

IL-1B Gene Polymorphism and Susceptibility to Rheumatoid Arthritis in Ethnic Saudi Patients

Adeel Gulzar Chaudhary

Department of Medical Technology, Faculty of Applied Medical Sciences,
P.O. Box 80216, King Abdulaziz University, Jeddah 21589, Saudi Arabia

Abstract: IL-1 is an inflammatory cytokine that has been widely implicated in Rheumatoid Arthritis (RA). In Saudi Arabia, limited work has addressed the genetic bases of RA. In this case-control study, association of single nucleotide polymorphisms (SNPs) in the IL-1 gene is investigated in a well characterized Saudi cohort. Keeping in view the genetic heterogeneity of Saudi population, blood samples from 100 ethnic Saudi RA patients and 200 matched healthy controls were analyzed. Genotyping for SNPs including IL-1A +4845, IL-1B -511, IL-1B +3954 and IL-1RA +2018 was performed by amplifying the polymorphic region using PCR followed by restriction enzyme analysis on polyacrylamide gel electrophoresis (PAGE). Comparison of RA patient data and healthy controls revealed significant increase in the carriage rate of rare (2/2) genotype for IL-1B +3954 in the RA cases (13% in RA vs 5% in controls, $P=0.022$). Subsequently the rare allele frequency (+3954T) was significantly increased in the RA patient group (frequency= 32.5% in RA patients $P=0.01$) compared to controls (frequency= 22.5%). No differences were observed for the remaining polymorphisms, indicating that the rare allele of IL-1B +3954 is associated with susceptibility to RA in ethnic Saudis. Due to known extracellular pro-inflammatory activity of secretory IL-1B, it is suggested that this bi-allelic polymorphism could be used as a prognostic marker for RA in ethnic Saudis.

Key words: IL-1 gene % SNPs % Rheumatoid arthritis % RFLP % Pro-inflammatory cytokine % Genotype % Heterogeneous population % Saudis

INTRODUCTION

Rheumatoid arthritis (RA) is the most common systemic autoimmune disorder characterized by chronic inflammation of multiple joints. This is mainly due to synovial cell proliferation with subsequent T-lymphocyte accumulation that leads to the destruction of both cartilage and bone [1, 2]. In fact RA is a complex multifactorial disease, where both environmental and genetic factors contribute to the disease, with the later being a substantial contributory factor in the Pathogenesis of the disease [3, 4]. It is widely recognized that susceptibility to RA is influenced by its association with human leukocyte antigen (HLA) complex alleles encoding the 'shared epitope' which account for 30% of RA cases [5]. Thus polymorphisms in HLA genes explain only part of the total genetic risk and this is where the role of IL-1, a pro-inflammatory cytokine and a potent transmitter between cells, plays a critical role in the pathogenesis of RA [6, 7].

The IL-1 candidate gene family clustered in a 430-kb region on the long arm of human chromosome 2 (2q13) is composed of two agonists and an antagonist. The agonistic effect of IL-1" and IL-1\$ has been implicated in joint destruction in RA, where as IL-1 receptor antagonist (IL-1RA) has an anti-inflammatory role in the disease process as a natural competitive inhibitor of IL-1 bioactivity and is generally found to be elevated in plasma of patients suffering from inflammatory disorders [8, 9]. Interestingly, several bi-allelic sequence variations in IL-1 gene cluster have been associated with susceptibility and/or severity to RA [10, 11]. In IL-1A gene, the common single nucleotide polymorphisms (SNPs) include the -889 and +4845 that are in complete linkage disequilibrium and have been associated with chronic iridocyclitis in juvenile chronic arthritis and chronic polyarthritis [12, 13]. In IL-1B the -511 and +3954 polymorphisms have been extensively analyzed and have shown to affect IL-1B processing and protein production [14, 15]. Where as the IL-1RA consists of variable number of tandem repeats

(VNTR) and IL-1 RA +2018 bi-allelic polymorphism where allele 2 has been implicated in several inflammatory diseases including early onset periodontitis, systemic lupus erythematosus, alopecia areata and ulcerative colitis [16-19].

