)		
Patient Group (n = 90)	33 (36.7)		57 (60.0)		
	$\chi^2 = 0.37$, $p = 0.54$		OR (95% CI) = 1.02 (0.73 - 1.42)		

Data are given as n (%) unless otherwise stated.

62.8% and Gln allele distributions were 36.5% and 37.2% for the control and patient groups respectively. In this distribution, however, there was not a statistically significant relationship, either ($\chi^2 = 0.02$, $p = 0.88$).

The Lys allele was 77% among the control group whereas it was 62.2% among the patient group. The Gln allele was 23.0% among the control group while it was 37.8% among the patient group. It can be argued that having a higher number of individuals in the patient group who has Gln amino acid might serve as a protective function against the risk of developing lung cancer with the genotype that includes Lys amino acid ($\chi^2 = 5.30$, $p = 0.02$; OR = 1.54, 95%) (Table 2).

Statistical Analyses of the XRCC1 Arg399Gln Polymorphism

The statistical analyses of the genotype distributions across the control and the patient groups were 58.0% and 40.0%, respectively, for the wild-type homozygote Arg/Arg allele distribution 38.0% and 44.4%, respectively, for the Arg/Gln heterozygote allele, and 4.0% and 15.6%, respectively, for the Gln/Gln homozygote allele. These figures show that there is a statistically significant difference between the healthy individuals and patients with lung cancer ($\chi^2 = 9.02$, $p = 0.01$).

Further, we found a statistically significant difference between the homozygote wild genotype Arg/Arg allele and the merged Gln allele group, composed of Arg/Gln and Gln/Gln alleles, and this difference stems from an increase in the frequency distribution of variant Gln allele ($\chi^2 = 6.13$, $p = 0.01$; OR = 2.22, 95% CI: 1.74 - 2.84) (Table 3).

Statistical Analysis of the XRCC1 Arg194Trp Polymorphism

In this polymorphism, Arg/Arg genotype was found 85.0% and 90.0% in the control and the patient groups, and Arg/Trp heterozygote genotype was found 15.0% and 10.0% in these groups respectively. The Trp/Trp homozygote variant was not found in either group. There was not a statistical difference of genotypes across the control and the patient groups ($\chi^2 = 1.07$, $p = 0.30$).

Table 3. Distribution of XRCC1 (Arg399Gln) polymorphisms across groups.

	XRCC1 (Arg399Gln) Genotypes			Allele Frequency (%)	
	Arg/Arg	Arg/Gln	Gln/Gln	Arg	Gln
Control Group (n = 100)	58 (58.0)	38 (38.0)	4 (4.0)	77.0	23.0
Patient Group (n = 90)	36 (40.0)	40 (44.4)	14 (15.6)	62.2	37.8
	$\chi^2 = 9.02$, $p = 0.01$			$\chi^2 = 5.3$, $p = 0.02$	
	Merged Genotypes				
	Arg/Arg	Arg/Gln + Gln/Gln			
Control Group (n = 100)	58 (58.0)	42 (42.0)			
Patient Group (n = 90)	36 (40.0)	54 (60.0)			
	$\chi^2 = 6.13$, $p = 0.01$	OR (95% CI) = 2.22 (1.74 - 2.84)			

Data are given as n (%) unless otherwise stated.

Since the Trp/Trp allele did not exist in either group, the genotypic risk analysis could not be performed by combining alleles, and there wasn't a statistically significant difference in alleles frequency distributions between the patient and the control groups ($\chi^2 = 0.35$, $p = 0.55$) (Table 4).

Statistical Analysis of the XRCC3 Thr241Met Polymorphism

In terms of genotypic distribution, we found the following three polymorphisms: Thr/Thr genotype (37.0% in the control group and 40.0% in the patient group), Thr/Met genotype (52.0% in the control group and 44.4% in the patient group), Met/Met genotype (11.0% in the control group and 15.6% in the patient group) but this distribution was not statistically significant ($\chi^2 = 1.41$, $p = 0.49$). Neither the comparison of the combined Thr/Met + Met/Met genotypes with the Thr/Thr homozygote genotype, nor the comparison of the Thr allele frequency and the Met allele frequency yielded a statistically significant difference between the control and the patient groups ($\chi^2 = 0.18$, $p = 0.67$ and $\chi^2 = 0.02$, $p = 0.88$ respectively) (Table 5).

Table 4. Distribution of XRCC1 (Arg194Trp) polymorphisms across groups.

