

INVESTIGATION OF THE FREQUENCY OF CRP GENE POLYMORPHISM IN RHEUMATOID ARTHRITIS AND ASSOCIATIONS WITH CRP LEVEL AND CLINICAL INVOLVEMENTS OF THE DISEASE

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ABSTRACT

Introduction: The aim of this study was to compare the frequency of rs1205, rs1130864 and rs1800947 single nucleotide polymorphisms (SNPs) in the C-reactive protein (CRP) gene in rheumatoid arthritis (RA) patients and healthy controls. In addition, the aim was to investigate the association between these polymorphisms and serum CRP levels, Disease Activity Score-28 (DAS28) and clinical findings, especially extra-articular involvement such as interstitial lung disease. **Methods:** This single-center study included 120 patients with RA and 100 healthy controls. DAS28-CRP was used to measure disease activity. Interstitial lung disease was confirmed by ground-glass opacities and interstitial thickening on chest computed tomography (CT) and other CT findings. Peripheral blood samples were collected in tubes with EDTA for genotyping. After DNA extraction, SNPs were analyzed using TaqMan probe-based polymerase chain reaction. **Results:** Among the three CRP SNPs (rs1130864, rs1205, rs1800947), a significant difference was observed in the distribution of rs1130864 between RA patients and controls. TT homozygous and CT heterozygous genotypes were significantly lower in the patient group ($p = 0.002$). Furthermore, the TT genotype of rs1130864 was significantly associated with lung involvement in patients with RA ($p = 0.017$). No significant association was found between SNPs and baseline CRP levels and DAS28 scores. **Conclusion:** In our study, we found a significant association between CRP rs1130864 gene polymorphism and lung involvement in RA patients. To the best of our knowledge, this is the first

study to evaluate this association. However, further studies with larger cohorts are needed to confirm these findings.

Key words: C-reactive protein, Genetic polymorphism, Inflammation, Interstitial lung disease, Rheumatoid arthritis

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune multisystem inflammatory disease of unknown etiology that targets synovial tissues. C-reactive protein (CRP) is encoded by the CRP gene located on chromosome 1q23. Single nucleotide polymorphisms (SNPs) in the CRP gene and haplotypes have been associated with variations in CRP levels during active inflammation [1]. Among these, the rs1205 (C>T) variant has shown the most consistent association with CRP levels. Other polymorphisms in the CRP gene include rs3093077, rs1130864, rs3093058, rs1800947, and rs3091244 [2].

Although the etiopathogenesis is not fully understood, multifactorial causes (environmental, genetic, etc.) are thought to play a role in the development of RA, as is the case with many autoimmune diseases. Genome-wide association studies (GWAS) have revealed that certain disease-associated variants may contribute to RA pathogenesis. Identifying these variants can be related to RA susceptibility and severity and may help guide treatment decisions [3]. The varying prevalence of RA among different ethnic groups and geographical regions, and its higher incidence among family members and twins, supports a genetic link. Delongui et al. found that the rs1130864 CRP polymorphism was associated with systemic lupus erythematosus (SLE) susceptibility, suggesting a potential role in its pathophysiology [4]. A similar relationship might exist in RA.

Studies investigating the relationship between CRP polymorphisms and CRP levels have shown mixed results.

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Some studies found significant associations, while others, such as the study by Plant et al., did not [5–7]. Danila et al. observed that the rs2808630 variant was linked to less radiographic damage in African Americans with RA [8]. However, the relationship between CRP polymorphisms and extra-articular clinical manifestations of RA, such as lung involvement, has not been adequately investigated.

The aim of this study was to investigate the frequency of rs1205, rs1130864 and rs1800947 polymorphisms among CRP SNPs in RA patients and healthy controls and to examine the association of these polymorphisms with serum CRP levels, DAS28 scores, extra-articular involvement and especially interstitial lung disease (ILD).

MATERIALS AND METHODS

Study group

This single-center retrospective study was conducted between January 2015 and December 2019 in patients diagnosed with RA according to the 2010 ACR/EULAR classification criteria [9]. Patient files at Çukurova University Faculty of Medicine Hospital were reviewed and eligible patients were included in the study. The control group consisted of 100 age- and gender-matched healthy volunteers without any symptoms or history of RA.

