ORIGINAL ARTICLE

Lack of association polymorphisms of the IL1RN, IL1A, and IL1B genes with knee osteoarthritis in Turkish patients

Melek Sezgin[1], MD
M. Emin Erdal[2], PhD
Zuhal Mert Altıntaş[2], MD
Handan Çamdeviren Ankaralı[3], PhD
İ. Ömer Barlas[1], PhD
Ebru Türkmen[3], MD
Günşah Şahin[3], MD

[1]Department of Physical Medicine and Rehabilitation, [2]Department of Medical Biology and Genetics, and [3]Department of Biostatistics, Medical Faculty of Mersin University, Mersin, Turkey

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Abstract

Purpose: To examine whether polymorphisms of the interleukin 1 receptor antagonist (IL1RN), interleukin 1 alpha (IL1A) and interleukin 1 beta (IL1B) genes are markers of genetic susceptibility to knee osteoarthritis in Turkish patients.

Methods: One hundred and seven patients with knee osteoarthritis and 67 controls were studied. Three polymorphisms of IL1A, IL1B, and IL1RN genes were typed from genomic DNA. Allelic frequencies were compared between patients and control subjects.

Results: No significant differences were observed in genotype and allele frequencies of the IL1RN VNTR, IL1A+4845, IL1B+3953 genes polymorphisms between patients and controls. Furthermore, we did not detect any association genotypes of the polymorphisms with the clinical, radiological, and laboratory profiles of patients.

Conclusions: The present study suggest that the IL1RN VNTR, IL1A+4845, IL1B+3953 genes polymorphisms are not genetic markers of susceptibility to knee osteoarthritis in Turkish patients, and are unrelated to the clinical, radiological, and laboratory characteristics of knee osteoarthritis.

Osteoarthritis (OA) is the most common form of arthritis and is among the leading causes of disability throughout the world[1]. Although many risk factors have been associated with OA, the pathogenesis of OA is still incompletely characterized.[2,3] Inflammatory mechanisms play a crucial role in the pathogenesis and evolution of cartilage degredation. Recent studies have suggested that OA might be considered a chronic inflammatory disorder, and elevated levels of IL-1, tumor necrosis factor-α, IL-6 and other acute phase proteins are found in patients with cartilage degradation.[4,5] Among the cytokines, IL-1 is one of the most potent pro-inflammatory agents, and it seems to play an important role in signal transmission between cells of the tissue where inflammatory reactions occur.[6] IL-1 acts through its signalling receptor, IL-1 receptor type 1 (IL-1R1), and the degree of expression of this receptor influences the response of cells to IL-1.[7] Three structurally related ligands can bind to the IL-1 receptors: IL-1α and the more abundant IL-1β functioning as agonists, and IL-1 receptor antagonist (IL-1Ra) as an antagonist that results in no intracellular signalling.[8,9]

Genes encoding for cytokines have been associated with susceptibility for joint OA. Recent studies have indicated a role for the IL-1 gene cluster and the IL1RN gene in the development of OA of the hand, hip and knee.[10-12] We investigated the effect of polymorphisms in the IL1A, IL1B, and IL1RN genes in relation to the occurence of knee OA in Turkish patients.

Methods

Informed consent was obtained from each patient. The study was designed as an open, case-control trial and conducted according to the Declaration of Helsinki. All subjects were Caucasians from southern Turkey.

One hundred and seven patients with knee OA and 67 controls were included in this case control study. Diagnosis of knee OA was made according to the criteria of American College of Rheumatology. For assessment of cinical of the patients, the Turkish version of the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) was used.[13] All patients underwent anterior-posterior and lateral knee radiographs using standard procedures. Severity of radiographs was graded using the Kellgren and Lawrence scale (grade 1-4). Radiographs were assessed by one expert reader. In addition, venous blood samples were taken, and erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels were established.

Controls were enrolled from subjects admitted to the outpatient clinic of the physical therapy and rehabilitation department. Control subjects underwent clinical (criteria of American College of Rheumatology) and laboratory evaluations to exclude OA (hip, knee, hand, and vertebral) and other arthritis. The WOMAC index was not used and because their radiographs were normal, was not graded using the Kellgren and Lawrence scale. Control subjects had no relationship with cases and no family history of OA.

DNA extraction and analysis

Venous blood samples were collected in ethylenediaminetetraacetic acid (EDTA) containing tubes. DNA was extracted from whole blood by salting out procedure.[14].