Limited work, if any, has addressed the genetic bases of RA in Saudi Arabia. This is the first case-control study in Saudi Arabia, where association of IL-1 cytokine was investigated by genotyping for IL-1A +4845, IL-1B -511, IL-1B +3954 and IL-1RA +2018 SNPs using PCR followed by restriction fragment length polymorphism (RFLP) analysis on polyacrylamide gel electrophoresis, establishing allele frequencies in ethnically well characterized Saudi RA patients.

MATERIALS AND METHODS

Patient Samples and Normal Controls: Blood samples were collected from 100 Saudi patients over a period of three years (2002-2005) with clinically established RA classified according to ARA criteria [20]. Since studies elsewhere have shown ethnicity to effect genotype carriage rates [21], stringent sample collection criteria were used to ensure ethnic integrity was maintained for both RA patients and subsequent healthy control group ($n=200$) in a ethnically heterogeneous Saudi population. The control group was free from RA symptoms and any other inflammatory disease. The patients were recruited from King Khalid National Guard Hospital in Jeddah, only after signing a consent form and a questionnaire concerning their tribal lineage and marital history. These RA patients and normal control subjects were the 2nd and 3rd generation of ethnic Saudi tribes from Western and Southern regions of Saudi Arabia.

All of the patients had been visiting their physician for more than two years, had positive RF titer, 65% had >6 inflammatory or tender joints and 66% had 1hr early morning stiffness. These patients were undertaking various treatments including methotrexate and corticosteroids that either reduce IL-1 production or blocked their potent affect.

Genomic DNA Isolation: Blood samples were collected from both RA positive patients and normal control samples in EDTA tubes and genomic DNA was isolated using Qiagen™ DNA extraction kit. Extracted DNA was stored at 4°C.

PCR Amplification and Genotyping for IL-1 Polymorphisms: The SNPs in the IL-1 gene cluster were

Table 1: PCR primer sequences used to amplify the SNP regions of the IL-1 gene

SNP	PCR primer sequences	
IL-1A +4845	Forward	ATG GTT TTA GAA ATC ATC AAG CCT AGG GCA
	Reverse	AAT GAA AGG AGG GGA GGA TGA CAG AAA TGT
IL-1B -511	Forward	TGG CAT TGA TCT GGT TCA TC
	Reverse	GTT TAG GAA TCT TCC CAC TT
IL-1B +3954	Forward	CTC AGG TGT CCT CGA AGA AAT CAA A
	Reverse	GCT TTT TTG CTG TGA GTC CCG
IL-1RA +2018	Forward	CTA TCT GAG GAA CAA CCA ACT AGT AGC
	Reverse	TAG GAC ATT GCA CCT AGG GTT TGT

screened using PCR/RFLP method followed by PAGE. The polymorphisms screened included IL-1A +4845, IL-1B-511, IL-1B +3954 and IL-1RA +2018. For each sample a 25 µl PCR master mix was prepared containing 10x reaction buffer, 1.5-2.5 mM MgCl₂, 0.2 mM dNTP, a pair of 0.75 M of each forward and reverse primer and a 1.25 U of *Taq* Polymerase (Promega). The PCR primer sequences used to amplify each of the four SNPs are described in Table 1.

IL-1A +4845 (G to T Variation): The optimized PCR conditions included denaturation at 95°C for 10 min followed by 30 cycles of denaturation at 95°C for 1min, annealing at 57°C for 1min and extension at 72°C for 1min. The PCR was completed with a final extension step of 5 min at 72°C. PCR product of 229 bp was size matched on 1.5% agarose gel before subjecting to 5 U of *Fnu* 4H1 restriction enzyme (New England Biolabs) at 37°C overnight. The restriction pattern was observed on 5% PAGE under UV light after staining with ethidium bromide. A constant band of 76 bp appeared for both alleles followed by two more bands of 24 bp + 124 bp representing allele-1. Whereas for allele-2 a single band of 153 bp was diagnostic.

