Interleukin-1 receptor antagonist (IL-1RN) and interleukin-1B gene polymorphisms in Turkish patients with rheumatoid arthritis

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Abstract

Objective

Interleukin 1 (IL-1) family is composed of two agonists, IL-1α and IL-1β and IL-1 receptor antagonist, IL-1Ra. The purpose of this study was to determine the relationship between polymorphisms of IL-1 receptor antagonist (IL-1RN), IL-1B promoter and IL-1B exon 5 genes and susceptibility to rheumatoid arthritis (RA) in Turkish population.

Methods

Polymerase chain reaction (PCR) was used to determine the genotype of the IL-1RN for 94 RA patients and 104 healthy controls. Genotyping of IL-1B polymorphisms at positions -511 (C/T) and +3953 (C/T) was detected by PCR followed restriction fragment length analysis.

Results

There was no significant difference in IL-1RN genotype and allele distributions between RA and the control groups. In addition, no significant association was observed in the allelic frequency (C or T) of IL-1B promoter (-511) between RA patients and the controls (P = 0,118), but the genotype distribution of 1/2 (C/T) at position -511 showed a significant difference (P = 0,038). Also, 2/2 genotype (T/T); (P = 0,028), and allele 2 (T) distribution (P = 0,011) of IL-1B (+3953) showed significant differences between RA patients and the control groups in the study population.

Conclusion

These results imply that 2/2 (T/T) genotype or allele 2 (T) of IL-B (+3953) are susceptibility factors for RA in Turkey. Also, 1/2 genotype (C/T) of IL-1 -511 can play a protective role for RA.

Key words

IL-1B and IL-1RN polymorphism, rheumatoid arthritis.

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Introduction

Rheumatoid arthritis (RA) is the most common systemic, autoimmune rheumatological disorder, characterized by chronic inflammation of multiple joints, synovial cell proliferation and the accumulation of T lymphocytes in synovial tissues, leading to the destruction and disability of joints (1, 2). The prevalence of RA in Turkey is 0.36% (3, 4). Different prevalences of the disease throughout the world indicate that genetic factors play an important role in RA pathogenesis (5, 6). It has been reported that human leukocyte antigen (HLA) "shared epitope" alleles have been frequently found in RA (30-60%) but their exact role in the etiopathogenesis is not clearly known (7). Among the other genetic factors that might be associated, Interleukin 1 (IL-1) which is a key mediator of inflammation and tissue destruction in RA, is a candidate gene. IL-1 family is composed of IL-1 α and β and IL-1 receptor antagonist (IL-1Ra). IL-1 α and β act as agonists and are implicated in joint destruction in RA (8). In contrast, IL-1Ra act as an antagonist in IL-1 signaling by inhibiting the binding of IL-1 to IL-1 R type I and has been shown to prevent joint erosions in RA (9). There are several high degree sequence variations in IL-1B and IL-1RN genes and these may be important for the susceptibility to RA. 86 bp-variable number tandem repeats (VNTR) in intron 2 has been discovered in the IL-1RN gene (10) and IL-1RN allele 2 is linked to autoimmune diseases (11). Other single nucleotide polymorphisms (SNPs) have been detected at position -511 C/T in IL-1B gene promoter (12) and at position +3953 C/T in the exon 5 of IL-1B (13) and these mutations are thought to influence IL-1 expression (12, 13). There are various reports showing a relationship between VNTR and SNPs on IL-1 gene family and diseases such as diabetic nephropathy, systemic lupus erythematosus (SLE) and RA (14-17). The purpose of this study was to investigate whether polymorphisms in IL-1RN and IL-1B (-511) and IL-1B (+3953) genes are associated with RA in Turkey. Our results showed that individuals carrying 2/2 (T/T) genotype of IL-1B (+3953) gene are susceptible to RA in the Turkish population. In addition, 1/2 (C/T) genotype of IL-1B (-511) may play a protective a role against RA.