The study protocol was in accordance with the Declaration of Helsinki and ethical approval was obtained from Çukurova University Local Ethics Committee (Date: 06.07.2018, Ref: 79/6). Informed consent was obtained from all participants. Demographic and clinical data including age, gender, smoking history, age at symptom onset, age at diagnosis, delayed diagnosis, family history and medication history were retrospectively analyzed and recorded. Laboratory data including rheumatoid factor (RF), anti-cyclic citrullinated peptide (anti-CCP) and antinuclear antibodies (ANA) were retrospectively reviewed and recorded from the hospital information system. Lung involvement was defined based on specific findings on chest computed tomography (CT) such as ground-glass opacities, interstitial changes such as reticulation or honeycombing, or fibrosis. CT results were recorded after retrospective review of the hospital's radiology image system.

CRP and erythrocyte sedimentation rate (ESR) levels were recorded before and after treatment. DAS28 scores were calculated using CRP values and matched to the time of CRP measurement. Patients with active infections, malignancies and active inflammation were excluded. Treatment regimens, including disease-modifying anti-rheumatic drugs (DMARDs) and biologics, were recorded. Blood ESR and CRP values at follow-up with these treatments were retrospectively analyzed to exclude confounding factors.

TagSNP Selection and Genotyping

Peripheral blood samples were collected from all participants for genetic analysis and stored in tubes containing ethylenediaminetetraacetic acid (EDTA). The tubes were stored at -70°C until DNA extraction. Genomic DNA was isolated from whole blood using a commercial DNA extraction method based on spin column technology. The amount and purity of DNA was assessed by spectrophotometry (Thermo Fisher Scientific) and only high-quality DNA (A260/A280 ratio between 1.8-2.0) was used for genotyping.

Genotyping of single nucleotide polymorphisms (SNPs) in the CRP gene was carried out using the TaqMan® SNP Genotyping Assay (Thermo Fisher Scientific, Waltham, MA, USA), a commercially available and validated real-time PCR-based method employing allele-specific fluorescent probes. Each reaction was performed in a final volume of 20 µL containing 10 ng genomic DNA, 10 µL TaqMan Universal PCR Master Mix, 0.5 µL specific TaqMan SNP assay mix and nuclease-free water. PCR amplification and allele separation were performed under thermal cycling conditions on a StepOnePlus™ Real-Time PCR System: Initial denaturation at 95°C for 10 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds and annealing/extension at 60°C for 1 minute. The SNPs investigated are listed in Table 1.

Statistical analysis

Categorical measurements were summarized as numbers and percentages, and numerical measurements were summarized as mean and standard deviation or median interquartile range (25-75 percentile values). Chi-square and Fisher tests were used to compare categorical data. Continuous variables were analyzed with Kolmogorov-Smirnov tests and histogram plots for the assumption of normal distribution. Mann-Whitney U test and Kruskal Wallis tests were used to compare measurement data that did not show normal distribution. For data showing normal distribution, T test and One-way Anova test were used in independent groups where appropriate. The statistical level of significance for all tests was considered as 0.05. All analyses were performed using the IBM SPSS version 20.0 software.

RESULTS

Our study was conducted with data from a total of 220 participants, 120 RA patients and 100 controls. There were 100 female and 20 male patients in the patient group, and 75 female and 25 male patients in the control group. The mean age of the patients in the patient group was 56.4 ± 9.7 years, and the mean age of the individuals in

Table 1. CRP gene polymorphisms

SNP ID	Localization	Context sequence	Phenotype	Polymorphism
rs1205	Chromosome 1:159712443	(C/T)	MIM:123260	C/T Transition Substitution
rs1800947	Chromosome 1:159713648	(C/G)	MIM:123260	C/G Transversion Substitution
rs1130864	Chromosome 1:159713301	(A/G)	MIM:123260	A/G Transition Substitution

Table 2. Socio-demographic and clinical characteristics of the patient-group

Age (years), mean $\pm$ SD	56,4 $\pm$ 9.7
Female sex, n (%)	100 (83.3)
The age of first symptom (years), mean $\pm$ SD	43,4 $\pm$ 9.7
The age of diagnosis (years), mean $\pm$ SD	47,2 $\pm$ 10.2
Smoking, n (%)	45 (37.5)
Family history, n (%)	21 (17.5)
Drug treatment, n (%)	
Biologic	13 (10.8)
DMARD	107 (89.2)
DAS-28 >5.1, n (%)	36 (30)
Presence of lung involvement, n (%)	15 (12.5)
The first CRP>0.8, n (%)	109 (91,2)
The last CRP>0.8, n (%)	32 (26,3)
Autoantibodies	
RF, n (%)	93 (77,5)
Anti-CCP, n (%)	78 (72,9)
ANA, n (%)	24 (22,0)

