



## Association of interleukin-1 receptor antagonist (*IL1RN*) genetic variants with severity of knee osteoarthritis

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### Abstract

Osteoarthritis (OA) is a chronic musculoskeletal disorder, characterized by degeneration of articular cartilage and decreased motion range of affected joints. It is regarded as the most common cause of disability among elderly population worldwide. This study aimed to detect interleukin-1 receptor antagonist (*IL1RN*) genotypes at single nucleotide polymorphism (SNP) rs9005 and rs315943, in patients with knee osteoarthritis, and to clarify their influence on susceptibility and severity of the osteoarthritic disease. Forty-seven unrelated Egyptian patients with primary knee OA and thirty-six control subjects, with matched age, sex and body mass index were included in this study. All patients were subjected to full history taking, general examination and complete knee and joint examination including assessment of the severity of knee OA clinically using the Lequesne's algofunctional Index and radiological grading by the Kellgren-Lawrence (KL) grade scale. Genotyping assays of *IL1RN* at SNP rs9005 and rs315943 were performed using real time PCR. *IL1RN* rs9005 AG, GG genotypes, G allele, as well as rs9005-rs315943 GC haplotype were associated with risk of OA development, while AChaplotype was associated with protective effect against OA development. Younger age of disease onset was associated with rs9005 GG genotype. Higher pain, maximum distance walked, activities of daily living (ADL), Lequesne's scores and KL grading were associated with rs9005 GG, rs315943 CC genotypes and GC haplotype. Whereas, lower pain, maximum distance walked, ADL, Lequesne's scores and KL grading were associated with rs9005 AA, rs315943 TT and AT haplotype.

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### Keywords

- Interleukin-1 receptor antagonist gene
- knee osteoarthritis
- SNP

## Introduction

Osteoarthritis (OA) is the most common type of arthritis. It is associated with continuous breakdown of the cartilage, pain, stiffness and eventually, decreased motion range of affected joint. It is regarded as the greatest cause of disability between elderly population worldwide (1). Although, some patients show a relatively stable course with slight symptomatic changes with time, others, rapidly develop severe structural disability leading to joint replacement (2).

OA is a multifactorial disease, which is attributed to interaction between several local and systemic factors(3). Currently, the inflammatory reaction is believed to play an important role in the development and progression of OA, even at the early stages, with production of variable amounts and types of cytokines, according to the disease stage and duration (4). Proinflammatory cytokines such as interleukin-1 alpha/beta (IL-1 $\alpha/\beta$ ) and IL6 represent the main players in OA. They cause cartilage degradation by increasing the expression and activity of proteolytic enzymes, matrix metalloproteinases (MMPs), chemokines, nitric oxide, prostaglandins and leukotrienes (5).

Interleukin-1 receptor antagonist (IL1RN or IL-1Ra) is a member of the IL-1 family, which also binds to IL-1RI but without initiation of the intracellular signaling cascade. Thus, it is regarded as a naturally occurring competitive inhibitor of IL-1 $\alpha$  and IL-1 $\beta$ . The imbalance between IL1 $\alpha/\beta$  and IL1RN has been implicated in progressive cartilage loss during OA course (6).

*IL1RN* belongs to *IL1* gene cluster on chromosome 2q13. It is a highly polymorphic gene that contains

different SNPs as well as variable numbers of tandem repeats (VNTR) of 86 bp (7). Genetic variations of the *IL-1RN* have been investigated in various chronic inflammatory diseases including rheumatoid arthritis, systemic lupus erythematosus, ulcerative colitis and OA with inconclusive results (8 and 9).

The aim of this work was to detect interleukin-1 receptor antagonist (*IL1RN*) gene SNP rs9005 and rs315943 genotypes in patients with knee osteoarthritis and clarify the influence of these SNPs on susceptibility and severity of the osteoarthritic disease.

