Potential influence of interleukin-1 receptor antagonist gene polymorphism on knee osteoarthritis risk

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Abstract. *Objectives:* Genes encoding for cytokines have been associated with susceptibility for joint osteoarthritis (OA) and interleukin (IL)-1 gene is supposed to be involved in the cartilage destruction process. In this regard, interleukin-1 receptor antagonist (IL-1RA) competing with IL-1 for binding to its receptor may act as an inhibitor of cartilage breakdown. We assessed the association of primary knee OA with IL-1RA region as a putative factor of susceptibility to knee OA in Egyptian patients.

Design and methods: Eighty patients with primary knee OA and 40 aged-matched healthy controls were included into the study. DNA samples were used to study genotypes of IL-1RN gene by polymerase chain reaction (PCR) in both groups.

Results: An increased frequency of the IL-1RN*1 and IL-1RN*2 alleles was found in OA patients relative to controls (60.5% vs. 39.5%, $P = 0.039$, 85.4% vs. 14.6%, $P = 0.002$, respectively) however, only the carriage rate of IL-1RN*2 allele was found to be significant when OA patients were compared to the controls. Significant higher frequencies of IL-1RN*1/*2 and IL-1RN*2/*2 genotypes in OA patients were observed as compared with controls. Both visual analogue scale (VAS) and radiographic score revealed significant correlation with both the allelic frequency and the carriage rate of IL-1RN*2 allele. Moreover, absolute frequency of IL-1RN*1/*2 genotype OA patients revealed severe VAS and high radiographic score.

Conclusion: These results suggest that IL-1RN*2 allele represent a significant factor influencing the severity and course of knee OA; thereby supporting the potential role of IL-1 in the pathogenesis of this disease.

Keywords: Knee osteoarthritis, interleukin-1, interleukin-1 receptor antagonist

1. Introduction

Osteoarthritis (OA) is one of the most common aging diseases characterized by degeneration of joint articular cartilage and remodeling of the subchondral bone [1]. The aetiopathogenesis of the disease is not yet fully understood; however, population-based studies and ge-

netic linkage studies in OA families suggest that OA is a multifactorial disease with genetic contribution [2].

Several cytokines are involved in cartilage metabolism and are synthesized by synovial cells and cartilage chondrocytes, among them interleukin-1 (IL-1) [3]. The IL-1 gene family contains three related genes, *IL-1A*, *IL-1B* and *IL-1RN*, which encode the pro-inflammatory cytokines IL-1 α , IL-1 β , and their naturally occurring antagonist, interleukin-1 receptor antagonist (IL-1RA), respectively. IL-1RA acts by competitive blockade of IL-1 receptor without affecting downstream signaling [4]. The polymorphic region within intron 2 of the IL-1RN* gene contains a variable numbers of tandem repeats (VNTR) of 86 bp; five alleles of the IL-1RN* have been reported (*1 to *5),

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corresponding to 2, 3, 4, 5 and 6 copies of the 86-bp sequence, respectively; the most frequent allele was designated as *1. Since three potential protein-binding sites are located in the 86-bp sequence, it is likely that the number of repeats may influence gene transcription and protein synthesis [5]. Although allele 2 (IL-1RN*2) was found to be more frequent among patients with autoimmune diseases such as systemic lupus erythematosus, Sjogren's syndrome or juvenile idiopathic arthritis [6,7], the data on its association with OA are still contradictory [2].

Finding a genomic region likely to contain pathogenic variants will be a big step toward explaining OA pathogenesis. We therefore examined the influence of a polymorphism within intron 2 of the IL-1 receptor antagonist gene (IL-1RN) on the susceptibility or severity of knee osteoarthritis (OA) among Egyptian patients.

