

## Association of IL2-330 Gene Polymorphism with Lung Cancer

IL2-330 Gen Polimorfizmin Akciğer Kanseri ile İlişkinin Araştırılması

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## ABSTRACT

Cytokines are secreted or membrane-bound proteins that like mediators of intercellular signaling to regulate homeostasis of the immune system. They are produced by cells of innate and adaptive immunity in response to microbes and tumor antigens. Although several studies showing that IL2 -330 (rs2069762) gene polymorphism is associated with many types of cancer, as far as we know, a few studies investigating the association between lung cancer and IL2 -330 gene polymorphism. In this study, the role of IL2 -330 gene polymorphism in the pathogenesis of lung cancer was investigated. 96 patients who were diagnosed with lung cancer and 96 age and sex-matched healthy subjects participated in the study. Genomic DNA was isolated using the blood DNA isolation kit and the IL2 -330 gene polymorphism was determined by polymerase chain reaction-confronting two pairs primer method. When analyzed for the lung cancer group and the healthy group according to IL2 -330 gene polymorphism, genotype and allele frequencies were found to be similar in both groups ( $p > 0,05$ ). As a result; there was no statistically significant difference between the groups. Considering the ethnic diversity of lung cancer, the study needs to be verified in other populations.

## ÖZET

Sitokinler, bağışıklık sisteminin homeostazını düzenlemek için hücreler arası sinyalleşmenin aracılığı olarak görev yapan, salgılanan veya zara bağlı proteinlerdir. Mikroplara ve tümör antijenlerine yanıt olarak doğuştan gelen bağışıklığa sahip hücreler tarafından üretilirler. IL2 -330 (rs2069762) gen polimorfizminin birçok kanser türü ile ilişkili olduğunu gösteren birkaç çalışma olmasına rağmen, bildiğimiz kadarıyla akciğer kanseri ile IL2 -330 gen polimorfizmi arasındaki ilişkiyi araştıran az sayıda çalışma vardır. Bu çalışmada IL2 -330 gen polimorfizminin akciğer kanseri patogeneziindeki rolü araştırılmıştır. Çalışmaya akciğer kanseri tanısı almış 96 hasta ile yaş ve cinsiyet bakımından uyumlu 96 sağlıklı, gönüllü birey katıldı. Genomik DNA izolasyon kiti kullanılarak izole edildi ve IL2 -330 gen polimorfizmi polimeraz zincir reaksiyonu ile iki çift primer yöntemiyle karşılaştırılarak belirlendi. Akciğer kanserinde IL2 -330 gen polimorfizmine göre hasta grubu ve sağlıklı grup incelendiğinde genotip ve allel frekanslarının her iki grupta benzer olduğu görüldü ( $p > 0,05$ ). Sonuç olarak gruplar arasında istatistiksel olarak anlamlı bir fark yoktu. Akciğer kanserinin etnik çeşitliliği göz önüne alındığında, çalışmanın diğer popülasyonlarda da doğrulanması gerekmektedir.

## Keywords:

Cytokines  
Interleukine 2  
IL2  
Lung Cancer  
Polimorfizm

## Anahtar Kelimeler:

Sitokin  
İnterlökin 2  
IL2  
Akciğer kanseri  
Polimorfizm

## INTRODUCTION

According to the World Health Organization (WHO) report, lung cancer is the most common cancer type in males worldwide, and the second most common cancer type in females also causes about 1.3 million deaths worldwide each year. The most common cause of lung cancer is long-term exposure to tobacco smoke, and nearly 15% of all lung cancer patients are non-smokers. Therefore, it is known that genetic factors play important role in the pathogenesis of lung cancer (1).

The immune system cells secrete a large number of reporter proteins, which regulate the division of the host cell and are involved in innate and acquired immune responses. These reporters are called cytokines (2). IL2, a potent immune regulatory cytokine involved in cell-mediated immune response, is produced by T cells when it is activated by mitogens, or by the interaction of antigen

with major histocompatibility complexes and also like as a T-cell growth factor (3).

The immune system is organized such that it does not respond to our antigens (4). However, as cancer cells acquire many mutations and changes (5), they express tumor-specific antigens with sporadic mutations, thereby activating the immune system, eventually leading to the killing of cancer cells. Thus, the immune system can prevent the formation of primary tumors (6).