## Subjects and Methods

This case-control study was performed on forty-seven (47) unrelated Egyptian patients with primary OA diagnosed according to the American College of Rheumatology (ACR) classification criteria of knee osteoarthritis(10). Patients were selected from among those attended or admitted to the Outpatients' Clinic and In-Patients' Department of Rheumatology, Rehabilitation and Physical Medicine, Benha University Hospitals. Thirty-six (36) subjects of matched age, gender and BMI were recruited as a control group. The exclusion criteria were: (i) Patients not fulfilling the (ACR) classification criteria of knee osteoarthritis (ii) BMI >25 (iii) History of repeated trauma or surgery to the joint structures (iv) crystal arthropathy as (gout, Calcium Pyrophosphate Deposition) (v) Previous inflammatory arthritis (e.g. rheumatoid arthritis or other autoimmune disease), (iv) Heritable metabolic causes (e.g. alkaptonuria, hemochromatosis, and Wilson's disease), (iiv) Hemoglobinopathies (e.g. sickle cell disease and thalassemia),(iiiv) Neuropathic

disorders leading to a Charcot joint (e.g. syringomyelia and tabes dorsalis), (ix) Underlying morphologic risk factors (e.g., congenital hip dislocation and slipped femoral capital epiphysis), (x) Disorders of bone (e.g. Paget disease and avascular necrosis), (xi) Previous surgical procedures (e.g. meniscectomy) and (xii) Diabetes mellitus.

This study was conducted at Molecular Biology and Biotechnology Unit after approval by the Research Ethics Committee of, Faculty of Medicine, Benha University. A written informed consent was obtained from each participant before enrollment in the study.

All patients were subjected to: Full history taking, general examination and complete knee and other joints examination. Body mass index: The body mass index (BMI) was calculated for all subjects included in the study as follows: the body mass (in kilograms) was divided by the square of the body's height (in meters). Indices of clinical severity of knee OA were assessed according to Lequesne's algofunctional Index for knee OA (11). Radiological investigations: Both knees: anteroposterior and lateral views. Grading of the radiological findings and severity was done according to grading of Kellgren and Lawrence (KL) (12). Blood sampling: 3 ml venous blood was obtained from each subject, collected into EDTA vacutainers for genotyping assays of *IL1RN* at SNP rs9005 and rs315943.

**Genotyping assays of *IL1RN*:** Whole blood samples were used for DNA extraction using the Genomic DNA Purification kit (Jena Bioscience, Germany) following the manufacturer's protocol. DNA concentration was

determined using Nanodrop 2000c spectrophotometer (Thermo Scientific, USA). Genotypes of *IL1RN*, SNP rs9005 and rs315943 were determined using 1µg genomic DNA, by TaqMan® Predesigned SNP Genotyping Assays and TaqMan Universal PCR Master Mix, No AmpErase UNG (Applied Biosystems, USA) following the manufacturer's instructions. Genotyping assays were performed and analyzed by StepOne real time PCR system (Applied Biosystems, USA).

#### **Statistical analysis:**

The clinical data were recorded on a report form. These data were tabulated and analyzed using SPSS (Statistical package for social science) program version 20. Quantitative data were expressed as mean and standard deviation. Qualitative data were expressed as number and percentage. For comparison between two groups, Student's *t*-test was used, while ANOVA test was used to compare more than two groups of quantitative data. Comparison of categorical data was performed using chi-square test and Fisher exact test. Odds ratio Deviations from Hardy-Weinberg equilibrium expectations were determined using the chi-squared test. The distribution of alleles and genotypes in the studied groups was tested for fitting to the Hardy-Weinberg equilibrium through comparing the observed and expected frequencies of genetic variants. Logistic regression analysis was applied to examine association between SNP variants and risk of OA. OR is given with 95 % confidence interval (CI). The Haploview program (*Barrett et al., 2005*) (version 4.2) was applied to estimate the haplotypes, linkage disequilibrium (LD) measure

(D') between studied SNPs. A  $p$  value  $<0.05$  was considered statistically significant at confidence interval 95%.

