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Original Article

Investigation of Glucocorticoid Receptor Gene Bcl-1 Polymorphism in Rheumatoid Arthritis

Romatoid Artritte Glukokortikoid Reseptörü Geni Bcl-1 Polimorfizminin Araştırılması

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Objectives: In this study, we investigated the association of the human glucocorticoid receptor gene (NR3C1) Bcl-1 CG polymorphism with clinical data and response to therapy among rheumatoid arthritis (RA) patients and healthy control subjects.

Patients and methods: We performed PCR-RFLP (Polymerase chain reaction-restriction fragment length polymorphism) method for identifying restriction of the Bcl-1 fragment length polymorphisms in the intron 2 region of the NR3C1 gene in 65 patients with RA (47 females, 18 males) and 70 healthy control subjects (47 females, 23 males). Clinical parameters and demographic characteristics of the patients along with previous history of drug treatments were also recorded. In addition, patients' response to treatment was monitored with the visual analog scale (VAS), Disease Activity Scores 28 (DAS28) and Health Assessment Questionnaire (HAQ) scores.

Results: The genotype frequencies of the NR3C1 Bcl-1 polymorphism did not differ between the patients with RA and the controls. We detected a deviation from the Hardy-Weinberg equilibrium in the RA group for the alleles tested (Rap=0.044, Kp=0.554). However, there was no significant difference between genotypes regarding age, gender, disease duration, or clinical parameters such as DAS28 scores, VAS and HAQ values ($p>0.05$).

Conclusion: Although the glucocorticoid receptor gene Bcl-1 polymorphism did not differ between the RA and control groups, we detected a deviation from the Hardy-Weinberg equilibrium in the RA group for the alleles tested. This indicates that the glucocorticoid receptor polymorphism, namely Bcl-1CG, may play a role in the ethiopathogenesis of RA and the treatment response with glucocorticoids.

Key words: Glucocorticoid; polymorphism; rheumatoid arthritis.

Amaç: Bu çalışmada romatoid artritli (RA) hastalarda ve sağlıklı kontrol deneklerinde insan glukokortikoid reseptor genindeki (NR3C1) Bcl 1-CG polimorfizminin klinik veriler ve tedaviye yanıtla ilişkisi araştırıldı.

Hastalar ve yöntemler: Bu çalışmada 65 RA'lı hasta (47 kadın, 18 erkek) ve 70 sağlıklı kontrol deneğinde (47 kadın, 23 erkek) PCR-RFLP (Polimeraz zincir reaksiyonu-restriksiyon parça uzunluk polimorfizmi) yöntemiyle NR3C1 geni intron 2 bölgesindeki Bcl I parça uzunluk polimorfizmleri çalışıldı. Hastaların önceki ilaç öyküsü ile birlikte klinik parametreleri ve demografik özellikleri de kaydedildi. Ek olarak hastalarda tedaviye yanıt görsel analog ölçeği (GAÖ), Hastalık Aktivitesi Skoru (DAS28) ve Hastalık Değerlendirme Anketi (SDA) skorlarıyla izlendi.

Bulgular: NR3C1 Bcl I polimorfizmi genotip frekansları RA'lı hastalarla kontroller arasında farklılık göstermedi. Romatoid artrit grubunda test edilen alellerde Hardy-Weinberg eşitliğinden bir sapma olduğu tespit edildi (Rap=0.044, Kp=0.554). Bununla birlikte genotipler arasında yaş, cinsiyet, hastalık süresi ve GAÖ, DAS28 ve SDA gibi klinik parametreler açısından fark saptanmadı ($p>0.05$).

Sonuç: Glukokortikoid reseptörü geni Bcl I polimorfizmi RA ve kontrol grubu arasında farklılık göstermese de RA grubunda test edilen alellerde Hardy-Weinberg eşitliğinden bir sapma tespit edildi. Bu durum glukokortikoid reseptörü geni polimorfizmi Bcl I-CG'nin RA etyopatogenezinde ve glukokortikoidlerle uygulanan tedaviye gelişen yanıtta rol oynayabileceğini göstermektedir.

Anahtar sözcükler: Glukokortikoid; polimorfizm; romatoid artrit.