However, mutational changes occurred as cancer cells continued to divide, accumulating either by chance or in response to immune-induced inflammation. Also, due to genetic instability, fixed tumor cell division can produce low immunogenicity that can escape immune elimination (7).

The human IL2 gene is localized on chromosome 4q26. In the human IL2 gene, two major polymorphisms have

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been identified: one is located in the promoter region at nucleotide -330 (8) and the other in the first exon at position +114 (9). G -330T (rs2069762), identified upstream of the IL2 promoter-enhancer domain, reportedly affects protein production. Individuals that were homozygous for the G allele (G/G) in the promoter region of the IL-2 gene produced more than three times more IL-2 than individuals that G/T and T/T genotypes (10).

We hypothesized that this SNP may be associated with lung cancer risk, as IL2 plays important role in the regulation of immune response and the process of inflammation-mediated carcinogenesis, and also because of its functional association with IL2-330 T/G. To test this hypothesis, we performed genotyping analyzes for this SNP in a case-control study of lung cancer patients and healthy controls in a Turkish population.

#### METHOD

##### Study Population

The case-control group consisted of 96 patients diagnosed with lung cancer in Zonguldak Bülent Ecevit University Medical Oncology Department and 96 healthy individuals. The patient group was diagnosed with chest X-ray, sputum cytology, and computed tomography imaging tests. This study was approved by the Zonguldak Bulent Ecevit University Clinical Research Ethics Committee (2017-05-11 / 01) in 2017, and written informed consent was obtained from each participant.

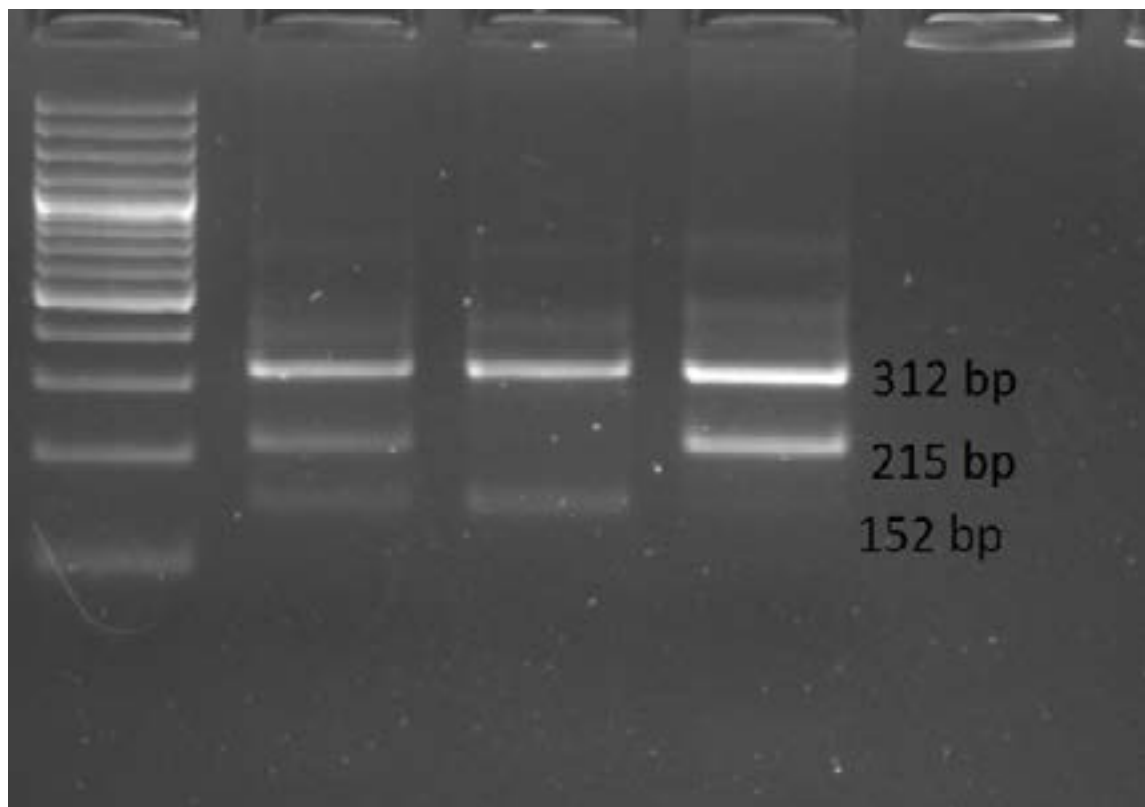
##### DNA Isolation and Genotyping

Genomic DNA was extracted from 200 microliter of peripheral blood using the E.Z.N.A Blood DNA isolation