2. Materials and methods

2.1. Study population

The study was approved by the Medical Ethics Committee of the Al-Azhar University, and written informed consent was obtained from all participants. The study population included 80 patients with OA recruited from the Orthopedic and Rheumatology Departments, Al-Azhar University Hospital. All the patients diagnosed with knee-OA were recruited according to the clinical criteria of Altman [8]. Patients were subjected to functional assessment of OA using Western Ontario and McMaster Universities (WOMAC) Osteoarthritis Index [9] and Joint pain assessment using Visual Analogue Scale (VAS) [10], BMI was calculated as prior described [11]. Family history was considered positive if first-degree relatives exhibited radiographically visible primary generalized OA disease. Radiographic views were done to all patients, and all films were assessed by one expert reader and scored for the following radiographic features: Joint space narrowing in both lateral and medial compartments, presence of Osteophytes, presence of subchondral sclerosis and cyst formation [12]. The symptoms best correlate with joint space narrowing, and sometimes the classification of OA into radiographic versus symptomatic OA is used. An overall OA grading was based on the Kellgren-Lawrence grading (KL) scale (grades 1-4) [13]. Exclusion criteria were the presence of malignancy, history of trauma, or any clinical evidence of bone or joint inflammation. Forty healthy volunteers without clinical

and radiological evidence of OA, were used as controls: all comprising individuals were admitted for ambulation at the Department of the Orthopedics for post-traumatic injuries or other symptomatic problems that did not correlate with OA. All control subjects underwent clinical examination and X-ray evaluation in order to exclude the presence of knee OA. Details of all demographic and clinical characteristics for the study population are summarized in (Table 1).

2.2. Blood sample and DNA isolation

Peripheral venous blood samples of 3 ml were drawn from each individual by standard venipuncture. Each blood sample was collected in sterile tubes with EDTA. Genomic DNA was isolated from buffy coats by using commercially available QIA amp DNA Blood kit (Qiagen, Hilden, Germany) [14]. Concentration of the extracted DNA was measured by NanoDrop ND-1000 (NanoDrop Tech., Wilmington, USA).

2.3. Genotyping of IL-1RN gene

Genomic DNA was assayed with polymerase chain reaction (PCR) for the detection of IL-1RN gene with *Taq* PCR core kit (Qiagen, catalogue no# 51104). To amplify the 86-bp tandem repeat region in intron 2 of IL-1RN gene, we used 2 flanking primers (Gene Bank accession number X64532) (sense 5' -CTCAGCCAACACTCCTAT-3' and antisense 5'-TCCTGGTCTGCAGGTAA-3') as described by Coskun and his colleagues [15].

Briefly, the PCR reaction was performed using a cocktail of 500 ng of genomic DNA, 0.5 μ M of each oligonucleotide primer, 0.2 mmol/l of dNTPs, 1.5 mmol/l $MgCl_2$, and 2.5 units of *AmpliTaq* polymerase in a final reaction volume of 50- μ l, in a Mastercycler gradient thermocycler (Eppendorf, Hamburg, Germany) with an initial denaturation step of 94 °C for 3 min and a final extension step 10 min at 72 °C. The following thermal profile was repeated for 35 cycles: denaturation for 1 min at 94 °C, annealing for 1 min at 60 °C, and extension for 1 min at 72 °C. PCR products were separated on 1% agarose gel and visualized by ethidium bromide with particular alleles being determined using a 100-bp size DNA ladder (Amersham Pharmacia Biotec); IL-1RN*0 (one repeat) is 150 bp, IL-1RN*1 (four repeats) is 410 bp, IL-1RN*2 (two repeats) is 240 bp, IL-RN*3 (three repeats) is 325 bp, IL-1RN*4 (five repeats) is 500 bp and IL-1RN*5 (six repeats) is 595 bp [16,17]. As shown in (Fig. 1).

Table 1
Characteristics of patients and control individuals

Demographic feature	Control group (n = 40)	OA group (n = 80)	P-value
Age (yrs)	50 ± 2.3	55.5 ± 5	0.0001 ^a
Gender status (males/females)	8/32	18/62	NS
BMI (kg/m ²)	283.4 ± 15.4	301.8 ± 45.5	NS
Family history (negative/positive)	32/8	16/64	0.0001 ^b
Visual analogue scale (VAS) (no.)			0.0001 ^b
No pain	40	–	
Mild	–	18	
Moderate	–	32	
Sever	–	30	

Significant at *P*-value < 0.05, ^ausing t-test and ^bChi-square test.