Genotypic Analysis of the IL1RN VNTR Polymorphism

Polymerase Chain Reaction (PCR) assays were used to determine IL1RN VNTR polymorphism in intron 2. The oligonucleotide primers used to determine the VNTR in intron 2 polymorphism within the IL1RN gene were described previously.[15,16] The primers, forward 5'-CTCAGCAACACTCCTAT-3'; reverse 5'-TCCTGGTCTGCAGGTAA-3' were used to amplify of the IL1RN gene. PCR products of the IL1RN; Allel 1; 410 bp (four repeats), Allel 2; 240 bp (two repeats), Allel 3; 500 bp (five repeats), Allel 4; 325 bp (three repeats), Allel 5; 595 bp (six repeats).

Genotypic Analysis of the IL1B +3953 C→T Polymorphism

Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) assay were used to determine IL1B +3953 C→T polymorphism. The oligonucleotide primers used to determine the +3953 C→T polymorphism within the IL1B gene were described previously[17]. The primers, forward 5'-GTTGTCATCAGACTTTGACC-3'; reverse 5'-TTCAGTTCATATGGACCAGA-3' were used to amplify of the IL1B gene. The Taq I restricted products of IL1B +3953, CC, CT and TT genotypes had band sizes of 135bp/114bp, 249bp/135bp/114bp and 249bp, respectively.

Genotypic Analysis of the IL1A +4845 C→T Polymorphism

Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) assay were used to determine IL1A +4845 C→T polymorphism. The oligonucleotide primers used to determine the +4845 C→T polymorphism within the IL1A gene were described previously.[18] The primers, forward 5'-ATGGTTTTAGAAATCATCAAGCCTAGGGCA-3'; reverse 5'-AATGAAAGGAGGGGAGGATGACAGA AATGT-3' were used to amplify of the IL1A gene. The Sat I restricted products of IL1A +4845, CC, CT

TABLE 1. Characteristics of the study subjects

Characteristics	Patients (n:107)	Controls (n:67)	P
Age, mean ± SD, yr	61.3 ± 8.9	51.5 ± 8.9	0.0001
BMI, mean ± SD, kg/m²	29,9 ± 4,5	26.8 ± 4.1	0.0001
Female/Male ratio, n	81/26	47/21	0.569

and TT genotypes had band sizes of 124bp/76/29bp, 153bp/124bp/76bp/29bp and 153/76bp, respectively.

All procedures were conducted blinded to the case status and other characteristics of the participants. Scoring of gels and data entry were conducted independently by two persons.

Statistical Analysis

The association between polymorphisms of the IL1RN, IL1A, IL1B genes and knee OA was tested using multiple logistic regression model**.** Odds ratios (ORs) for this model with the corresponding 95 % confidence intervals (95 % CIs) were computed. Allele frequencies were assessed by counting alleles and calculating sample proportions. The allelic frequency distributions in the control and OA groups were compared by the Pearson chi-squared test. In each group the allele distribution was checked for deviations from Hardy-Weinberg equilibrium using an exact test. The associations between genotypes of IL1B+3953, IL1A+4845 and IL1RN VNTR polymorphisms with clinical, laboratory and radiological characteristics of knee OA were investigated using one way ANOVA, Kruskal-Wallis and Pearson chi-squared tests. Statistical analyses were performed with SPSS software, version 12. Results were considered statistically significant when the probability of findings occurring by chance was less than 5% ($P< 0.05$).

Results

The demographic characteristics of the study population are shown in Table 1. There were differences between groups in terms of age and BMI (P:0.0001) but no difference in terms of sex (P:0.569). The study population were adjusted for age (yr), and BMI (kg/m²) using multiple logistic regression model. Thereafter, the genotypes distribution of IL1RN VNTR, IL1A+4845 and IL1B+3953 genes polymorphisms were compered between OA patients and the control group. There were no differences between groups (Table 2).

TABLE 2. Genotypes distribution of IL1RN, IL1A, and IL1B genes polymorphisms between groups

Genotypes	Patients (%)	Controls (%)	P	OR	95 % CI
IL1RN VNTR					
4/4*	74 (69.2)	47 (70.1)	1.000	1	(-)
4/2	19 (17.8)	12 (17.9)	0.978	0,98	0,32-2,95
2/2	4 (3.7)	3 (4.5)	0.805	1,27	0,18-8,63
5/4	7 (6.5)	2 (3.0)	0.793	0,75	0,08-6,47
5/2	1 (0.9)	1 (1.5)	0.821	0,71	0,04-12,70
4/3	1 (0.9)	1 (1.5)	0.943	0,89	0,04-18,53
5/5	0 (0.0)	1 (1.5)	1.000	IR	IR
5/3	1 (0.9)	0 (0.0)	1.000	IR	IR
IL1A+4845					
CC*	53 (50.0)	34 (50.7)	0.482	1	(-)
CT	47 (44.3)	26 (38.8)	0.763	1,16	0,43-3,12
TT	6 (5.7)	7 (10,4)	0.314	0,39	0,06-2,42
IL1B+3953					
CC*	68 (63.6)	41 (61.2)	0.586	1	(-)
CT	32 (29.9)	22 (32.8)	0.608	0,77	0,29-2,06
TT	7 (6.5)	4 (6.0)	0.458	2,15	0,28-16,35