Kit (Omega Bio-Tek, Norcross, GA, USA) following the manufacturer's instructions. The polymorphism in the -330 region of the IL2 gene was analyzed by the Confronting two pairs primer method (8). The PCR mix contains 20 pmol from each of the primers (Table 1), 125 mM dNTP, 125 mM Taq DNA buffer, 125 mM MgCl<sub>2</sub>, 15 mM Taq DNA Polymerase in a final volume of 25 µl. PCR reaction conditions; 1 cycle 95 ° C for 5 minutes, then 35 cycles of 95 ° C for 1 minute and 57 ° C for 1 minute, 72 ° C for 1 minute followed 1 cycle 72 ° C for 7 minutes. PCR products were visualized on 3% agarose gel electrophoresis. For the G allele, a band of 152 bp was obtained and for the T allele, a band of 215 bp was obtained. A 312 bp band is common in all samples (Figure 1).

**Table 1:** Confronting two pairs primer sequence

Primers	Sequence
F1	5'-CTG ACA TGT AAG AAG CAA TCT AT-3'
R1	5'-CTC AGA AAA TTT TCT TTG TCC-3'
F2	5'-TTC ACA TGT TCA GTG TAG TTT TAT-3'
R2	5'- TGT TAC ATT AGC CCA CAC TTA-3'



**Figure 1:** Genotyping by electrophoresis: (right to left) GG, TT, and GT genotype. The first well is a 100bp DNA ladder. Base pairs are indicated in the figure.

### Statistical Analysis

A case-control study was performed and the allelic frequency of the polymorphism was calculated in both cases and controls. Deviations from Hardy-Weinberg equilibrium were evaluated by comparing observed and expected genotype frequencies. The  $\chi^2$  test was used to compare the genotype frequency of IL2 gene polymorphism in lung cancer patients and controls. The odds ratio (OR) and 95% confidence interval (CI) were calculated to compare the lung cancer risk for the alleles. p values <0.05 were considered to indicate statistical significance. The SPSS software was used (ver. 18.0; SPSS Inc., Chicago, IL).

### RESULTS

The mean age of the lung cancer patient group was 43.26±10.54, the mean age of the control group was 45.8±10.62. The patient and control groups were compared in terms of gender. There was no statistically significant difference was found between the groups (p>0.05) (Table 2).

**Table 2:** Demographic features of patients and control group

	Control	Patient	p-value
<b>Age (years, Mean±SD)</b>	45.8±10.62	43.26±10.54	0.097
<b>Sex</b>			
<b>Male</b>	89 (50.3%)	88 (49.7%)	1.0
<b>Female</b>	7 (46.7%)	8 (53.3%)	

**Table 3:** Association between lung cancer and IL2 -330 T/G polymorphism according to genotype frequency

IL2 -330	Case n (%)	Control n (%)	Total n (%)	p	OR (95% CI)
<b>TT</b>	21 (21.9%)	17 (17.7%)	38 (19.8%)	0.100	Reference
<b>GT</b>	65 (67.7%)	76 (79.2%)	141 (73.4%)	0.317	0.692 (0.337-1.423)
<b>GG</b>	10 (10.4%)	3 (3.1%)	13 (6.8%)	0.177	2.698 (0.639-11.389)

**Table 4:** Association between lung cancer and IL2 -330 T/G rs2069762 polymorphism of the allele frequency

IL2 -330	Case n (%)	Control n (%)	Total n (%)	p	OR 95% CI
<b>T</b>	107 (55.7%)	110 (57.3%)	217 (56.5%)	0.757	Reference
<b>G</b>	185 (44.3%)	82 (42.7%)	167 (43.5%)	0.837	1.066 (0.712-1.59)
<b>Total</b>	292 (100%)	192 (100%)	384 (100%)	0.837	

Similarly, with our results, the study did not find any correlation between genotype and lung cancer risk for the IL2 rs2069762 and rs2069763 polymorphisms (13).