Fig. 1. Interleukin-1 receptor antagonist genotypes. Agarose gel (1%) displaying IL-1RN* genotypes in eight knee OA patients: heterozygotes *1/2 (lanes 1-5, 7), homozygotes *1/1 (lanes 6) and heterozygotes *1/3 (lane 8). Lane M: molecular weight DNA ladder standard (Amersham Pharmacia Biotec).

2.4. Statistical analysis

Demographic and clinical data between groups were compared by Chi-square (X^2) test and t test. Genotype and allele frequencies were compared by Chi-square (X^2) test. Odds ratios were calculated with 95% CI. To estimate the association between genotype and OA, a logistic regression analysis was used. In addition, we evaluated the associations between genotype and radiographic severity, as measured by KL scores. Statistical analysis of the data was performed with Statistical Package for Social Science software (SPSS INC.,

Chicago, IL). Two-tailed analyses were performed, and *P* values less than 0.05 were considered statistically significant.

3. Results

Among the cohort of 80 patients with OA and a control group of 40 healthy individuals analyzed; the demographic characterized for OA patients and the control groups are shown in (Table 1). IL-RN genotyping, allelic and carriage frequencies studied were found

Table 2
Genotypic frequencies of the IL-RN in OA patients and controls

IL-RN* genotypes	OA patients (N = 80)		Control (N = 80)		Odd ratio	Relative risk	95% CI	P
	N	Freq.%	N	Freq.%				
*1/*1								
–ve	56	70	12	30	0.184	2.33	0.08–0.42	0.0001
+ve	24	30	28	70				
*1/*2								
–ve	22	27.5	32	80	10.55	0.276	4.215–26.315	0.0001
+ve	58	72.5	8	20				
*2/*2								
–ve	68	85	38	95	3.353	0.333	0.713–15.777	0.108
+ve	12	15	2	5				
*1/*3								
–ve	80		40	–	–	–	–	–
+ve	–		–					

Table 3
Allelic frequencies of the IL-RN in OA patients and controls

IL-RN* alleles	OA patients (N = 80)		Control (N = 40)		Odd ratio	Relative risk	95% CI	P
	N	Freq.%	N	Freq.%				
IL-RN*1	98	60.5	64	39.5	0.395	1.306	0.210–0.745	0.003
IL-RN*2	70	85.4	12	14.6	4.407	0.343	2.214–8.775	0.0001
IL-RN*3	0	0	0	0	0	0	0	0

to be in accordance with the Hardy-Weinberg-Castle equilibrium.

3.1. Genotyping frequencies of IL-RN among the studied groups

The observed frequencies of individual IL-RN genotypes are shown in (Table 2). An increased frequency of the IL-RN*1/*1 genotype was found in patients with knee OA in comparison with controls ($P = 0.003$). In addition, a similar pattern was seen for the frequency of the IL-RN*1/*2 genotype, which was significantly increased in OA patients as compared to controls ($P = 0.0001$). Although the frequency of the IL-RN*2/*2 genotype was slightly higher in patients with knee OA as compared to controls; the frequency did not reach a statistically significant value.

3.2. Allelic and carriage frequencies of IL-RN

An increased allelic frequency of IL-RN*1 was found in patients with knee OA in comparison with controls (60.5% vs 39.5% at $P = 0.039$). Similarly, the allelic frequency of IL-RN*2 was increased in patients with knee OA in comparison with controls (85.4% vs 14.6% at $P = 0.002$), as shown in (Table 3). Regarding the carriage frequency of IL-RN*, a significant higher carriage rate of allele IL-RN*2 was detected in the

group of patients as compared to controls (72.5% vs 25% at $P = 0.0001$) (Table 4).