	XRCC1 (Arg194Trp) Genotypes			Allele Frequency (%)	
	Arg/Arg	Arg/Trp	Trp/Trp	Arg	Trp
Control Group (n = 100)	85 (85.0)	15 (15.0)	0	92.5	7.5
Patient Group (n = 90)	81 (90.0)	9 (10.0)	0	95.0	5.0
	$\chi^2 = 1.07$, $p = 0.30$			$\chi^2 = 0.35$, $p = 0.55$	
	Merged Genotypes				
	Arg/Arg	Arg/Trp + Trp/Trp			
Control Group (n = 100)	85 (85.0)	15 (15.0)			
Patient Group (n = 90)	81 (90.0)	9 (10.0)			
	$\chi^2 = 1.07$, $p = 0.30$	OR (95% CI) = 1.26 (0.81 - 1.97)			

Data are given as n (%) unless otherwise stated.

Table 5. Distribution of XRCC3 (Thr241Met) polymorphisms across groups.

	2D-XRCC3 (Thr241Met) Genotypes			Allele Frequency (%)	
	Thr/Thr	Thr/Met	Met/Met	Thr	Met
Control Group (n = 100)	37 (37.0)	52 (52.0)	11 (11.0)	63.0	37.0
Patient Group (n = 90)	36 (40.0)	40 (44.4)	14 (15.6)	62.2	37.8
	$\chi^2 = 1.41$, $p = 0.49$			$\chi^2 = 0.02$, $p = 0.88$	
	Merged Genotypes				
	Thr/Thr	Thr/Met + Met/Met			
Control Group (n = 100)	37 (37.0)	63 (63.0)			
Patient Group (n = 90)	36 (40.0)	54 (60.0)			
	$\chi^2 = 0.18$, $p = 0.67$	OR (95% CI) = 0.8 (0.3 - 2.5)			

Data are given as n (%) unless otherwise stated.

Statistical Analysis of the APE1 Asp241Gln Polymorphism

In terms of the Asp241Gln polymorphism in APE1 Gene, distribution of Asp/Asp genotype was found 63.0% in the control group and 50.0% in the patient group, Asp/Glu genotype was 25.0% in the control group and 33.3% in the patient group, and Glu/Glu was 12.0% in the control group and 16.7% in the patient groups respectively. The allele and genotype distributions across the patient and the control groups are not statistically significant ($\chi^2 = 3.27$, $p = 0.19$).

When we combined the heterozygote variant genotype Asp/Glu and homozygote variant genotype Glu/Glu and compared it with Asp/Asp genotype, their distribution across the control and patient groups were not significant but the p value was very close to the threshold value of 0.05 ($\chi^2 = 3.26$, $p = 0.07$) (Table 6).

In evaluating the allele frequency; Asp was observed in 75.5% of the control group and in 66.5% of the patient group, Glu amino acid was present in 24.5% of the control group and in 33.9% of the patient group. Nevertheless, the distribution of these figures across the control and the patient groups was not significant ($\chi^2 = 1.94$, $p = 0.16$) (Table 6).

Statistical Analysis of the PARP Val762Ala Polymorphism

In the PARP Val762Ala polymorphism, the genotype distributions in the control and the patient groups were 35.0% and 22.5% for the Val/Val, 54.0% and 36.9% for the Val/Ala, and 11.0% and 21.6% for the Ala/Ala. There was not a statistically significant difference between the patient and the control groups in terms of the distribution of these genotypes ($\chi^2 = 7.76$, $p = 0.02$).

The crosstabulation of the combined Val/Ala + Ala/Ala genotypes with the homozygote Val/Val genotype across the study groups showed that Val/Val genotype was present in 35.0% of the control group and 22.5% in the patient group, the combined Val/Ala + Ala/Ala genotypes were present in 65.0% of the control group and 77.5% of the patient group. In this distribution, two groups show very close frequency distributions ($\chi^2 = 1.14$, $p = 0.28$).

Table 6. Distribution of APE1 (Asp241Glu) polymorphisms across groups.

	2E-APE1 (Asp241Glu) Genotypes			Allele Frequency (%)	
	Asp/Asp	Asp/Glu	Glu/Glu	Asp	Glu
Control Group (n = 100)	63 (63.0)	25 (25.0)	12 (12.0)	75.5	24.5
Patient Group (n = 90)	45 (50.0)	30 (33.3)	15 (16.7)	66.5	33.9
	$\chi^2 = 3.27$, $p = 0.19$			$\chi^2 = 1.94$, $p = 0.16$	
	Merged Genotypes				
	Asp/Asp		Asp/Glu + Glu/Glu		
Control Group (n = 100)	63 (63.0)		37 (37.0)		
Patient Group (n = 90)	45 (50.0)		45 (50.0)		
	$\chi^2 = 3.26$, $p = 0.07$		OR (95% CI) = 1.8 (0.9 - 3.6)		

Data are given as n (%) unless otherwise stated.