In several studies, genetic polymorphisms of the IL2 -330 T/G have been implicated in the susceptibility to a range of inflammatory diseases and cancer, including gastric atrophy from *Helicobacter pylori* infection, myelogenous leukemia (14, 15, 16, 17), gastric cancer (18), lung cancer (19), and breast cancer (20). However, other studies have shown that IL2 -330T / G polymorphism is not associated with gastric cancer or cutaneous malignant melanoma. The first meta-analysis which provided comprehensive information of the association on IL2 -330 T/G polymorphism and cancer risk was performed by

In this study, the association between lung cancer and IL2 -330 (rs2069762) polymorphism was investigated and it was found that the frequencies of TT, GT, and GG genotypes were 21.9%, 67.7%, and 10.4% in the patient group and were 17.7%, 79.2% and 3.1% in the control group, respectively. There was no statistically significant difference between these two groups (Table 3).

For IL2 -330 T / G polymorphism, T allele frequencies were 55.7% in lung cancer patients and 56.5% in the control group. G allele frequency was determined as 44.3% in lung cancer patients and 43.5% in the controls (Table 4). There was no statistically significant difference between the groups.

### DISCUSSION

Cytokines are small glycoproteins that bind to cell surface receptors and regulate the development, survival, and function of immune cells (11). IL2 is one of the key cytokines with pleiotropic effects on the immune system. This immunoregulatory cytokine IL2 plays an important role in the antitumor immunoreactivity of T cells, and the polymorphism in the -330 promoter region is the most investigated (12). However, in the present case-control study, our results indicated that there was no association between IL2 -330 rs2069762 gene polymorphism and lung cancer. Few reports regarding the association between the IL2 gene polymorphism and lung cancer have been published. A case-control study by Eaton KD et al., it was investigated the role of genetic polymorphisms in inflammation-related genes in the risk for development of lung cancer in 625 cases and 625 matched controls.

Hongyu Zhao et al. and this study showed that IL2 -330 T/G polymorphism is strongly associated with lymphoma risk, whereas the association between gastric cancer and IL2 -330 T/G polymorphism is not significant (21).

Song N. et al. investigated the genetic susceptibility of childhood lymphoma and reported that sixteen SNPs in six genes (IL1RN, IL2, IL12RB1, JAK3, TNFRSF13B, and XRCC3) were significantly associated with the risk of lymphoma (p<0.05). The most important association was found in the IL2 (-330 T/G) polymorphism (22). Also, Torres-Mejía G. et al. (2012) showed that IL2 and IL2RA were associated with the risk of breast cancer (4).

In addition, Wei YS et. al. investigated whether the IL2 gene polymorphism and serum levels were related to

nasopharyngeal carcinoma in the Chinese population. It was reported that there was a significant difference in the IL2 -330 T/G polymorphism genotype distributions between patients with nasopharyngeal carcinoma and the control group ( $p < 0.005$ ). Moreover, there was a decrease in serum IL2 levels when compared with the wild-type allele in patients with nasopharyngeal carcinoma carrying the IL2 -330 G variant allele (23).

There are some limitations to this study. First, we did not adjust other compounding variables for risk factors of lung cancer, such as tobacco smoke, environmental

factors, and family history. Second, the number of samples was relatively small for the investigation of genetic polymorphisms. Further studies with large samples and including environmental, other host genetic factors will be needed.

#### CONCLUSION

Our study is the first study investigating the association between lung cancer and IL2 rs2069762 polymorphism. Because lung cancer shows ethnic variation, repeating the study with more samples in different populations will contribute to the understanding of the relationship.

**Ethics:** This study was also approved by the Zonguldak Bulent Ecevit University Clinical Research Ethics Committee (2017-05-11/01) in 2017 and written informed consent was obtained from each participant.

**Conflict of Interest:** No conflict of interest was declared by the authors.

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