



## ARAŞTIRMA / RESEARCH

# Relationship between interleukin-2 promoter polymorphism and preeclampsia

İnterlökin-2 promotörü polimorfizmi ile preeklampsi arasındaki ilişki

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*Cukurova Medical Journal 2017;42(4):730-734*

### Abstract

**Purpose:** One of the cytokines involved in the immune system is interleukin-2 (IL-2). It is contemplated that polymorphisms in the promoter region of the IL-2 gene may be one of the determining factors in the maternal immunization system associated with abnormal placentation resulting in preeclampsia. This study tried to determine the protective genotypes for preeclampsia by genotyping the -384 polymorphisms in the promoter region of interleukin 2 gene in preeclamptic Turkish women.

**Material and Methods:** 146 blood samples, 57 normal and 89 preeclampsia, were collected from unrelated pregnant women. -384 promoter region of IL-2 gene is amplified by using PCR and Restriction Fragment Length Polymorphism method is applied by using Bfa-1 enzyme to identify the polymorphisms.

**Results:** Of the 89 preeclamptic women included in the study, 38 had TT, 36 had GT, 15 had GG alleles and of the 57 normal pregnant women, 28 had TT, 22 had GT, and 7 had GG alleles.

**Conclusion:** Considering the close relationship of preeclampsia with the immune system and the response to inflammation, this study aimed to investigate the relationship between IL-2 gene -384 promoter region polymorphism and preeclampsia. No statistically significant relationship was found between the genotypes. We think that comprehensive genetic studies including all negative factors that are effective for placenta formation would be useful.

**Key words:** Preeclampsia, interleukin-2, RFLP, polymorphism.

### Öz

**Amaç:** İmmün sistemde rol alan sitokinlerden biri de interlökin-2'dir. IL-2 geninin promotör bölgesindeki polimorfizmlerin preeklampsi ile sonuçlanan anormal plasentasyon ile ilişkilendirilen maternal savunma mekanizmasındaki belirleyici faktörlerden biri olabileceği ihtimali düşünülmektedir. Bu çalışmada preeklampşik Türk kadınlarında interlökin 2 geninin promotör bölgesindeki -384 polimorfizmi çalışılarak genotiplendirme yapılmış ve hangi genotipin koruyucu olduğu belirlenmeye çalışılmıştır.

**Gereç ve Yöntem:** 57 normal, 89 preeklampsi olmak üzere toplam 146 birbiri ile akraba olmayan kadından kan alındı. IL-2 geninin -384 promotör bölgesi PCR yöntemi ile amplifiye edildi ve polimorfizmlerin tespiti için Restriction Fragment Length Polymorphism yöntemi Bfa-1 enzimi kullanılarak gerçekleştirildi.

**Bulgular:** Çalışmaya dahil edilen 89 preeklampşik kadından 38'inin TT, 36'sının GT, 15'inin GG; 57 normal gebe kadından 28'inin TT, 22'sinin GT ve 7'sinin GG alleleline sahip olduğu tespit edilmiştir.

**Sonuç:** Preeklampsi hastalığının immün sistem ve inflamasyon cevabıyla olan yakın ilişkisi göz önünde bulundurularak, bu çalışmada IL-2 geni -384 promotör bölgesi polimorfizmi ile preeklampsi ilişkisinin araştırılması amaçlanmıştır. Genotipler arasında istatistiksel olarak anlamlı bir fark bulunamamıştır. Plasenta oluşumuna etkili olan tüm olumsuz faktörlerin dâhil edildiği genetik tabanlı kapsamlı araştırmaların yararlı olacağını düşünmekteyiz.

**Anahtar kelimeler:** Preeklampsi, interlökin-2, RFLP, polimorfizm.

## INTRODUCTION

Preeclampsia, seen in 3-5% of all pregnancy, is a disease which results from pregnancy and progresses with proteinuria and oedema as well as hypertension. It is one of the most important causes of perinatal mortality and morbidity and even maternal mortality<sup>1</sup>. It emerges after the 20th week of your pregnancy and disappears after birth. Because of this feature, the most appropriate treatment is delivery<sup>2-4</sup>.

The etiology and pathogenesis of preeclampsia has not yet been fully elucidated. In recent years, important advances have been made in understanding the pathophysiology of the disease by drawing attention to other terms such as protein in urine instead of high blood pressure. It is known that placental and maternal factors are involved in the preeclampsia pathophysiology. It is thought that placental and maternal factors such as vascular endothelial dysfunction and chronic hypertension play an important role in preeclampsia. It is considered that the balance between placental and maternal factors causing preeclampsia is diverse in different pregnancies. Oxidative stress is an important cause of preeclampsia pathophysiology, which is present in both placental and maternal factors. In addition, recent studies have focused on immunological and genetic factors, and these factors are thought to be the leading causes behind the formation of preeclampsia. Genetic factors are effective in almost all human diseases and the understanding of the role of genetic factors in disease provides an understanding of non-genetic, environmental factors. Despite the obstacles that arise in the genetic study of preeclampsia, significant improvements are made in this area. Research for understanding the genetic basis of preeclampsia has focused on family studies, twin studies, effects of fetal genetic aberration, paternal genotype contribution, changes in pregnancy interval and molecular studies<sup>3</sup>.