Materials and methods

Patient and controls

Ninety-four Turkish patients with RA (76 females and 18 males, mean age 49 years) and 104 healthy controls (80 females and 24 males, mean age 47 years) were included in this study. RA patients that were evaluated regularly at 4 monthly intervals in the RA Clinic of the Rheumatology Division of Marmara University, School of Medicine, Istanbul by the same physcian (NI) were included into the study consecutively. All patients fullfilled 1987 Classification Criteria of the American College of Rheumatology (18). All RA patients had established disease with more than 2 years of disease duration (mean 8.8 (2-31) years). Among the 94 RA patients 65% were RF positive and mean RF titre was 124.1 (8.6-652) IU. The mean DAS28 score of patients at the time of inclusion was 3.1 (1.2-7.5). Ninety-six percent of RA patients were on methotrexate (mean maximum dosage 14.7 mg/week (0-25)) and 87% of RA patients were on corticosteroids (mean maximum prednisolone dosage 8.9 mg/day (0-60)). More than half of RA patients had erosive disease (56 %) and 28.9% had extra-articular manifestations. The study was approved by the Institutional Review Board and informed consent was taken.

Genomic DNA isolation

Genomic DNAs were isolated from whole blood collected in EDTA containing tubes from RA patients and the control groups. The genomic DNAs were extracted using salting out method (19), followed by ethanol precipitation and then stored at 4ºC.

Genetic analysis of polymorphisms

Genetic analysis of polymorphisms at IL-1RN (VNTR), IL-1B (+3953 C/T, exon 5), and IL-1B (-511 C/T, promoter region) was investigated.

IL-1RN polymorphism region was amplified by PCR using forward and reverse primers respectively, forward

primer: (5' CTCAGCAACACTCCTAT 3' and reverse primer: 5' TCCTGG-TCTGCAGGTAA 3'). PCR cycles: (94ºC, 3 min) x1; (94ºC, 30 sn, 55ºC, 30 sn, 72ºC, 45 sn) x35; (72ºC, 10 min) x1, and the size of PCR products was determined by 1.5 % agarose gel stained with ethidium bromide (Fig. 1A). Genotype distribution was evaluated based on repeating unit size of PCR products.

IL-1B (-511 region) also was amplified by PCR using forward and reverse primers respectively, (5' GTTTAG-GAATCTTCCCACTT 3' and 5' TGGCATTGATCTGGTTCATC 3'). PCR cycles: (94ºC, 3 min) x1; (94ºC, 30 sn, 54ºC, 30 sn, 72ºC, 45 sn) x35; 72ºC, 10 min) x1. Restriction digestion was done with 5 U AvaI at 37ºC for overnight. Then IL-1B genotype was done based on size of digested PCR product on 2.5% agarose gel (Fig. 1B). AvaI cut yielded 190 and 114 bp fragments when allele C was present; however, 304 bp fragment was seen in presence of allele T.

IL-1B (3953 exon 5) polymorphism was determined by PCR using forward and reverse primers respectively, (5' GTTGTCATCAGACTTTGACC 3' and 5' TTCAGTTCATATGGACCA-GA 3'). PCR cycles: (94ºC,1 min) x1; 94ºC, 30 sn, 54ºC, 30 sn, 72ºC, 45 sn) x35; 72ºC, 10 min) x1. Restriction digestion was performed with 5U Taq1 at 65ºC for overnight followed by 3 % agarose gel electrophoresis analysis (Fig. 1C). TaqI digestion yielded 135 and 114 bp fragments in presence of allele C, whereas 249 bp fragment was seen in presence of allele T.

Statistical analysis

The genotype and allele frequency of RA patients for IL-1 RN, IL-1B (-511) and IL-1B (+3953) were compared to control groups using the chi-squared test. P values less than 0.05 were accepted to be significant.

Results

Genomic DNAs were isolated from 94 RA patient and 104 controls, PCR was performed and PCR amplifications were used for analysis of Taq 1 and AvaI restriction fragment length polymorphisms. All 198 patient and controls were genotyped based on PCR product size or digestion products. Genotype and allelic frequencies of IL-1 RN are shown in Table I. Three of five alleles at IL-1 RN gene were observed in Turkish RA and the control groups. Alleles 1 and 2 were found frequently in Turkish population; however, 1/3 and 2/2 genotype and allele 3 of IL-1 RN were less frequently present. There were no significant differences based on genotype distribution and allele frequency of IL-1 RN gene between the RA patients and the control group (Table I).