3.3. Correlation between VAS and alleles, carriage and genotype of IL-RN

Based on the visual analogue scale (VAS) for pain score; there were 18 OA patients with mild VAS, 32 patients with moderate and 30 patients with severe VAS. As shown in (Table 5), the carriage and the allele frequencies of IL-RN*2 were detected in all OA patients (100%) with severe VAS ($P = 0.0001$). Similarly, the frequency of the IL-RN*1/*1 and IL-RN*1/*2 genotypes were highest in OA patients with severe VAS ($P = 0.0001$). When considering the radiographic score, 34 patients were KL [1,2] scale and the remaining were KL [3,4] scale. Patients with high radiographic scores were significantly associated with OA patients having carriage and the allele frequencies of IL-RN*2, as shown in (Table 5). All demographic factors were included in the linear regression analysis, only family history and VAS for pain score revealed significant results. Family history showed significant relation with the carriage and the allele frequencies of IL-RN*2 at ($R = 0.252$, $P = 0.024$) for both. Similarly, VAS for pain score revealed significant relation with the carriage and the allele frequencies of IL-RN*2 at ($R = 0.711$, $P = 0.0001$) for both. The frequency of IL-RN*1/*2

Table 4
Carriage rates of the IL-RN in OA patients and controls

IL-RN* carriage rates	OA patients (N = 80)		Control (N = 40)		Odd ratio	Relative risk	95% CI	P
	N	Freq.%	N	Freq.%				
*1	74	92.5	36	90	1.37	0.973	0.364–5.163	NS
*2	58	72.5	10	25	7.909	0.345	3.321–18.836	0.0001
*3	0	0	0	0	0	0	0	0

Table 5
Alleles and genotypes frequencies in OA patients regarding the visual analogue scale (VAS) and radiographic OA score

	Visual analogue scale (VAS) (%)			P	Radiographic score		
	Mild (n = 18)	Moderate (n = 32)	Severe (n = 30)		KL 1–2 (n = 34)	KL3–4 (n = 46)	P
Allele frequency							
1	16 (88.9)	28 (87.5)	30 (100)	0.141	32 (94.1)	42 (91.3)	0.637
2	2 (11.1)	26 (81.3)	30 (100)	0.0001	19 (55.9)	39 (84.8)	0.004
Carriage frequency							
1	16 (88.9)	28 (87.5)	30 (100)	0.141	32 (94.1)	42 (91.3)	0.637
2	2 (11.1)	26 (81.3)	30 (100)	0.0001	19 (55.9)	39 (84.8)	0.004
Genotype frequency							
1/*1							
–ve	4 (22.2)	22 (68.8)	30 (100)	0.0001	20 (58.8)	36 (78.3)	0.061
+ve	14 (77.8)	10 (31.3)	–		14 (41.2)	10 (21.2)	
1/*2							
–ve	16 (88.9)	6 (18.8)	–	0.0001	15 (44.1)	7 (15.2)	0.003
+ve	2 (11.1)	26 (81.3)	30 (100)		19 (55.9)	39 (84.8)	
2/*2							
–ve	18 (100)	28 (87.5)	22 (73.3)	0.038	14 (41.2)	8 (17.4)	0.004
+ve	–	4 (12.5)	8 (26.7)		20 (58.8)	38 (82.6)	

genotype was significantly related with family history and VAS for pain score at ($R = 0.252$, $P = 0.024$, and $R = 0.711$, $P = 0.0001$, respectively), while the frequency of IL-RN*1/*1 genotype revealed negative correlation with VAS for pain score at ($R = -0.632$, $P = 0.0001$). Using multivariate logistic regression analysis, among the epidemiological factors, age has strong association with prevalence and severity of knee OA (Wald 6.7, $p < 0.0001$). Family history showed a significant association with the severity of knee OA (Wald 4.3, $p < 0.028$). Moreover, the severity of OA was significantly associated with polymorphism (Wald 3.2, $p < 0.043$).

4. Discussion

The present study was performed to delineate the suspected role of the cytokines IL-RN in the pathogenesis of OA. Overall, a significant difference was observed between the two studied groups (80 OA; 40 controls) regarding the age; the mean age for the OA patients was higher than the control group. This finding agrees with previous reports that OA was strongly correlated with

age, with an almost exponential increase in prevalence after the age of 50 years [18]. Regardless of how OA is defined, it is uncommon under the age of 40 years but prevalent in persons older than 60 years [18–20]. On the subject of the gender status, OA was increased in females (77.5%) as compared to males (22.5%), a result that is consistent with previous findings [21] which show that OA has a higher prevalence and is more often generalized in women than in men. In addition to age and sex as systematic risk factors for OA, family history was concerned. According to our results, sixty-four out of eighty (80%) OA patients had a positive family history of OA as compared to sixteen (20%) had a negative family history. This finding agreed with previous reports that the prevalence of OA among relatives is 2–3-fold increases in risk of OA relative to controls [22].