DMARD: Disease-modifying anti-rheumatic drugs, DAS-28: Disease activity score-28, CRP: C-reactive protein, RF: rheumatoid factor, anti CCP: Anti-cyclic Citrullinated Peptide, ANA: Antinuclear antibody

Table 3. Distributions of CRP gene polymorphisms and alleles of CRP gene polymorphisms in study groups

		Patient group n (%)	Control group n (%)	P value ^a
rs1205 polymorphism	TT homozygous CT heterozygous CC homozygous	15 (12.5) 55 (45.8) 50 (41.7)	17 (17.0) 38 (38.0) 45 (45.0)	0.429
rs1130864 polymorphism	TT homozygous CT heterozygous CC homozygous	6 (5) 42 (35) 72 (60)	14 (14) 48 (48) 38 (38)	0.002
rs1800947 polymorphism	CC homozygous CG heterozygous	11 (9.2) 109 (90.8)	7 (7) 93 (93)	0.559
rs1205 allele	T C	85 (35.4) 155 (64.6)	72 (36) 128(64)	0.899
rs1130864 allele	T C	54 (22.5) 186 (77.5)	76 (38) 124 (62)	<0.001
rs1800947 allele	G C	109 (45.4) 131 (54.6)	93 (46.5) 107 (53.5)	0.82

^aChi-square test.

the control group was 54.4 ± 5.4 . The sociodemographic and clinical characteristics of the patient group are summarized in Table 2.

The CRP genotype and allele of the patient and control groups were in Hardy-Weinberg equilibrium. When the

distributions of CRP gene polymorphisms were examined according to the study groups, a significant difference was found with the rs1130864 polymorphism. The ratio of TT Homozygous and CT Heterozygous cases in the patient group was found to be significantly lower than in the

Table 4. Associations between the rates of rs1130864 polymorphism and sociodemographic characteristics, clinical features and laboratory results

	RS1130864 polymorphism			P value
	TT homozygous	CT heterozygous	CC homozygous	
The age of first symptom (years), median (IQR)	50(48-52)	43.5(37-50)	42(38-49.5)	0.154 ^a
The age of diagnosis (years), median (IQR)	56(52-58)	47(38-54)	47(42-52.5)	0.061 ^a
Delay time of diagnosis (years), median (IQR)	5.5(3-8)	2(1-5)	3(1-6)	0.279 ^a
Smoking, n (%)				
+	3(6.7)	18(40)	24(53.3)	0.448 ^b
-	3(4)	24(32)	48(64)	
Family history, n (%)				
+	1(4.8)	9(42.9)	11(52.4)	0.714 ^b
-	5(5.1)	33(33.3)	61(61.6)	
Drug treatment, n (%)				
Biologic	1(7.7)	4(30.8)	8(61.5)	0.775 ^b
DMARD	5(4.7)	38(35.5)	64(59.8)	
DAS-28 >5.1, n (%)				
+	2(5.6)	11(30.6)	23(63.9)	0.793 ^b
-	4(4.8)	31(36.9)	49(58.3)	
The first CRP>0.8, n (%)				
+	5(4.6)	38(34.9)	66(60.6)	0.625 ^b
-	1(9.1)	4(36.4)	6(54.5)	
The last CRP>0.8, n (%)				
+	1(3.6)	12(42.9)	15(53.6)	0.659 ^b
-	5(5.4)	30(32.6)	57(62)	
RF, n (%)				
+	5(5.4)	34(36.6)	54(58.1)	0.807 ^b
-	1(3.7)	8(29.6)	18(66.7)	
Anti-CCP, n (%)				
+	3(3.8)	29(37.2)	46(59)	0.804 ^b
-	2(6.9)	11(37.9)	16(55.2)	
Presence of lung involvement, n (%)				
+	3(20)	4(26.7)	8(53.3)	0.017 ^c
-	3(2.9)	38(36.2)	64(61)	
DAS 28 score, mean ± SD	4.8±0.65	4.7±0.55	4.9±0.5	0.140 ^d
The first ESR (mm/h), median (IQR)	40.5(21-59)	34.5(24-51)	39.5(28.5-53)	0.314 ^a
The final ESR (mm/h), median (IQR)	11.5(3-15)	11(5-17)	11.5(6-17.5)	0.943 ^a
The first CRP, (mg/dl), median (IQR)	7.5(4.3-30.7)	2.6(1.7-5.1)	2.8(1.4-4.9)	0.162 ^a
The final CRP(mg/dl), median (IQR)	0.2(0.1-0.5)	0.5(0.3-0.9)	0.5(0.2-0.7)	0.339 ^a
RF (IU/ml), median (IQR)	69(22-159)	137(30-324)	61(10-215)	0.214 ^a
Anti CCP (IU/ml) median (IQR)	122(11-74)	84(15-815)	20(18-1000)	0.664 ^a