## Results

The present study included 47 unrelated Egyptian patients with primary knee OA and 36 apparently healthy volunteers as controls.

This sample of individuals was selected randomly from the population in Benha, Qualubiya Governorate, Egypt. Applying Hardy-Weinberg equation, revealed that the studied genotypes of *ILIRN* in both cases and control subjects were independent (i.e., they are in HW equilibrium). There is no evidence to reject the assumption of HWE in the sample. Patients' characteristics are shown in Table (1). No significant differences were found between OA and control groups concerning gender, age and BMI ( $p>0.05$ ).

Comparison of distribution of genotypes and alleles of both *ILIRN* SNPs between OA cases and controls revealed that rs9005 AG, GG genotypes and G allele were associated with risk of OA development (Table 2). Otherwise, no genotypes, alleles or haplotypes were associated with risk of OA.

Studying rs9005- rs315943 haplotypes' frequencies showed that AC haplotype had the highest frequency in control group (0.38%), while AT showed the highest frequency in OA group (0.32%). GT was the least in both groups (0.08% in OA cases and 0.17% in control). GC haplotype showed significantly higher ( $p=0.026$ ), while AC haplotype showed significantly lower ( $p=0.018$ ) distribution in OA as compared to the control group.

The current results also indicate that there were non-significant differences between various genotypes of either SNPs regarding age, gender and BMI in OA patients ( $p>0.05$  all). However, an earlier disease onset was significantly associated with rs9005 GG genotype only ( $p=0.022$ ). Interestingly, higher pain, maximum distance walked, ADL, Lequesne's scores and KL grading were associated with rs9005 GG, rs315943 CC genotypes, while lower pain, maximum distance walked, ADL, Lequesne's scores and KL grading were associated with rs9005 AA and rs315943 TT. (Table 3).

Finally, we found that higher pain, maximum distance walked, ADL, Lequesne's scores and KL grading were significantly associated with rs9005-rs315943 GC haplotype ( $p=0.044$ , 0.012, 0.015, 0.010 and 0.015, respectively), while lower pain ( $p=0.045$ ), maximum distance walked ( $p=0.008$ ), ADL ( $p=0.007$ ), Lequesne's scores ( $p=0.005$ ) and KL grading ( $p=0.031$ ) were associated with AT haplotype with statistical significance. Otherwise, none of the haplotypes showed statistically significant differences in frequency distribution regarding age, gender, BMI and age at disease onset ( $p>0.05$ , all).

## Discussion

In our study, 24 (66.7%) of the controls had the AA genotype of the *ILIRN* gene (SNP rs9005), 9 (25.0%) had AG genotype and 3 (8.3%) had GG genotype. Meanwhile, among OA patients the frequencies were 14 (29.8%), 25 (53.2%) and 8 (17.0%) for the AA, AG and GG genotypes, respectively.

**Table (1):** Patients' characteristics

	OA patients N=47
Age (years) Mean± SD	52.81±3.66
Males N (%)	5 (10.6%)
Females N (%)	42 (89.4%)
BMI (kg/m <sup>2</sup> ) Mean± SD	24.304±1.70
Age at disease onset (years) Mean± SD	47.32±3.46
Pain score Mean± SD	3.74±1.26
Maximum distance walked score Mean± SD	2.85±0.72
ADL score Mean± SD	2.62±0.70
Lequesne's score Mean± SD	9.17± 1.81
KL grading Mean± SD	1.06±0.32

N: number, BMI: body mass index, ADL: activities of daily living, KL: Kellgren-Lawrence