The distribution of genotype and allele frequencies of IL-1B -511 in RA and controls is shown in Table II. IL-1B -511 genotype showed a significant difference between RA and controls, 1/2 genotype (C/T) of IL-1B -511 is found to be higher than expected in the control group (52,9% in RA vs 35,1% in controls; P = 0,038). This may imply that 1/2 (C/T) genotype of IL-1B -511 can have a protective effect for RA. However, 1/1 (C/C) genotype of IL-1B -511 is increased in RA patients (48,9% in RA vs 33,7% in controls), but did not reach a statistically significant difference. In contrast, no significant difference was observed in allelic distribution between RA and the controls (P = 0,188); (Table II).

The allelic frequency and genotype distribution of IL-1B +3953 in RA patients and controls have been shown in Table III. When genotype distribution (C/C, TT or C/T) of IL-1B +3953 was compared, a significant difference was observed in 2/2 (T/T) genotype of IL-B +3953 between RA and the control groups (16 % in RA vs 4,8 % in controls; p = 0.028). Also, the frequency of allele 2 (T) for +3953 showed a significant difference between RA and control groups (Table III, 31,4 % in RA vs 20,2 % in controls; P = 0,011). In addition, 1/1 (C/C) genotype and allele 1 (C) of IL-1B +3953 increased in healthy controls, but did not reach a statistical significance (P = 0,118).

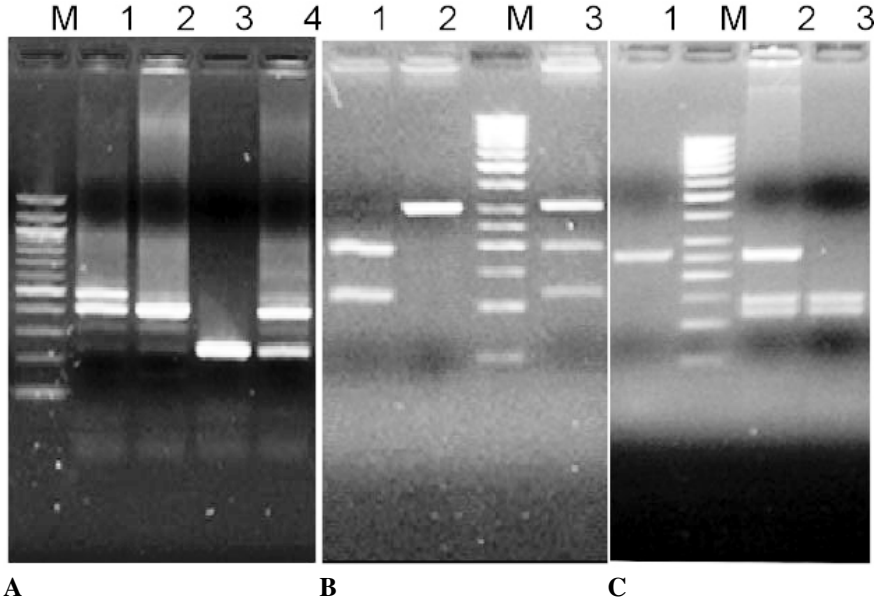

Fig.1. Genotyping of IL-1RN VNTR and -511 (C/T) and +3953 (C/T) SNPs. **A**: M is 100 base pair (bp) marker, lane 1 represents heterozygous for alleles 1 and 3 (410 and 500 bp), lane 2 is homozygous for allele 1 (410 bp), lane 3 shows homozygous for allele 2 (240 bp), lane 4 is heterozygous for alleles 1 and 2 (410 and 240 bp); **B**: lane 1 shows homozygous for allele 1 (C/C); (190 and 114 bp), lane 2 represents homozygous for allele 2 (T/T) (304 bp), M is 50 bp DNA marker and lane 3 shows heterozygous for alleles 1 and 2 (C/T); (190, 114 bp and 304 bp); **C**: lane 1 shows homozygous for allele 2 (T/T); (249 bp), M shows 50 bp DNA marker, lane 2 is heterozygous for alleles 1 and 2 (C/T); (135, 114, and 249 bp), lane 3 represents homozygous for allele 1 (C/C); (135 and 114 bp).