To investigate the genetic factors behind the formation of preeclampsia, more than 50 genes have been investigated so far and it has been determined that 8 of them may be candidate genes<sup>5</sup>. Previous studies have identified a link between preeclampsia and changes in these genes, but the results are contradictory and there is no definite conclusion in

the studies of different polymorphism types in various regions within these candidate genes<sup>6</sup>.

A successful pregnancy depends on the mother's genetically stable immune system. One of the cytokines involved in the immune system is interleukin-2 (IL-2). It is contemplated that polymorphisms in the promoter region of the IL-2 gene may be one of the determining factors in the maternal immunization system associated with abnormal placentation resulting in preeclampsia. In this context, the polymorphisms in the promoter region of IL genes were examined in different populations and different results were obtained regarding their relation with preeclampsia<sup>7,8</sup>.

The polymorphism of the IL-2 -384 promoter region for preeclampsia has not yet been studied in the Turkish population. Preeclampsia is associated with polymorphisms related to various genes such as Tumor Necrosis Factor alpha (TNF $\alpha$ )<sup>9,10</sup>. The IL-2 gene has been studied in various diseases<sup>11,12</sup>. In a study conducted with the TNF $\alpha$  gene in the Turkish population, promoter regions of this gene was genotyped at positions G-308A and C-850 and it was stated that AA genotype at 308 position was higher in preeclamptic patients and TT genotype at 850 position was lower in preeclamptic patients<sup>10</sup>. These results suggest that the 308AA genotype increases the risk for preeclampsia and the 850TT genotype is protective.

In a study conducted with the microsomal epoxide hydrolase gene in the Dutch population, homozygous Tyrosine 113 genotype showed high enzyme activity and was associated with preeclampsia<sup>13</sup>.

This study tried to determine the protective genotypes for preeclampsia by genotyping the -384 polymorphisms in the promoter region of interleukin 2 gene in preeclamptic Turkish women. Thus, authors aimed to fill the gap in the literature and to reveal the genotype distribution in Turkish population.

## MATERIAL AND METHODS

### Blood sample collection and DNA isolation

The study groups were formed from the patients diagnosed with preeclampsia who were followed in Çukurova University Faculty of Medicine Department of Obstetrics and Gynecology and

normal pregnant women. Total of 146 pregnant women, 57 normal and 89 preeclamptic women, were investigated. All women participating in the study were measured for systolic and diastolic blood pressures and protein levels in the urine. Age, weight, number of pregnancies, gestational week, chronic hypertensive disease cases of all subjects were questioned. Patients with gestational hypertension and superimposed preeclampsia were not included in the study. Blood samples were collected from subjects and DNA was obtained from the blood samples by salt precipitation. The study was approved by the Cukurova University Ethical Committee and all participants were informed about the study and signed patient consent form.

#### DNA amplification and PCR

PCR reactions for amplification of the IL-2 gene -384 promoter region was prepared by using; 2.5 µl (10X) PCR buffer, 0.60 µl dNTP (10 mM), 0.75 µl Taq Polymerase (5U/ µl), 1 µl Forward primer (10 pmol) (5'-ATTCACATGTTTCAGTGTAGTTCT-3'), 1 µl Reverse primer (10 pmol) (5'-GTGATAGCTCTAATTCATGC -3'), 3 µl DNA (100-200 ng) and 16.15 µl distilled water. The denaturation, annealing and extension steps were performed for 35 cycles at 94 ° C (20 sec), 52 ° C (40 sec) and 72 ° C (20 sec), respectively, after 2 min. of initial denaturation of the prepared reaction tubes at 94 ° C. Finally, amplification was completed by applying a 10 min extension at 72 ° C.

#### Application of RFLP (restriction fragment length polymorphism) method

The PCR amplified 131-bp region of the -384 promoter region of the IL-2 gene was cut by the bfa-1 restriction enzyme using the RFLP method. The Bfa-1 enzyme is a type II enzyme that cuts in the presence of the normal genotype in the IL-2 -384 promoter region. The primers were designed accordingly. DNA cut with Bfa-1 enzyme was expected to be displayed in two fragments of 110 and 21 bp in Homozygous GG genotyped individuals, in three fragments of 131, 110 and 21 bp in heterozygous GT genotyped individuals and in one fragment of 131 bp in homozygous TT genotyped individuals.