OR: Odds ratio, 95 % CI: 95 % confidence interval, IR: Inefficient results (simple size is too small), *: Reference genotype

Similarly, we detected no differences in allelic frequencies of IL1RN VNTR, IL1A+4845 and IL1B+3953 genes polymorphisms between the patients with knee OA and the controls (Table 3).

The relationships between genotypes of IL1RN VNTR, IL1A+4845 and IL1B+3953 genes polymorphisms and clinical characteristics (such as WOMAC pain, stiffness and physical function scores), laboratory measures (ESR, CRP), and K&L radiological

TABLE 3. Allelic frequency distribution of IL1RN, IL1A, and IL1B genes polymorphisms between groups

Alleles	Patients (%)	Controls (%)	P
IL1RN VNTR			
Allele 2	28 (13.1)	19 (14.2)	0.985
Allele 3	2 (0.9)	1 (0.7)	
Allele 4	175 (81.8)	109 (81.3)	
Allele 5	9 (4.2)	5 (3.7)	
IL1A+ 4845			
C	153 (72.2)	94 (70.1)	0.685
T	59 (27.8)	40 (29.9)	
IL1B +3953			
C	168 (78.5)	104 (77.6)	0.844
T	46 (21.5)	30 (22.4)	

scores are shown in table 4 and 5. In addition, we did not find any significant differences between genotypes of IL1RN, IL1A, and IL1B genes polymorphisms with respect to the clinical characteristics, laboratory findings, and radiological scores in patients with knee OA.

Discussion

In the present study, three polymorphisms (VNTR in intron 2, +4845, and +3953 respectively) of the IL1RN, IL1A IL1B genes were assessed, genotypic and allelic frequencies were compared between patients and controls. No differences were found, which suggested that the IL1RN, IL1A, IL1B genes polymorphisms were not markers of genetic susceptibility to knee OA in Mersin. Furthermore, our study also suggested that IL1RN, IL1A, IL1B genes polymorphisms were unrelated to the clinical, radiological and laboratory characteristics of knee OA.

In contrast to our results, some researchers have suggested association between the IL-1 gene cluster and occurence of OA.[0-12] Previously, Moos et al. investigated the distribution of polymorphic alleles of four different genes encoding TNF-α, IL1RN, IL1B and IL-6 in knee or hip OA patients with controls. The

TABLE 4. The relationship between genotypes of IL1RN, IL1A, IL1B polymorphisms and clinical, laboratory findings of patients with knee OA

	Genotypes	WPS mean±SD	WSS mean±SD	WPFS mean±SD	ESR(mm/h) mean±SEM	CRP(mg/L) mean±SEM
IL1RNVNTR	4/4	14.4±3.5	5.0±2.1	46.1±12.8	23.4±2.2	3.5±0.4
	4/2	13.5±4.6	4.5±2.8	45.3±15.2	21.6±4.7	2.9±0.4
	2/2	13.0±3.5	4.5±2.6	55.0±14.8	13.2±5.1	1.5±0.3
	5/4	15.4±2.6	5.0±1.7	57.7±21.5	31.1±3.3	4.3±1.5
	5/2	12.0	5.0	52	6.0	0.5
	4/3	16.0	4.0	53	6.2	3.4
	5/3	12.0	3.0	39	5.0	1.2
P		0.802	0.917	0.373	0.207	0.544
IL1A+4845	CC	13.9±3.8	4.6±0.3	44.4±14.0	23.2±2.3	3.1±0.3
	CT	14.0±3.2	4.9±0.2	48.7±13.1	23.3±3.0	3.1±0.4
	TT	16.6±1.9	5.3±0.8	53.8±14.0	26.8±8.4	7.2±3.8
P		0.198	0.683	0.132	0.907	0.519
IL1B+3953	CC	13.7±0.4	4.8±2.2	46.3±1.5	25.0±2.3	3.4±0.4
	CT	14.8±0.6	4.9±2.1	48.5±2.8	19.4±3.0	2.4±0.3
	TT	16.5±1.3	5.1±3.0	48.2±6.3	24.1±8.2	6.7±3.2
P		0.085	0.988	0.743	0.384	0.147