However, when the allele frequencies were examined, there was a statistically significant difference among alleles across study groups ($\chi^2 = 5.8$, $p = 0.01$; OR = 2.1, 95% CI: 1.45 - 7.13) (Table 7). Val allele was observed in 62.0 % of the control group and in 45.5% of the patient group and the Ala allele was found in 38% of the control group and in 54.5% of the patient group.

4. Discussion

Since cancer stems from errors in cells' molecular-level regulatory functions, it needs to be examined at the molecular level. It is a well-known fact that the toxic chemicals in cigarettes cause a host of damages to genomic DNA, one- and double-strand breakages being the leading examples. These damages are corrected by the DNA repair mechanism which is highly instrumental in correctly rendering genetic knowledge and transferring them to the next generations.

Healthy functioning of these mechanisms has a significant place in protection against cancer and a lot of other diseases. Many genes in charge of DNA repairs might go through structural changes. A part of these changes is constituted of various polymorphic structures in these genes, such as single nucleotide polymorphism and tandem repeating double sequence polymorphisms. Since they change the aminoacid sequences of synthesized proteins and enzymes, the polymorphic structures in nucleotide sequences of repair genes might happen to be functional polymorphic structures. For this reason, the polymorphic changes with these genes might cause significant functional changes in their DNA repairing capacity and influence the individuals' sensitivity, namely their tendency or resistance, to different types of cancer diseases [2] [5] [6] [7].

Even though up to 70% of patients of lung cancer, which is a multifactorial disease, are known to be cigarette smokers, we do not know why not all cigarette smokers do not develop any cancer type. One might speculate that the reason for this is the molecular-level individual differences in repairing the harmful effects of cigarettes to the organism. Perhaps, in some individuals, such damages are

Table 7. Distribution of PARP (Val762Ala) polymorphisms across groups.

	2F-PARP (Val762Ala) Genotypes			Allele Frequency (%)	
	Val/Val	Val/Ala	Ala/Ala	Val	Ala
Control Group (n = 100)	35 (35.0)	54 (54.0)	11 (11.0)	62.0	38.0
Patient Group (n = 90)	25 (22.5)	41 (36.9)	24 (21.6)	45.5	54.5
	$\chi^2 = 7.76$, $p = 0.02$			$\chi^2 = 5.8$, $p = 0.01$	
	Merged Genotypes				
	Val/Val		Val/Ala + Ala/Ala		
Control Group (n = 100)	35 (35.0)		65 (65.0)		
Patient Group (n = 90)	25 (22.5)		65 (77.5)		
	$\chi^2 = 1.14$, $p = 0.28$		OR (95% CI) = 1.66 (1.13 - 2.42)		

fully repaired, while in others these damages are only partially repaired or not repaired at all. Because of these possibilities, it is important to identify the effects of polymorphic structures in functional DNA repairing genes in cases of lung cancer, in whose etiology of cancerogenic effects of cigarettes is well-known [8].

In this study, we have found that there is not a statistically significant difference in XPD Lys751Gln polymorphism between the patient and healthy control groups. In a review article published in the American Journal of Genetic Epidemiology, the authors examined lung cancer cases among people with different social, ethnic, and racial backgrounds by using the Human Genome Epidemiology (HuGE) Review methodology and found similar results to our study: XPD Lys751Gln polymorphism is not a risk factor [9].

In a study on 352 healthy individuals and 288 patients with cancer diseases in Iran, the authors found that Lys/Gln allele was more prevalent among lung cancer patients, and as a result, it could be a risk factor in lung cancer. However, the statistical difference was marginal (p = 0.047) and the heterozygote difference was not found to be at the same level of risk with homozygote variant alleles [10].

In a study in China, the same polymorphic structure was not found to be a risk factor for patients with lung cancer. Nevertheless, in another study in Northern China, the Gln allele was proposed as a risky allele [11].

In our current study, after the evaluation of the Arg399Gln and Arg194Trp polymorphism of XRCC1 and Thr241Met polymorphism of XRCC3, which are important genes in the BER (base excision repair) mechanism, we have found that there is a significant relationship between Arg399Gln and lung cancer. However, we have not found the other two polymorphisms as risk factors in developing lung cancer.