**Table (2):** Distribution of *IL1RN* gene SNP rs9005 and rs315943 genotypes and alleles between OA cases and controls

		Control N=36	Cases N=47	<i>p</i>	OR	95% CI
rs9005	<i>Genotype</i>				R	
	N (%)	AA 24 (66.7%)	14 (29.8%)			
		AG 9 (25.0%)	25 (53.2%)	0.002	4.762	1.739-13.041
		GG 3(8.3%)	8 (17.0%)	0.044	4.571	1.039-20.114
	<i>Allele</i>	A 57 (79.2%)	53 (56.4%)	0.002	2.940	1.460-5.918
N (%)	G 15 (20.8%)	41 (43.6%)				
	HW <i>p</i>	0.146	0.577			
rs315943	<i>Genotype</i>				R	
	N (%)	TT 7 (19.4%)	13(27.7%)			
		TC 17 (47.2%)	20(42.5%)	0.562	0.724	0.243-2.159
		CC 12 (33.3%)	14 (29.8%)	0.686	0.783	0.240-2.556
	<i>Allele</i>	T 31 (43.1%)	46 (48.9%)	0.451	0.789	0.426-1.463
N (%)	C 41 (56.9%)	48(51.1%)				
	HW <i>p</i>	0.824	0.308			

N: number, HW *p*, Hardy Weinberg *p* value. R, reference genotype according to National Center for Biotechnology Information (NCBI), *p*<0.05: significant

**Table (3):** Comparison between genotypes of *IL1RN* gene SNP rs9005 and rs315943 regarding the clinical and radiological data of OA patients

	rs9005			<i>p</i>	rs315943			<i>P</i>
	AA N=14	AG N=25	GG N=8		TT N=13	TC N=20	CC N=14	
Pain score Mean±SD	2.36± 0.55	4.04± 1.97	5.25± 1.01	<b>0.007</b>	3.23± 1.08	3.25± 1.12	4.93± 1.98	<b>0.042</b>
Maximum distance walked score Mean±SD	1.57± 0.34	3.08± 1.10	4.38± 1.67	<b>0.002</b>	2.15± 0.95	2.50± 0.61	4.00± 1.92	<b>0.021</b>
ADL score Mean±SD	1.57± 0.34	2.80± 0.38	3.88± 1.23	<b>0.005</b>	1.92± 0.40	2.40± 0.50	3.57± 1.16	<b>0.028</b>
Lequesne's score Mean±SD	5.50± 1.09	9.84± 1.85	13.50± 2.76	<b>0.004</b>	7.31 1.34	8.05± 1.01	12.50± 2.32	<b>0.032</b>
KL grading Mean±SD	0.71± 0.11	1.12± 0.37	1.50± 0.31	<b>0.042</b>	0.77±0.1 3	0.90± 0.14	1.57± 0.26	<b>0.016</b>

*P*<0.05: significant, N: number, ADL: activities of daily living, KL: Kellgren-Lawrence

Regarding the *ILIRN* gene SNP rs315943, 7 (19.4%) of the controls had the T\T genotype, 17 (47.2%) had TC genotype and 12(33.3%) had C\C genotype, while in OA group, 13(27.7%) patients were carriers of the TT genotype, 20 (42.5%) patients had TC genotype, while 14 (29.8%) had the CC genotype. Such frequency distribution of genotypes revealed that control subjects had significantly higher percentage of rs9005 AA genotype, while the GG genotype was significantly higher in OA patients. We observed that these percentages were not clearly defined in previous studies (8 and 14-17).

Importantly, we found that the presence of rs9005 G allele in heterozygous, homozygous or as haplotype with the C allele of rs315943, implies an increased risk of OA development. To our knowledge, earlier studies have been inconclusive regarding the role of *ILIRN* genotypes and/or haplotypes in risk of knee OA. However, Wu and colleagues (17) showed that, none of the studied *ILIRN* polymorphisms was implicated in the susceptibility for knee OA. This discrepancy may be explained by the small sample size of our study.