^aKruskal-Wallis, ^bChi-Square Test, ^cFisher's test, ^dOne-way ANOVA, IQR: Interquartile Range (25-75 percentile)

DMARD: Disease-modifying anti-rheumatic drugs, DAS-28: Disease activity score-28, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, RF: rheumatoid factor, anti CCP: Anti-cyclic Citrullinated Peptide.

healthy control group (p=0.002). When the distributions of alleles in the CRP gene polymorphisms were examined according to the study groups, the rate of rs1130864 T allele in the patient group was found to be significantly lower than in the healthy control group (p<0.001). Distributions of CRP gene polymorphisms and alleles of CRP gene polymorphisms in study groups are shown in Table 3.

RS1205 polymorphism rates were analyzed according to sociodemographic characteristics, clinical features

and laboratory results, and no statistically significant relationship was observed with the parameters examined. RS1130864 polymorphism rates were analyzed according to sociodemographic characteristics, clinical features and laboratory results, and a significant correlation was found with lung involvement. The rate of TT homozygous cases was higher in patients with lung involvement compared to patients without lung involvement, and the rate of CT heterozygous and CC homozygous individuals was lower

($p=0.017$). No statistically significant difference was found with the other parameters (Table 4). No significant relationship was detected between sociodemographic characteristics, clinical features, laboratory results, and rs1800947 polymorphism.

DISCUSSION

In this study, we examined the frequency of CRP single nucleotide polymorphisms rs1205, rs1130864, rs1800947 and their association with serum CRP levels, DAS28 scores and clinical manifestations of the disease in RA patients and healthy controls. In our study, no statistically significant correlation was found between CRP SNPs rs1130864, rs1205, rs1800947 and baseline CRP levels and DAS28 scores. We found a higher frequency of the rs1130864 TT homozygous genotype in patients with lung involvement. This finding may significantly affect the follow-up and treatment decisions, especially in terms of lung involvement.

CRP is an important acute phase protein that increases in response to inflammation. [10] Acute phase proteins are mainly produced by hepatocytes upon stimulation by cytokines (e.g. interleukin-6, interleukin-1 and tumor necrosis factor [11]). RA is a chronic, autoimmune, multisystem inflammatory disease of unknown etiology targeting synovial tissues. Serial measurements of ESR and CRP are valuable for monitoring the level of inflammation in RA patients. ESR and CRP levels are the most commonly used markers of inflammation and their levels decrease with effective treatment [12]. When clinically relevant, elevated CRP and ESR levels suggest that the disease is active. Therefore, CRP levels are frequently used in diagnosis and treatment follow-up.

CRP is encoded by the CRP gene located on chromosome 1q23 [13]. There are many studies examining whether there is a relationship between CRP levels and CRP gene polymorphism. Danik et al. conducted a study for evaluating the mean CRP level after acute ischemic events and found higher CRP levels in individuals with rs3091244 AA genotype [14]. In the review of Rhodes et al., relationship between CRP level and CRP gene polymorphism was emphasized. One of the polymorphisms showing the most consistent association with CRP levels is the rs1205 (C>T) variant, which is associated with low CRP levels. Other polymorphisms identified in the CRP gene include rs3093077, rs1130864, rs3093058, rs1800947, and rs3091244 [2]. Delongui et al. showed that rs1130864 CRP polymorphism was associated with SLE susceptibility [4]. In our study when the distributions of CRP gene polymorphisms were examined according to the study groups, a significant difference was found with the

rs1130864 polymorphism. The ratio of TT Homozygous and CT Heterozygous cases in the patient group was found to be significantly lower than in the healthy control group. The fact that rs1130864 polymorphism is low in patients suggests whether it might be protective from RA disease.