The reaction tubes prepared by using 2.0 µl buffer, 0.5 µl of restriction enzyme (Bfa-1), 12.5 µl of PCR product and 5 µl of distilled water were allowed to

stand for 17 hours at 37 ° C for the cleavage reaction. After the cleavage process, 5 µl of Bromphenol blue (6X) was added to the products and electrophoresis was performed on 2% agarose gel and the results were obtained.

#### Statistical analysis

Genotype distributions and allele frequencies of the patient and control groups were determined by using Pearson's Chi square test and the results were analyzed at  $p < 0.05$  confidence interval.

### RESULTS

Of the 89 preeclamptic women included in the study, 38 had TT, 36 had GT, 15 had GG alleles and of the 57 normal pregnant women, 28 had TT, 22 had GT, and 7 had GG alleles. The genotype distributions and allele frequencies of the -384 promoter region of the IL-2 gene were analyzed using the Chi-Square test. In the IL-2 gene -384 promoter region, the frequency of the patient group with the TT allele was 42.7% and the frequency of the control group with the TT allele was 49.1%. No statistically significant relationship was found between the groups. The T allele frequency was determined as 62.9% in the patient group and 67.8% in the control group, but there was no statistically significant relationship between them (Table 1).

**Table 1. Genotype distribution of polymorphisms**

N(%)	Patient (n=89)	Control (n=57)	p
GG	15 (16.9)	7 (12.3)	0.660
GT	36 (40.4)	22 (38.6)	
TT	38 (42.7)	28 (29.1)	

In the patient group, frequency of GG allele was 16.9% and this rate was 12.3% in the control group, and statistically no significant relationship was found between the groups. The G allele frequency was determined as 37.1% in the patient group and 32.2% in the control group, but there was no significant relationship between them. As a result, no statistically significant relationship was found between T and G alleles and preeclampsia (Table 1).

### DISCUSSION

It has been determined that preeclampsia affects approximately 5% and 8% of pregnant women on a global scale and this ratio does not differ among different populations<sup>14</sup>. Risk factors for

preeclampsia include; chronic hypertension, renal disease, diabetes, obesity, placental hypoxia and low placenta arterial pressure<sup>15-17</sup>.

Messengers such as Interleukin, Interferon, TNF, and Chemokine, which play a role in communication between cells of the immune system, are members of the cytokine group. IL-2, which is a cytokine released by macrophages and T lymphocytes, providing specific interaction between leukocytes, is produced by active T-lymphocytes and serves to antigen presentation by macrophages at the time of infection, providing both cellular and humoral response modulation<sup>18</sup>.

Considering the close relationship of preeclampsia with the immune system and the response to inflammation, a number of studies based on cytokine-encoding genes, especially polymorphisms of these genes, have been performed. Some of these studies are; TNF-alpha (-308 GA), IL-6 promoter (-174 GC), interferon gamma intron-1 (674 AT), IL-10 promoter (-1082 AG), TGF Beta-1 codon 10 (869 TC) and codon 25 (915 GC) mutations and/or polymorphisms<sup>19-21</sup>. Genotype distributions and allele frequencies of the -384 promoter region of the IL-2 gene were analyzed using the Chi-Square test. There was no statistically significant difference between genotypes of patient and control groups ( $p = 0.660$ ).

In 2013, in meta-analysis related to preeclampsia it is evaluated 2965 articles. 542 of these articles addressed the association between genetics and preeclampsia and 163 of these articles emphasized 22 polymorphisms related to 15 genes. According to the evaluations made, the relationship between polymorphisms and preeclampsia was reported only in the promoter region of the IL-10 gene, (1082A-G), (819C-T) and (592C-A)<sup>19,22,23</sup>. However, since the candidate genes identified in the linkage and association studies have been shown to give conflicting results in different studies, no gene has been identified that is precisely associated with preeclampsia<sup>5,6,24-26</sup>. These candidate genes take role in oxidative stress (EPHX, GST), lipid metabolism (LPL, ApoE), thrombophilia (MTHFR), endothelial injury (AGTR1, AGTR2) and immunogenetics (HLA-G) mostly<sup>27</sup>.

Preeclampsia disease is a multifactorial disease, but it also suggests imprinting, which is based on ethnicity difference but does not constitute reproductive barriers. The only approach to combine the most

accepted immunological and genetic association of preeclampsia is the imprinting structure and the matching of the related genes between the couples<sup>28</sup>.

Considering the main causes of preeclampsia are immune system, abnormal immune tolerance of the mother during pregnancy, HLA incompatibility between partners and imprinting, we think that comprehensive genetic studies including all negative factors that are effective for placenta formation would be useful.

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