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Rheumatoid arthritis (RA) is a chronic inflammatory disease of unknown etiology characterized by chronic synovitis which often results in joint destruction.[1] Glucocorticoids are used to treat a wide variety of inflammatory diseases, including RA, auto-immune diseases, allergies, and asthma.[2,3] Glucocorticoids bind the glucocorticoid receptor (GR), also denoted as NR3C1, in the cytosol. They activate the cytosolic GR which translocates to the nucleus to regulate the target gene transcription and determine the reduction of synthesis and release of pro-inflammatory cytokines.[4,5] In clinical observations, a considerable variability among subjects is seen in their sensitivity to glucocorticoid therapy, both with regard to efficacy and to the prevalance and severity of side effects.

The human GR gene is one locus on chromosome 5q31-32. An NR3C1 diallelic single nucleotide polymorphism, Bcl-1, has been reported to distinguish individuals with the highest GC sensitivity.[6] The aim of the present study was to investigate the association between the NR3C1 gen (Bcl-1-CG) polymorphism and the response to therapy among RA patients and healthy control subjects.

PATIENTS AND METHODS

Sixty-five patients with RA and 70 unrelated healthy controls were enrolled into this study. The patients with RA were divided into two groups as anti-TNF-alpha and DMARD groups. Thirty-five patients received anti-TNF-alpha therapy (either etanercept 25 mg subcutaneous twice weekly or infliximab 3 mg/kg infusion at weeks 0, 2, 6, and then every 8 weeks and 30 patients received disease-modifying antirheumatic drugs (DMARDs). Corticosteroids and non-steroidal anti inflammatory drugs (NSAIDs) are allowed as adjuvants at the treatment period. The study was approved by the local ethics committee, and informed consent was obtained from all patients. Rheumatoid arthritis patients were diagnosed according to the American College of Rheumatology (ACR) criteria. Disease activity was determined on the basis of defined parameters. The number of tender and swollen joints (28-joint count), duration of morning stiffness (in minutes), global health and disease activity on a 0-100 mm visual analog scale (VAS) and the Disease Activity Score 28-joint assessment (DAS28) were calculated. Functional assessment was carried out using the Health Assessment Questionnaire (HAQ) at baseline and after six months of therapy.[7]

The distribution of the NR3C1 Bcl-1 polymorphism in the intron 2 polymorphism were evaluated in RA patients and healthy control populations by using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Blood samples were obtained from all subjects. Genomic DNA was extracted from mononuclear cells obtained from ethylenediaminetetraacetic acid (EDTA)-treated peripheral venous blood using the salting-out method.[7] The Bcl-1 polymorphism in the intron 2 of NR3C1 was amplified using the following primer pair: Bcl1F-5'-TGCTGCCTTATTTGTAAATTCGT-3' and Bcl1R-5'-AAGCTTAACAATTTTGGCCATC-3'. DNA was amplified by a single PCR program consisting of one cycle of 95 °C for seven min, 40 cycles of 94 °C for one min, one cycle of 56°C for one min, one cycle of 72 °C for 1 min, and one cycle of 72 °C for seven min. The genotyping was determined by PCR-RFLP (Figure 1).[8,9]

Statistical analysis

The data analyses were performed using the computer software SPSS for Windows version 13.0 (SPSS Inc., Chicago, Illinois, USA). The statistical significance of the differences between the patient and control groups was estimated by logistic regression analysis. Adjusted odds ratios (ORs) were calculated with a logistic regression model that controlled for gender and age and was reported at 95% confidence intervals (CI). Differences in Bcl-2 allele frequencies between the control group and patients were compared with a chi-square test and, when needed, Fisher's exact test was used. The Hardy-Weinberg equation was used to calculate the estimated genotype frequency and experienced genotype frequency. For statistical comparison of groups, the Mann-Whitney U-test was used. A p-value of less than 0.05 was considered statistically significant.

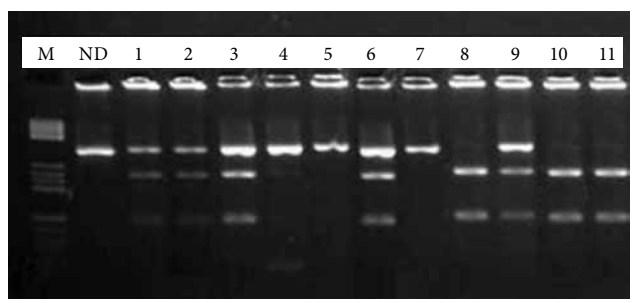

Figure 1. Agarose gel electrophoresis of NR3C1-Bcl1 fragments stained with ethidium bromide. M: DNA size standard, ND: Non-digest PCR product. The results regarding patients with RA; 1-4,6,9: GC/8,10,11: CC/5,7: GG.