WPS: WOMAC pain subscale, WSS: WOMAC stiffness subscale, WPFS: WOMAC physical function subscale

TABLE 5. The relationship between genotypes of IL1RN, IL1A, IL1B polymorphisms and radiological findings of patients with knee OA

Genotypes	The Kellgren Lawrence Scale				P
	Grade 1 (n, %)	Grade 2 (n, %)	Grade 3 (n, %)	Grade 4 (n, %)	
IL1RN VNTR					
4/4	19 (25.7)	26 (35.1)	24 (32.4)	5 (6.8)	0.565
4/2	3 (15.8)	12 (63.2)	4 (21.1)	0 (0.0)	
2/2	1 (25,0)	2 (50.0)	0 (0.0)	1 (25.0)	
5/4	1 (14.3)	2 (28.6)	3 (42.9)	1 (14.3)	
5/2	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	
4/3	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	
5/3	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	
IL1A+4845					
CC	12 (22.6)	23 (43.4)	15 (28.3)	3 (5.7)	0.871
CT	10 (21.3)	17 (36.2)	17 (36.2)	3 (6.4)	
TT	1 (16.7)	3 (50.0)	1 (16.7)	1 (16.7)	
IL1B+3953					
CC	13 (19.1)	31 (45.6)	21 (30.9)	3 (4.4)	0.306
CT	9 (28.1)	8 (25.0)	11 (34.4)	4 (12.5)	
TT	2 (28.6)	4 (57.1)	1 (14.3)	0 (0.0)	

analysis of genotype frequencies for the IL1B gene, more OA patients than controls were homozygous for allele 2, although any significant diffferences for the TNF-α, IL1RN and IL-6 polymorphisms were found.[11]

In the later study by Loughlin et al, seven single nucleotide polymorphisms (SNPs) and a VNTR polymorphism from within the IL-1 ligand genes IL1A (-889), IL1B (3954, 5810, -31, -511) and IL1RN (VNTR, 9589, 11100) were genotyped in knee or hip OA cases and controls. No association was detected with any of the variants. When they stratified their cases by site of disease (hip or knee), they detected a marginal association for SNP –889 in knee cases.[12] In contrast to Moos et al, there was no evidence for an association of this gene cluster to hip OA, which may reflect heterogenity of susceptibility between these different joint sites.

Recently, Meulenbelt et al, two polymorphisms of the IL1B gene (3953 and –511) and one polymorphism of the IL1RN gene (VNTR in intron 2) were assessed for the occurence of radiographic OA (ROA) of the hips, knees, hands, and disc dejeneration of the spine in a cohort. In contrast to results of Moos et al, a protective effect for hip ROA was observed in carriers of allele 2 of the 3953 polymorphism. Similar to Moos et al, a predisposing effect for hip ROA was observed in carriers of allele 2 of the -511 and VNTR polymorphisms. Moreover, when combining the risk alleles, an additive effect was observed in subjects carrying in creasing numbers of risk alleles of either the –511 or the VNTR polymorphism with a linear by linear association.[19]

Much of our research has focused on individual polymorphisms or small numbers of polymorphisms in single genes. This approach may be oversimplistic due to the complex interaction of the ligands and receptors involved in the transduction an inhibition of IL-1 signalling. Smith et al. found no association between promoter haplotypes in the IL1R1 gene and knee OA but they identified an extended IL1A, IL1B, IL1RN risk haplotype (2C-CTG-1TT), which was more frequent in patients with knee OA. In an analysis of IL1B-IL1RN region haplotypes, one of these (CCA-1TT) was associated with a protective effect against knee OA.[20] Their results may help to explain

previous reports of linkage from some groups between markers in IL-1 region and OA in genome-wide-scans.

OA is a complex genetic disease that is among the most difficult to analyze, due to its high frequency in the general population and its extensive clinical heterogeneity. The degree to which clinical heterogeneity translates into genetic heterogeneity is unknown. However, a number of epidemiological studies have highlighted potential differences in the degree of OA heritability between different joint groups.[21,22] Recent studies support the approach of stratifying for site of disease when attempting to identify OA susceptibility genes.[12]

Our study has some limitations. First, the size of the study population is small and our findings need to be confirmed with larger samples in Turkey. Second, in this study we did not make haplotype analysis of IL-1 gene cluster and this may be the reason for lack of association with knee OA. Recent reports have indicated that polymorphic IL-1 haplotypes, as opposed to single locus polymorphisms may provide better genetic markers of transcriptional activity and disease association.[20,23] Another reason for the lack of association may be different effectively polymorphisms within IL-1 region on knee OA in Turkish patients. Finally, we cannot exclude evidence for an association of a haplotype within the IL-1 ligand gene cluster to knee OA. Further studies would be necessary to evaluate the real role of the polymorphic IL-1 haplotypes or extended haplotypes in Turkish patients with knee OA.