There are some studies showing that CRP gene polymorphisms are associated with CRP levels in rheumatological diseases such as Ankylosing Spondylitis (AS) and RA [15,16]. In the study by Kasapoğlu et al., it was determined that CRP levels and therefore disease activity were higher in the AS patient group with the C allele at the rs3091244 CRP gene locus [17]. In the study conducted by Rhodes et al. in England, the relationship between CRP level and polymorphism in RA patients was evaluated. In particular both rs1205 and rs1800947 have been widely associated with lower basal CRP expression [5]. However, in a study conducted in the Chinese population, no significant association was found between CRP gene polymorphisms and CRP levels in patients with Takayasu's arteritis [18]. In our study, no statistically significant correlation was found with rs1130864, rs1205, rs1800947 CRP SNPs and initial CRP levels. This may be due to ethnic and geographical differences.

Serum CRP level is widely used to evaluate disease activity in RA patients and is part of the DAS28 score [12,19]. Ammitzbohl et al. investigated the relationship between CRP genotype associations and CRP levels and DAS28 scores of 315 RA patients in Denmark using seven CRP SNPs. The seven CRP SNPs studied were rs11265257, rs1130864, rs1205, rs1800947, rs2808632, rs3093077, and rs876538. No correlation was found between basal serum CRP levels and SNPs. No significant correlation was observed between the seven CRP SNPs and the DAS28 score. [6] Therefore, this study demonstrates that DAS28, the key parameter for inflammatory activity in RA, can be used in clinical decision making without adjustment for CRP gene variants. Similarly, in our study, no statistically significant correlation was found between rs1130864, rs1205, rs1800947 CRP SNPs and DAS28 score.

Danila et al. investigated the role of CRP polymorphism in radiological severity and its relationship with CRP level by studying seven SNPs. SNPs studied were rs3093059, rs3093062, rs3091244, rs1417938, rs3093066, rs1205, rs2808630. CRP rs2808630 has been associated with reduced radiographic damage. In this study, it was stated that it has important implications for the assessment of disease activity and risk of erosive disease in African Americans with RA [8]. In our study, the rs1130864 polymorphism was examined and a significant correlation was found with lung involvement. Compared to patients without lung involvement, the rate of TT homozygous

cases was higher, and the rate of CT heterozygous and CC homozygous individuals was lower. We did not find a significant relationship with lung involvement in rs1205 and rs1800947 polymorphisms. The high incidence of rs1130864 polymorphism in TT homozygotes in patients with lung involvement might significantly affect the follow-up of the disease and the decision of treatment. Especially these patients might be followed closely in terms of lung involvement. Our study has been the only study investigating the relationship between RA lung involvement and CRP gene polymorphism so far.

In this article, we investigated the frequency of CRP gene polymorphism in RA patients and its association with clinical manifestations of the disease. We found a significant association between CRP gene polymorphism and RA lung involvement. The low frequency of rs1130864 polymorphism in patients suggests that it may be protective against RA disease. To our knowledge, this is the first study showing the relationship between CRP gene polymorphism and RA lung involvement. Studies including more patients and different ethnic groups are needed to confirm our findings. No significant association was found between CRP gene polymorphism and CRP level and DAS28. This result supports the use of DAS28 in disease activity independent of CRP gene polymorphism. However, our study has some limitations. We had a small number of patients, and the number of CRP gene polymorphisms analyzed was limited due to the difficulty in accessing and affording validated SNP genotyping assays. Therefore, the results in this study cannot be generalized to all RA patients. The difference between the studies was related to both the small number of patients and ethnic and geographical differences.

In conclusion, our study shows that there is a significant association between rs1130864 CRP gene polymorphism and RA-associated lung involvement. Further studies with larger cohorts are needed to confirm our findings and investigate potential clinical applications.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

Our article represents original, unpublished material that has not been subject to editorial consideration elsewhere. Ethical rules were followed in the conduct of the research and the study was conducted in accordance with the original text of the Declaration of Helsinki and its later amendments or similar ethical standards and was approved by the local ethics committee (Date: 6.7.2018, Reference number: 79/6).

DECLARATION OF CONFLICTING INTERESTS

The authors declared no potential conflicts of interest for the research, authorship, and/or publication of this article.

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