Table 1. Main demographic and clinical characteristics of the rheumatoid arthritis and control subjects

	Anti TNF-alpha treatment						DMARD treatment						Control		
	n	%	Range	Mean±SD	Med.	(min.-max.)	n	%	Range	Mean±SD	Med.	(min.-max.)	n	Range	*p*
No. of patients	35						30					70			
Age	50		38-65				47		29-78				50	29-76	NS
Gender															
Male	8						10						23		NS
Female	27						20						47		NS
Disease duration				9.5±10.4	8.2	(0.6-15.5)				8.7±7.5	7.8	(0.6-13.8)			NS
RF +	41	82					37	79							NS
DAS28 scores															
Week 0				4.9±1.6	4.63	(2.33-7.89)				5.3±1.1	5.17	(3.4-7.32)			NS
Week 24				3.4±1.3	3.20	(1.36-5.94)				4.3±1.1	4.14	(1.87-6.79)			0.004
Improvement in DAS28 scores															
Week 0/24				1.5±1.0	1.49	(-0.17-3.67)				1.0±1.0	0.82	(-0.9-3.3)			0.051 NS
VAS															
Week 0				39.4±20.0	55	(20-90)				45.7±17.2	70	(20-90)			NS
Week 24				24.6±15.7	40	(10-80)				44.8±15.3	62	(30-85)			<0.001
HAQ															
Week 0				0.9±0.8	0.87	(0-2.25)				0.7±0.6	0.56	(0-2)			NS
Week 24				0.7±0.6	0.5	(0-2)				0.7±0.6	0.5	(0.2)			NS

TNF: Tumor necrosis factors; DMARD: Disease-modifying antirheumatic drugs; RF: Rheumatoid arthrites; SD: Standard deviation; Med.: Median; DAS28: Disease Activity Score 28; VAS: Disease activity score; HAQ: Health assessment questionnaire; NS: Non significant; p: Mann-Whitney U-test.

RESULTS

The demographic characteristics of the patients and their responses to treatment after 24 weeks of therapy are shown in table 1. The mean age of the patients in the anti-TNF-alpha, DMARD, and control groups did not differ significantly [50 (38-65) in anti-TNF-alpha the etanercept group, 47 (29-78) in the DMARD group and 50 (29-76) in the control group]. Mean disease durations were similar in the anti-TNF group (9.5±10.4 years) and in the DMARD group (7.5±8.7 years). The mean VAS and HAQ score were not significant in either group. At six months, the mean DAS28 improvement was not significant among the groups (p=0.051).

Digestion of PCR product by the Bcl-1 enzyme generated one fragment for homozygous G/G, three fragments for heterozygote G/C, and two fragments for homozygous C/C (Figure 1).

Genotype frequencies of the NR3C1 Bcl-1 polymorphism did not differ between the patients with RA and the controls (table 2). We detected a deviation from the Hardy-Weinberg equilibrium in the RA group for the alleles tested (Rap=0.044, Kp=0.554).

When we considered the correlation between allelic distribution of the NR3C1 Bcl-1 polymorphism and clinical parameters such as the DAS28 scores, VAS, and HAQ values, we didn't find also any statistical significance among the groups (table 3).

DISCUSSION

Although glucocorticoids are successfully used in the treatment of a wide range of rheumatic and other inflammatory diseases, few patients with these diseases show a poor response or absence of response to even high doses of glucocorticoids.[8-10] The molecular basis of generalized glucocorticoid resistance has been ascribed

Table 2. Frequency of Bcl-1 genotypes in control and rheumatoid arthritis cases

NR3C1 gene	Rheumatoid arthritis (n=65)		Healthy control (n=70)		OR	95% CI	*p*
	n	%	n	%			
BCL-1 polymorphism Genotype							
CC	35	53.8	42	60	3.023*	0.870-10.506*	0.082*
CG	20	30.8	24	34.3	2.933*	0.795-10.821*	0.106*
GG	10	15.4	4	5.7	3.000†	0.891-10.096†	0.090†

High expression; † Low expression; * OR (95%CI) was adjusted by age and sex; † Fisher's Exact Test.

