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## **MYD88 Gene Polimorphism in Patients With Chronic Lenfoid Leukemia**

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**Abstract:** Leukemia is a type of cancer that manifests itself with the excessive proliferation of blood cells, especially white blood cells, and affects the blood production system in the body. Leukemias are classified as acute or chronic and according to the extent and development of the tumor. In chronic lymphocyte leukemia (CLL), cancer cells are found in the bone marrow, blood, and lymph nodes. Therefore, in this study, the effects of polymorphisms in the MYD88 gene of CLL patients were investigated. For this purpose, 100 patients diagnosed with CLL and 70 healthy individuals without cancer history were included in the study as the control group. DNA was isolated from CLL patients and control groups. Polymorphisms in the MYD88 (L265p T/C) promoter regions were studied by RT-PCR. Genotype and allele frequencies were calculated directly. Statistical significance of genotypic distributions between CLL patients and control groups was evaluated with Z Ratios test and Fisher's Exact test. For the MYD88 L265p T/C region in 100 CLL patients, 95 wild type TT, 3 TC and 5 mutant type CC genotypes were detected. No statistically significant difference was found between CLL patients and control groups in the MYD88 L265p T/C region ( $p > 0.05$ ). According to the allele frequencies of the region, it was determined as 0.965 and 0.065 for T and C, respectively. According to Fisher's Exact results, it was calculated as 0.078 for TT, 0.078 for TC and 0.269 for Mutant, respectively. The MYD88 genotype frequencies of the patients included in the study do not appear to be statistically correlated with CLL patients. The research we have done as a result of our study will shed light on the studies with different SNPs to be made with the increase in the number of CLL patients..

**Keywords:** KLL, MYD88, Polymorphism, Real-Time PCR

### **Introduction**

Leukemia is a type of cancer that manifests itself with the excessive proliferation of blood cells, especially white blood cells, and affects the blood production system in the body. Leukemias are classified as acute or chronic and according to the extent and development of the tumor. In chronic lymphocyte leukemia (CLL), cancer cells are found in the bone marrow, blood, and lymph nodes. (Montserrat et al., 2008).

Polymorphisms in the structure of cytokines that play a role in cell proliferation, differentiation, apoptosis mechanisms and immune system regulation are another important genetic factor in the emergence of CLL. (Caporaso et al., 2007). MYD88 variations affect 2-5% of CLL patients. Interleukins are important immune

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system signaling molecules in the class of cytokines. The role of interleukin 10 polymorphism in cancer formation is remarkable. According to some data, polymorphisms in the 1082 A/G promoter region cause changes in the expression of the gene and promote the development of some malignant tumors, including non-Hodgkin's lymphoma. (Cunningham et al., 2003; Kang et al., 2010). Variations of MYD88 cause the activation of the Nuclear factor kappa B (NF- $\kappa$ B) pathway, thus allowing proliferation and survival of variant CLL cells. (Alsagaby et al., 2016).

In one study MYD88 CLL patients with gene variation are seen in a younger population and overall survival is better than those without variation (Martínez-Trillos et al., 2014). In another study MYD88 It is stated that although patients with gene variation are diagnosed at a younger age than patients without variation and in a more advanced clinical stage, no difference was observed between these two groups in terms of disease progression and survival rates. (Puente et al., 2011). In the study of Martínez-Trillos et al. for CLL patient groups MYD88 It is stated that variations in genes have prognostic importance. (Martínez-Trillos et al., 2014; Mitsui et al., 2016). In the study conducted by Xia et al., it is stated that these variations do not show a significant difference in terms of prognosis and overall survival time compared to the cases without variation. (Dai et al., 2014).

Several studies have analyzed clinical outcomes in lymphoma patients found to have MyD88 mutations: in WM, somatic mutations in MyD88 have been found to be important predictors of clinical presentation and may also be associated with overall survival. In the absence of MyD88 L265P, patients with WM are more likely to be female, with splenomegaly and express CD27, a member of the tumor necrosis factor (TNF) receptor family that may be involved in B-cell activation and memory (Mori et al., 2013; Poulain et al., 2013). Patients with MyD88 wild type (MyD88WT) have also been shown to be older (Treon et al., 2014), however, the effect of MyD88 mutation status on survival is controversial. Italy (Varettoni et al., 2013) and from India (Patkar et al., 2015) Independent studies have shown that patients with the MyD88 L265P mutation are more likely to have a greater burden of disease and a higher risk of disease progression. In addition, it has been determined that the probability of developing progressive disease after treatment is higher. In contrast, a study from the USA reported that MyD88WT patients had a significantly higher mortality from progressive disease at follow-up. (Treon et al., 2014). Therefore, in our study MYD88 It was aimed to investigate the effects of variations in genes on the prognosis and overall survival of CLL disease.