Comparison of age between different studied genotypes of rs9005/rs315943 revealed that there were nonsignificant within either patients or control groups. However, there was a significant association between the ages at disease onset and the GG genotype of SNP rs9005. Such results indicate that OA patients with GG genotype are more liable to develop disease at younger age than those with A\A genotype. Of interest, this association did not apply to SNP rs315943 genotypes.

In line with our observations, Attur and colleagues (2) , found that patients with rs9005 GG of older ages had significantly more severe form of OA disease. A more recent study (18) also found that carriers of two copies of the haplotype TTG (rs41958, rs315952, rs9005) had a more severe form of OA than those with zero copies of the same age, which is consistent with earlier onset of the disease in the haplotype carriers.

Moreover, there were non-significant differences regarding BMI between OA patients and controls with different *ILIRN* SNP rs9005 and /or rs315943 genotypes. On the contrary, Wu and colleagues reported a BMI association with OA progression only in subjects carrying the TGC haplotype of *ILIRN*SNPs (rs419598, rs9005, rs315943) (17). Such contradiction may be attributed to the differences in study design, haplotype combination, as well as to different sample size.

In the present study, patients carrying SNPs rs9005 GG, rs315943 CC genotypes and GC haplotype had significantly higher pain, maximum distance walked, ADL, Lequesne's scores and KL grading, while those who carry rs9005 AA, rs315943 TT and AT haplotype showed lower pain, maximum distance walked, ADL, Lequesne's scores and KL grading.

Our results, in this regard, are consistent with those of Wu and his group (17) , who revealed that radiologic progression and severity associated with TGC haplotype of 3 *ILIRN* SNPs (rs419598/rs9005/rs315943). Consistently, former studies concluded that the haplotype CTA (rs419598, rs315952 and rs9005) of *ILIRN*, was significantly associated with decreased risk of severe OA (2). Furthermore, a large scale meta-

analysis of CTA haplotype (rs419598, rs315952 and rs9005, respectively) suggested its involvement in pathogenesis of less severe OA phenotype (8). In addition, it was recently found that presence of TTG haplotype (rs41958, rs315952, rs9005) was significantly associated with increased radiographic severity and narrower joint space width (18).

The mechanisms underlying the pathological changes of OA associated with *IL1RN* SNPs are not yet clearly understood. Nevertheless, analysis by an earlier study suggested that *IL1RN* genotype modulates IL10, IL1 $\beta$  release in synovial fluid (SF). They found that the *IL1RN* CTA haplotype, corresponding to (rs419598/rs315952/rs9005) was significantly associated with IL10 lower levels in SF of the patients. Furthermore, in the same patient group the levels of IL1 $\beta$  and IL6 showed a tendency to decrease in SF. Therefore, the presence of CTA haplotype confers a protective effect by increasing the IL1RN/(IL10, IL1 $\beta$  and IL6) ratio. On the other hand, absence of this haplotype may disturb the balance in favor of the proinflammatory cytokines (IL10, IL1 $\beta$  and IL6), which promote cartilage degradation and OA progression(2). This hypothesis was further supported by a recent finding of significantly lower plasma IL-1Ra levels in OA patients who had 2 copies of TTG haplotype (rs419598/rs315952/rs9005) compared with those with zero copies (18).

### Conclusion

In conclusion, GG and CC genotypes and GC haplotype of *IL1RN* SNP rs9005 and rs315943, respectively, were associated with increased clinical and radiological severity of OA. Additionally, carrying rs9005 AG and GG

genotypes may increase the risk of susceptibility to OA.

The current findings suggest that *IL1RN* genotyping may be useful for identification of patients at increased risk of poor prognosis at early stages, who would be candidates for more aggressive therapeutic approaches. Markers based on *IL1RN* genetic polymorphisms may also represent hoping targets for drug advances. Though, our study was limited by the very small number of subjects, yet results may provide the basis for large sized randomized control studies on Egyptian patients with OA. These studies are recommended to establish the role of our studied *IL1RN* SNPs in susceptibility, clinical course and management of OA disease.

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