Table 3. Bcl-1 genotypes of rheumatoid arthritis patients in association with clinical parameters

	DAS28 scores*				VAS*				HAQ*			
	Mean±SD	Med.	(min.-max.)	*p*†	Mean±SD	Med.	(min.-max.)	*p*†	Mean±SD	Med.	(min.-max.)	*p*†
BCL-1												
CC[a] (n=35)	4.9±1.4	4.85	(2.48-7.9)	0.627[ac]	55.0±15.9	65	(20-80)	0.510[ac]	0.9±0.7	0.87	(0-2.25)	0.226[ac]
CG[b] (n=20)	5.4±1.3	4.87	(3.75-7.7)	0.282[ab]	58.8±18.9	68	(20-90)	0.197[ab]	0.8±0.8	0.5	(0-2.2)	0.400[ab]
GG[c] (n=10)	5.1±1.5	5.27	(2.33-7.33)	0.965[bc]	56.7±28.4	70	(20-85)	0.965[bc]	0.6±0.4	0.56	(0-2.25)	0.710[bc]

DAS28: Disease Activity Score 28. VAS: Disease activity score; HAQ: Health Assessment Questionnaire; SD: Standard deviation; Med.: Median; * Mean-week 0; p† Mann-Whitney U-test; ac: Statistics between CC genotype and GG genotype; ab: Statistics between CC genotype and CG genotype; bc: Statistics between CG genotype and GG genotype.

to mutations in the hGR gene, which impair one or more of the molecular mechanisms of hGR action, thereby altering tissue sensitivity to glucocorticoids. Several polymorphisms of the gene which might have an impact on glucocorticoid sensitivity have been reported.[11-14]

In this report, we didn't determine any significant differences in the NR3C1 Bcl-1 polymorphism among the RA patients or healthy controls. Also, there was no correlation between the clinical parameters of RA and the polymorphisms. The results of this analysis may be related to the fact that the patients belonged to the same area of Turkey. Most of the RA patients in our group had high disease activity which may be related to the lack of regular admittance of patients to clinics or to inappropriate drug use.

Derijk et al,[15] reported a newly identified polymorphism in the hGR gene in exon9beta, in an "ATTTA" motif, was significantly associated with RA. In another study, however, no significant difference was obtained among 198 RA patients and the control subjects when three GR polymorphisms (intron B Bcl-1polymorphism, a ttg insertion/deletion within intron F (rs2307674) and the single nucleotide polymorphism (SNP) lying in the 3' untranslated region of exon 9b).[16] Chatzikyriakidou et al,[17] explored the association among RA susceptibility and the GR-α polymorphisms rs33388, rs33389, Bcl-1, and GR-β variant rs6198 in 136 RA patients. They concluded that the GR-α and GR-β polymorphism are potentially associated with RA susceptibility. In another study, the hGR +647G/C genetic polymorphism was investigated in Korean patients with RA, but no difference between the RA and control groups was reported.[18] Our results support most of the previous reports, but, as far as we know, this is the first study which compares the clinicial severity of RA and NR3C1 gene polymorphisms.

Another heavily-studied autoimmune disease is systemic lupus erythematosus (SLE). Lee et al,[19] showed a single mutation in exon 9 of the GR gene in 11/132 (8.3%) of 66 patients with SLE whereas no mutations were detected in 52 healthy individuals.

The variation in time required to obtain the cessation of proteinuria in children with nephrotic syndrome represents one aspect of the variations shown by these children in response to glucocorticoid therapy. In a study which consisted of 118 children with nephrotic syndrome, the Bcl-1 polymorphism was assessed, and the GTA haplotype was found to be associated with a higher sensitivity to steroid treatment as determined by time to proteinuria resolution.[20]

The glucocorticoid receptor gene NR3C1 has been widely studied in other inflammatory and autoimmune diseases, such as cystic fibrosis, asthma, and Graves ophthalmopathy.[21-23]

In conclusion glucocorticoid resistance is important in several inflammatory diseases, including RA, and this complicates their clinical management. The glucocorticoid receptor polymosrphism Bcl1-CG may be one of the gene candidates for ethiopathogenesis and response to treatment with glucocorticoids. The authors suggest that further studies are needed to better elucidate the relationship between GR variants and RA.

Declaration of conflicting interests

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