## **MATERIALS AND METHODS**

### **Material**

Genotyping will be done by determining the polymorphic distributions of some regions of the gene MYD88, which has not been studied before, in chronic lymphoid leukemia (CLL) patients. A group of 100 patients over the age of 18 who were diagnosed with Chronic Lymphoid Leukemia, who applied to the Hematology Department of Sanko University Research and Application Hospital between 2018 and 2020, and 70 healthy individuals over the age of 18 without any cancer history were included as the control group. This study was carried out in Gaziantep University biology department laboratory. When the study process started, manual DNA isolation was performed using the salt precipitation method for the control group and patient group blood samples (Carpi et al., 2011). Quantification and purity of DNA samples were determined by taking readings at 260 and 280 nm of the diluted samples in a UV spectrophotometer. Absorption values measured at 260 nm (A260) show the total nucleic acid amount and absorption values measured at 280 nm (A280) show the total protein amount. The purity of the DNA sample was determined by calculating the ratio (OD value) (A260/A280) between the values read at 260 and 280 nm (Sambrook et al., 1989).

## **RESULTS AND DISCUSSION**

The gender distribution and age distribution of the patient and control groups in the study are given in the Table. Patient and control group DNA samples were studied by Real Time PCR method.

### **Evaluation of L265p T/C Polymorphism Results**

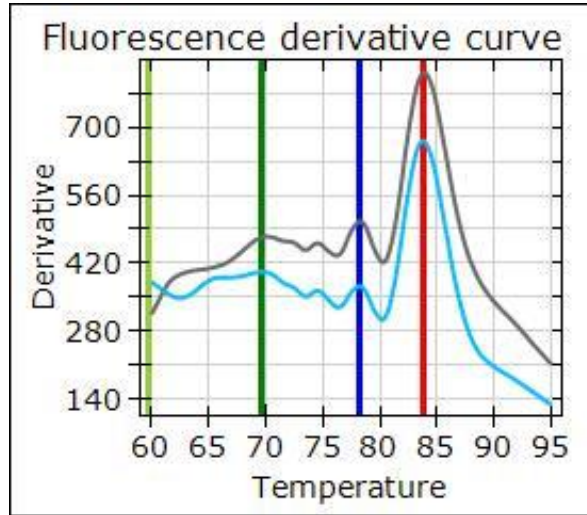


Figure 1. Homozygous-Heterozygous Analysis is shown.

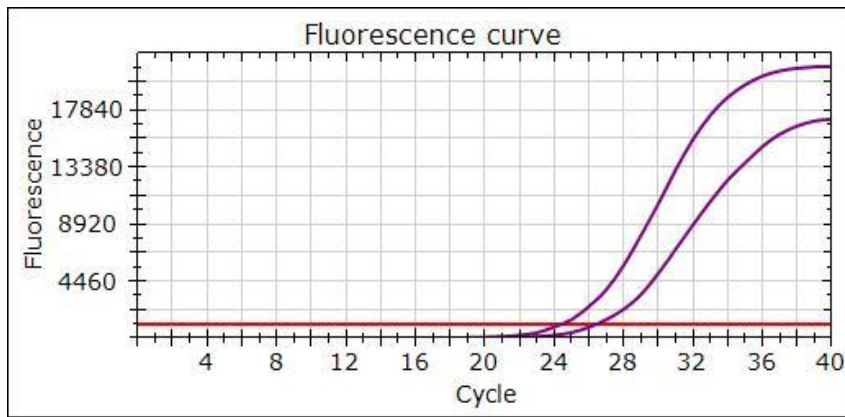


Figure 2. Parameters of the Homozygous-Heterozygous Analysis are shown.

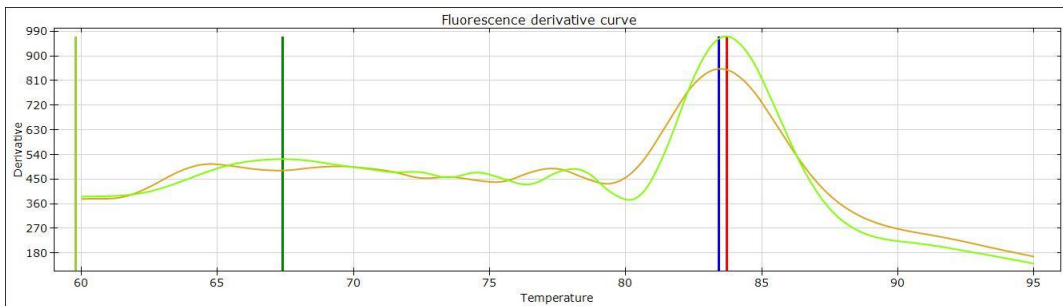


Figure 3. The parameters of the Wild Type- Mutant Analysis are shown.