Cytokines are key players in pathogenesis of synovitis and cartilage destruction associated with OA [23]. Difference in levels of various cytokines among different individuals can be a possible explanation for differences in disease susceptibility and severity. These variations are mostly due to polymorphism in the genes encoding for cytokines. IL-1, a cytokine produced by monocytes mediates cartilage and bone destruction in

OA. IL-1 α , IL-1 β and their naturally occurring antagonist, IL-1RA are coded by genes located within a 430 KB region. IL-1RA counteracts the action of IL-1 by binding to IL-1 receptor without activating it [24].

In this study, the genotypic frequencies of IL-RN1*/1* and IL-RN1*/2* were significant when OA patients were compared to control group. Similarly, the allelic frequencies of IL-RN*1 and IL-RN*2 were found to be significantly increased in patients with knee OA in comparison with controls. Understanding the genetic contribution to OA has two important clinical implications. First, by finding genes involved in disease risk or involved in progression, we will better understand the molecular pathogenesis of OA, which may open areas for therapeutic intervention. Second, by identifying sets of genetic variants associated with risk for disease or with progression of OA, it will be possible to detect individuals at high risk and to monitor disease progression better [25]. To provide further insight into the role of IL-1RA in Knee OA, we also assessed the carriage rate of IL-RN*1 and IL-RN*2 where the number of individuals with at least one copy of a specific allele divided by the total number of individuals within the group. Accordingly, the carriage rate of IL-RN*2 was significantly increased in the knee OA patients when compared with the control group. The possible mechanisms of association of the IL-1RN*2 allele to the knee OA are still unclear. The functional significance of the IL-1RN*2 allele is not known yet, particularly with respect to disturbances of the IL-1/IL-1RA balance, which influences physiological and pathophysiological effects of IL-1. IL-1 represents one of the most important local mediators of cartilage destruction. Articular chondrocytes are very sensitive both to IL-1 β stimulation and to induction of IL-1 β production by long-lasting mechanical stress [26]. In this regard, the IL-1RA protein (encoded by the IL-1RN gene) might serve as a potent local inhibitor of cartilage degradation, which restores IL-1/IL-1RA balance directly within the affected joint [2].

One might consider the influence of using Visual Analogue Scale (VAS) clinical criteria for knee OA to do a knee radiograph. As the control group were examined and classified as healthy normal lacking VAS clinical criteria but not subjected to radiograph. While knee OA patients were classified according to the VAS score into mild ($n = 18$), moderate ($n = 32$) and severe ($n = 30$). In our study, positive correlation was reported between VAS score and radiographic score with both the allelic frequency and the carriage rate of IL-1RN*2 as well as the genotypic frequency of IL-RN1*/

2*. Moreover, the distribution of both the allelic frequency and the carriage rate of IL-1RN*2 were found absolute in patients with severe knee OA ($P = 0.0001$), also all patients with positive genotypic frequency of IL-RN1*/2* were reported to be with severe knee OA ($P = 0.0001$). On the other hand negative correlation was reported between VAS score and genotypic frequency of IL-RN1*/1* as all patients with negative genotypic frequency of IL-RN1*/1* were considered as severe knee OA ($P = 0.0001$). Accordingly IL-1 network may be important in OA progression and thus severity because IL-1 stimulates cartilage degradation and induces production of other substances that also serve to foster cartilage breakdown, an IL-1-related locus represents a biologically reasonable candidate gene for OA [27].

In conclusion, an increased frequency and carriage rate of the IL-1RN*2 allele, as well as the frequency of the IL-1RN*1/*2 genotype, were found in Egyptian patients with knee OA which might rather suggest a purely predisposing role of this allele in the development of knee OA. Although we recognize that the limited number of patients in this study comprise just a retrospective study, and future studies based on large number of patients to confirm these findings are highly recommended. Once this is achieved, this will supports the hypothesis that variations of genes encoding for inflammatory cytokines, such as IL-6, may play an important role in the series of events responsible for the pathophysiology of OA.

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