Discussion

RA is a multifactorial disease and both genetic and environmental factors influence the susceptibility to RA. Although "shared epitope" alleles have been reported to be associated with

Table I. Genotype distribution and allele frequency of IL-1RN in Turkish RA patients and healthy samples.

IL-1RN Genotype	RA Patients (n = 94) (%)	Healthy controls (n = 104) (%)
1/1	58 (61.7)	64 (61.5)
1/2	24 (25.5)	27 (26)
1/3	4 (4.3)	5 (4.8)
2/2	8 (8.5)	8 (7.7)
IL-1RN Alleles	RA patients (n = 188) (%)	Controls (n = 208) (%)
1	144 (76.6)	160 (76.9)
2	40 (21.3)	43 (20.7)
3	4 (2.1)	5 (2.4)

*P value < 0,05 was taken as significant.

Table II. Genotype distribution and allele frequency of IL-1B (-511) found in Turkish RA patients and healthy samples.

IL-1B (-511) Genotype	RA Patients (n = 94) (%)	Healthy Controls (n = 104) (%)
1/1	46 (48.9)	35 (33.7)
1/2*	33 (35.1)	55 (52.9)
2/2	15 (16)	14 (13.5)
IL-1B (-511) Alleles	RA patients (n = 188) (%)	Controls (n = 208) (%)
1	125 (66.5)	125 (60.1)
2	63 (33.5)	83 (39.90)

*P value < 0,05 was taken as significant.

Table III. Genotype distribution and allele frequency of IL-1B +3953 found in Turkish RA patients and healthy samples.

IL-1B (+3953)	RA Patients (n = 94) (%)	Healthy Controls (n = 104) (%)
1/1	50 (53.2)	67 (64.4)
1/2	29 (30.8)	32 (30.8)
2/2*	15 (16)	5 (4.8)
IL-1B (+3953) Alleles	RA patients (n = 188) (%)	Controls (n = 208) (%)
1	129 (68.6)	166 (79.8)
2*	59 (31.4)	42 (20.2)

*P value < 0,05 was taken as significant.

RA, their presence is not sufficient. Other genes and their polymorphisms thought to be involved in the pathogenesis of RA are investigated. Disease activity of RA and the progression of joint destruction are correlated with IL-1 levels in plasma and synovial fluid (20) and polymorphisms on IL-1 gene locus can influence the susceptibility to RA. One of the candidate genes is IL-1 family, consisting of IL-1 A, B and RN (21). IL-1RN gene has a variable of number of tandem repeats (VNTR) in intron 2 and it includes 6 VNTRs depending on the number of repeats of 86 base pair (bp) fragment. Six alleles (0, 1, 2, 3, 4, 5) corresponding to 1, 4, 2, 3, 5, and 6 copies of repeated sequence have been identified (22, 23). It has been reported that allele 2 of this gene was involved in the production of IL-Ra (24). Also, a relationship between allele 2 of IL-1RN and several diseases such as diabetes nephropathy (14), systemic lupus erythematosus (15) ulcerative colitis (25) and multiple sclerosis (26) was reported. Allele 2 was increased also in ankylosing spondylitis (AS)(27) and in juvenile arthritides (28). However, there are controversial reports for the possible function of allele 2 of IL-1RN. One report showed that allele 2 reduces mRNA expression in tissues from ulcerative colitis (25), whereas other studies suggest that allele 2 increases IL-1Ra production (11, 29). We examined intron 2 region of IL-1RN gene of 94 RA patients and 104 controls. Our results showed that no statistical significance was observed between IL-1RN gene variations and RA. Although some studies indicated that IL-1RN VNTR is not associated with RA (22, 26, 30), an association has been shown with patients living in East Asia. Allele 4 of IL-1RN was increased RA patients in Taiwan (31), whereas another study found an association with allele 2. However, allele 2 of IL-1RN was lower in RA patients in Korea compared to the control group (32).