### TT Wild Type Results

Table1. Results of TT wild type

TT Wild Type	Patient		Healthy	
	N	%	N	%
No	5	5.0	0	0.0
Yes	95	95.0	70	100.0

### CC Mutant Results

Table2. Results of CC mutant type

CC Mutant	Patient		Healthy	
	N	%	N	%
No	95	95.0	70	100.0
Yes	5	5.0	0	0.0

### TC Heterizygot Results

Table5. Results of TC heterizygot type

TC Heterizygot	Patient		Healthy	
	N	%	N	%
No	97	97.0	70	100.0
Yes	3	3.0	0	0.0

### L365p T/C Real Time PCR Analysis

SPSS package program was used to analyze the data. A z-ratio test was used to compare L265p polymorphism rates in healthy and patient numbers, and Fisher's Exact was used to compare frequencies. The study included 100 patients diagnosed and followed up in 2018-2020 and 70 healthy adult individuals voluntarily. The mean age of CLL patients who voluntarily participated in the study was 63 (31-88) and the mean age of healthy control group individuals was 46 (18-85).

### Discussion

B-cell CLL disease is the most common type of leukemia in Western countries and in individuals over 50 years of age. The disease is characterized by the accumulation of mature-appearing monoclonal B cells in peripheral blood, bone marrow, and lymphoid organs. (Rodriguez-Vicente et al., 2013). At the same time, CLL disease is not characterized by excessive proliferation of B-CLL cells, but by accumulation of cells as a result of the cessation of the apoptosis mechanism in the G0/G1 stage. (Kitada et al., 1998). Clinical heterogeneity is the hallmark of CLL, with some patients surviving for years without the need for treatment, while others show early progression and short survival if left untreated. (Bagacean et al., 2017). Because CLL greatly alters the clinical course, staging systems have been studied to categorize patients into different risk groups to predict survival. Rai and Binet staging systems are generally used in staging. Both systems consist of parameters obtained by clinical examination and standard laboratory tests.

The etiology of CLL is still unknown. In researches for the etiology of CLL disease, the relationship between exposure to chemicals and radiation, environmental factors and CLL has not been fully determined. (Blair et al., 2007). The lower frequency of CLL in individuals of Eastern origin and a higher incidence in family members than other mature B-cell neoplasms (5-10%) reflect the importance of the genetic factor. (Rodrigues et al., 2016). In studies conducted with the development of NGS technique in recent years, MYD88 genes are stated among the most recurrent somatic variations in CLL. (Guièze et al., 2015).

MYD88 gene plays an important role in the innate and adaptive immune response. is gene. MYD88It acts as an important signal converter in TLR and IL-1R pathways. gets (Amaya-Chanaga & Rassenti, 2016). MYD88 L265P missense variation in CLL patients although it is seen with a low frequency, it is known as one of the most common variations. (Rossi et al., 2013). These variations have been IGHV (Mutational clonotypic immunoglobulin heavy variant), is associated with low levels of ZAP-70 and CD38 expression and normal levels of  $\beta$ 2M and occurs in younger patients regardless of clinical stage. (Amaya-Chanaga & Rassenti, 2016). In a study by Puente et al. MYD88 It is stated that L265P gene variation has no effect on advanced clinical stage and overall survival. (Puente et al., 2011). MYD88 variations, also wild type MYD88 in younger patients and all variations IGHV observed in cases with variation. In a study of samples from 587 CLL patients, MYD88 L265P was noted as the most common variation. MYD88 variations, often undergoing variation IGHV gene, del (13q), CD38 and ZAP-70 factors were observed in cases with low expression. In this study MYD88 patients with variation were significantly younger than patients without variation, and MYD88 variation represents a population with positive outcomes in young CLL patients. (Martínez-Trillos et al., 2014).

## CONCLUSION

In our study, patients with CLL MYD88 the p.L265P variation was not detected. The reason for this is the insufficient number of patients. Therefore, no statistically significant result was obtained in our study. With this study we have done, among CLL patients who applied to Sanko University Research and Application Hospital Hematology Polyclinic. MYD88 We aimed to elucidate how often the genes change, and their relationship with treatment and overall survival. We examined the variational status of MYD 88 genes, as a result of our study, only five patients which has been L265p mutant. Therefore, it is thought that the possible relationship between L265p rs387907272 T/C SNPs and CLL can be statistically clarified in future studies that will be planned by increasing the number of samples.

## RECOMMENDATIONS

This study can be considered as a starting point for studies to be designed to increase the number of patients with CLL and to examine different polymorphisms.

## Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the authors.

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