In their meta-analysis of 18 studies that investigate XRCC1 Arg399Gln polymorphism and 9 case studies that focused on XRCC1 Arg194Trp polymorphism, Kiyohara and his colleagues found that there was a statistically significant relationship between XRCC1 Arg399Gln and lung cancer but XRCC1 Arg194Trp was not related to lung cancer [12]. These findings are in line with our findings. On the other hand, in another meta-analysis of 30 studies on XRCC1 Arg399Gln and 16 studies on XRCC1 Arg194Trp, Wang and his colleagues found that XRCC1 Arg194Trp polymorphism might be a risk factor in lung cancer in addition to XRCC1 Arg399Gln structure [13]. In another meta-analysis by Dai and his colleagues on 39 studies that focus on XRCC1 Arg399Gln polymorphism and 22 studies on XRCC1 Arg194Trp, the author combined all the statistical findings of these studies and found that both gene polymorphisms were significant genetic markers in predicting an individual tendency to lung cancer [15].

There are many meta-analysis studies on Thr241Met polymorphism in the XRCC3 gene, which is an important gene in BER repair mechanism, and their findings are confirming our results. For example, Sun and his colleagues' study analyzes 14 studies [16], Zhan and his colleagues' study evaluated 17 studies [17], and Xu and his colleagues' study examines 17 controlled studies [18], all

these meta-analysis studies, the authors concluded that Thr241Met polymorphism is not a risk factor for lung cancer and it is not a tendency indicator for any cancer type. These findings are also corroborating our results.

Another BER protein in our study was PARP, which detects single sequence fractures in DNAs, binds with the terminal DNA-binding domain identifies the damaged region by increasing poly ADP-ribose activity and causing other repair agents to gather in that region. PARP-1 Val762Ala is an important polymorphism that triggers the repairing of damaged DNA regions. PARP-1 protein provides stability to the genome and plays a highly critical role in the continuity of Genom. This protein maintains a balance between apoptosis and cancerogenesis, particularly through a complex structure composed of proteins such as XRCC1 ve DNA-PK [19] [20].

In a Chinese study on 1000 patients and 1000 healthy individuals, PARP-1 Val762Ala polymorphism was found to be an important risk factor among patients with lung cancer in terms of Ala/Ala genotype. Moreover, Arg399Gln polymorphism in XRCC1 gene was not a significant factor alone. However, in correlation with PARP-1 Val762, it was found to be a risk factor in terms of Ala/Ala genotype [20]. In our current study, we have found that Ala/Ala allele was detected more in the patient group than in the control group.

The region where the XRCC1 Arg399Gln polymorphism exists is important because it is the region where poly-ADP-ribose polymerases (PARP) molecule, which is responsible for detecting DNA fractures, and the XRCCA gene combines to create a complex structure [20] [21] [22] [23]. In our study, these regions are also important because we have found that both of the polymorphisms in these gene regions are risk factors for lung cancer.

Just like a host of variations in DNA other than changes that can be directly related to pathology, such as mutations, studying polymorphisms in repair genes on a limited number of individuals and then coming to definitive conclusions based on this data shall not be a very accurate analysis. In our study, XRCC1 (Arg399Gln) and PARP (Val762Ala) polymorphisms yielded statistically significant differences between the patient and the control groups. Because of the increasing variant alleles in these polymorphisms, these two synchronically functioning genes attracted our attention. In the literature, there are several studies that draw attention to the same gene regions that we have identified as risk factors for patients with lung cancer [13] [14] [15] [20].

Limitations

Our research also has some limitations. Factors other than smoking such as family susceptibility and diet were not evaluated in the study. We believe that further studies with larger samples and following meta-analysis studies to evaluate past research on this topic will be helpful in understanding the effect of these polymorphic structures on lung cancer.

5. Conclusion

In this study, we have investigated the DNA repair gene polymorphisms in the

Turkish population, namely the genes of ADPRT/PARP-1 V762A, ERCC2/XPD Lys751Gln, XRCC1 Arg194Trp, XRCC1 Arg399Gln, XRCC3 Thr241Met, and APE1 Asp148Glu, and their functions in the development of lung cancer. The findings reveal that none of these genes except for XRCC1 (Arg399Gln) and PARP (Val762Ala) constitute a risk factor in lung cancer development in the Turkish population.

Conflicts of Interest

The authors have no conflicts of interest to declare.

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Abbreviations

SNP: Single Nucleotid Polymorphism
DNP: Tandem repeating double nucleotid polymorphism
PCR: Polymerase chain reaction
TAE: Tris Acit, Asetic Acit, and EDTA
PARP: Poly-ADP-ribosis polymerasis