IL-1β plays an important a role in the pathogenesis of autoimmune diseases. The expression of metalloproteinase genes such as collagenase, elastases and adhesion molecules involved in joint destruction are induced by IL-1 (33). Also, bone resorption and cartilage degradation *in vitro* are promoted by IL-1 (8). The polymorphisms in IL-1 B gene can affect severity or susceptibility to different diseases. Two important single nucleotide polymorphisms (SNPs) at -511 C/T in the promoter and +3953 C/T in the exon 5 of IL-1B genes are shown respectively (12, 13). It has been reported that individuals who have IL-1B (-511) allele 2 show higher levels of IL-1Ra (29). Also, LPS-induced IL-1β production was increased 2-3 fold by T allele at -511 position (34). -511 allele of IL-1B is associated with RA in Swiss patients (35). However, Camargo *et al*. did not find any relationship between -511 alleles of IL-1B and RA (36). Our study showed that 1/2 genotype of IL-1B -511 showed a significant difference between RA and controls (P = 0.038). However, we can not observe any significant differences in allele 1 and 2 or 1/1 and 2/2 genotypes of IL-1

-511 in our study group. Our results imply that 1/2 genotype of IL-1B -511 can play a protective role for healthy people from RA (35.1% in RA vs 52.9% in controls). A possible explanation for the protective role of 1/2 genotype of IL-1B -511, is the role of heterozygous promoter region which can influence IL-1β gene expression in a negative way by inhibiting transcription factors' binding. We also found that 1/1 genotype of IL-1B -511 is increased in RA patients (48.9% in RA vs 33.7 % in controls) but did not reach a statistical significance. In this case, transcription factors can bind to 1/1 (C/C) genotype region of IL-1 B promoter and activate gene expression positively. Thus higher levels of IL-1β might be produced and might affect the course of RA.

IL-1B polymorphisms are studied also in other autoimmune diseases such as primary Sjögren's syndrome (pSS) and systemic lupus erythematosus (SLE). -511 T allele and CT genotypes are found to be associated with SLE in African American people; however, this association was not seen in Caucasian people living in the south-east of the United States (37) and in Chinese patients (38).

Another IL-1B polymorphism is located at position +3953 in exon 5 and thought to influence IL-1 expression. There are controversial reports of the affect of IL-1B +3953 allele on IL-1B protein expression levels. Some studies found an association between IL-1B +3953 and increased plasma levels of IL-1β (13, 34), but others found no affect or reduction of IL-1 levels (16, 36, 39). An association was seen between +3953 T allele (allele 2) and a more severe RA course in Chinese, Sweden, French and Polish patients (16, 17, 40, 41) but no association in Colombian, Dutch and Taiwanese (31, 36, 42). Also other studies showed no relationship between IL-1B +3953 polymorphism and psoriatic arthritis (PsA) (43). +3953 T alelle was also found to be protective in Colombian SLE patients (36). Our results showed a significant difference in 2/2 genotype (T/T) of IL-1B +3953 between RA and the control group (P = 0,028). Also, allele frequency of allele 2 (allele T) for IL-1B +3953 showed a significant difference between the groups (Table III, P = 0,011). These results imply that allele 2 (T) and 2/2 (T/T) genotype of IL-1B +3953 may be susceptibility factors for RA in Turkish patients. It could be possible that allele 2 (T) or genotype homozygous T/T in exon 5 at located +3953 can act as a enhancer for transcription activation for IL-1 B. However, this enhancer could be tissue and cell specific based on different studies.

1/1 (C/C) genotype and allele 1 (C) of IL-1B +3953 were increased in healthy controls, but not reaching a statistical significance. This may imply that indicate allele 1 (C) and 1/1 (C/C genotype of IL-1B +3953 polymorphism may be protective for RA. Our results also showed that allele 1 (C) or 1/1 (C/C) genotype region of IL-1B +3953 may act as regulatory elements for suppressor proteins for the reduction of IL-1β expression.

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