References

1. Elders MJ. The increasing impact of arthritis on public health. J Reumatol 2000;60: 6-8
2. Sharma L. Local factors in osteoarthritis. Curr Opin Rheumatol 2001;13: 441-6
3. Sowers M. Epidemiology of risk factors for osteoarthritis: systemic factors. Curr Opin Rheumatol 2001;13: 447-51
4. Nakamura H, Yoshino S, Kato T, et al. T-cell mediated inflammatory pathway in osteoarthritis. Osteoarthritis Cartilage 1999;7: 401-2
5. Fernandes JC, Martel-pelletier J, Pelletier JP. The role of cytokines in osteoarthritis pathophysiology. Biorheology 2002;39: 237-46
6. Eisenberg SP, Evans RJ, Arend WP. Primary structure and functional expression from complementary DNA of a human interleukin 1 receptor antagonist. Nature 1990;343: 341-6
7. Sims JE, Gayle MA, Slack JL, et al. Interleukin 1 signaling occurs exclusively via the type 1 receptor. Proc Natl Acad Sci USA 1993;90: 6155-9
8. Sims JE, Dower SK. Interleukin-1 receptors. Eur Cytokine Network 1994;5: 539-46
9. Taylor SL, Renshaw BR, Garka KE et al. Genomic organisation of the interleukin-1 locus. Genomics 2002;79: 726-33
10. Leppavuori J, Kujala U, Kinnunen J, et al. Genome scan for predisposing loci for distal interphalangeal joint osteoarthritis: evidence for a locus on 2q. Am J Hum Genet 1999;65: 1060-7
11. Moos V, Rudwaleit M, Herzog V, et al. Association of genotypes affecting the expression of interleukin-1beta or interleukin-1 receptor antagonist with osteoarthritis. Arthritis Rheum 2000;43: 2417-22
12. Loughlin J, Dowling B, Mustafa Z, et al. Association of the interleukin-1 gene cluster on chromosome 2q13 with knee osteoarthritis. Arthritis Rheum 2002;46: 1519-27
13. Tuzun EH, Eker L, Aytar A, et al. Acceptability, reliability, validity and responsiveness of the Turkish version of VOMAC osteoarthritis index. Osteoarthritis Cartilage 2005;13: 28-33
14. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16:1215
15. Katila H, Hanninen K, Hurme M. Polymorphisms of the interleukin-1 gene complex in schizophrenia. Mol Psychiatry 1999;4: 79-81
16. Bajnok E, Takacs I, Vargha P, et al. Lack of association between interleukin-1 receptor antagonist protein gene polymorphism and bone mineral density in Hungarian postmenopausal women. Bone 2000;27:559-62
17. Hurme M, Santtila S. IL-1 receptor antagonist (IL-1Ra) plasma levels are co-ordinately regulated by both IL-1Ra and IL-1beta genes. Eur J Immunol 1998;28:2598-602
18. Yucesoy B, Vallyathan V, Landsittel DP, et al. Association of tumor necrosis factor-alpha and interleukin-1 gene polymorphisms with silicosis. Toxicol Appl Pharmacol 2001;172:75-82
19. Meulenbelt I, Seymour AB, Nieuwland M, et al. Association of the interleukin-1 gene cluster with radiographic signs of osteoarthritis of the hip. Arthritis Rheum 2004;50:1179-86
20. Smith AJ, Keen LJ, Billingham MJ, et al. Extended haplotypes and linkage disequilibrium in the IL1R1-

IL1A-IL1B-IL1RN gene cluster: association with knee osteoarthritis. Genes Immun 2004;5:451-60
21. MacGregor AJ, Spector TD. Twins and the genetic architecture of osteoarthritis. Rheumatology (Oxford) 1999;38: 583-90
22. Chitnavis J, Sinsheimer JS, Clipsham K, et al. Genetic influences in end-stage osteoarthritis: sibling risks of hip and knee replacement for idiopathic osteoarthritis. J Bone Joint Surg Br 1997;79:660-4
23. Cox A, Camp NJ, Nicklin MJ, et al. An analysis of linkage disequilibrium in the interleukin-1 gene cluster, using a novel grouping method for multiallelic markers. Am J Hum Genet 1998;62:1180-8

Correspondence to:

Dr. Melek Sezgin
Liparis Plaza-1, H Blok, 7/15 Mezitli, Mersin, Turkey
Email: msezgin@mersin.edu.tr