IL-1B -511 (C to T Variation): This promoter region polymorphism in IL-1B was amplified using site specific primers listed in Table 1. PCR conditions included denaturation at 95°C for 10 min followed by 30 cycles of denaturation at 95°C for 1min, annealing at 54°C for 1min and extension at 72°C for 1min. The PCR was completed with a final extension step of 5 min at 72°C. PCR product of 304 bp was size matched on 1.5% agarose gel before subjecting to 2.5 U of *Ava*1 restriction enzyme (New England Biolabs) at 37°C overnight. The restriction pattern was observed on 5% PAGE under UV light after staining with ethidium bromide. The resulting fragments of 190 bp + 114 bp were diagnostic for allele-1 whereas allele-2 remained uncut resulting in a single 304 bp fragment.

IL-1B +3954 (C to T Variation): The optimized PCR conditions included denaturation at 95°C for 2 min followed by 30 cycles of denaturation at 95°C for 30 sec, annealing at 54°C for 30 sec and extension at 72°C for 30 sec. The PCR was completed with a final extension step of 5 min at 72°C. PCR product of 194 bp was size matched on 1.5% agarose gel before subjecting to 10 U of *Taq I* restriction enzyme (Promega) at 65°C overnight. The restriction pattern was observed on 5% PAGE under UV light after staining with ethidium bromide. The enzyme cuts a constant band of 12 bp (the absence of which indicates incomplete digestion) followed by two more bands of 85 bp and 97 bp diagnostic for allele-1, whereas for allele-2, a 12 bp and 182 bp were visualized.

IL-1Ra +2018 (C to T Variation): The optimized PCR conditions included denaturation at 96°C for 1 min followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 57°C for 30 sec and extension at 70°C for 30 sec. The PCR was concluded with a final extension step of 5 min at 70°C. PCR product of 154 bp was size matched on 1.5% agarose gel before subjecting to 5 U of *Alu I* restriction enzyme (Promega) at 37°C overnight. The restriction pattern was observed on 5% PAGE under UV light after staining with ethidium bromide. For allele-1 two products of 126 bp and 28 bp were visualized where as a single uncut band of 154 bp was diagnostic for allele-2.

RESULTS AND DISCUSSION

Genomic DNA was extracted from 100 Saudi RA patient group and 200 normal controls. The samples were genotyped for the four common IL-1 SNPs using site specific primers to amplify the gene fragment of interest that was subjected to restriction enzyme fragmentation.

In order to determine possible association with susceptibility to Rheumatoid arthritis, genotype carriage rates and subsequent allele frequencies for each of the bi-allelic polymorphism were matched in both the patient and control groups. The common homozygous was designated 1/1, followed by 2/2 as the rare homozygous and 1/2 being the heterozygous genotype.

Statistical Analysis: All statistical analysis was performed on SPSS 13.0 software for windows (SPSS, Chicago, III). Comparison of RA patient and normal control groups showed no significant differences in the genotype carriage rate and allele frequencies for IL-1! +4845 (9% in RA vs. 8% in controls, $P=0.87$), IL-1# -511 (10% in RA vs. 11% in controls, $P=0.93$) and

IL-1RA +2018 (8% in controls vs. 7% in RA, $P=0.87$) as seen in Table 2 and 3. However, for IL-1B +3954, the genotype carriage rate and allelic frequency of the two groups was significantly different. There was an increase in the rare (2/2) genotype carriage rate in RA patients (13% in RA vs. 5% in controls $P=0.022$) as shown in Table 2. Subsequently, the frequency of rare allele (+3954T) was significantly higher in RA patients compared to the control group (32.5% in RA vs. 22.5% in controls, $P=0.01$) as seen in Table 3.

The genotype distribution for all SNPs in both the groups followed the Hardy Weinberg equilibrium and therefore was in agreement with commonly reported IL-1 genotype distribution spectrum elsewhere.

IL-1 and Other Diseases: RA is a multifactorial autoimmune disease where environmental and genetic factors play role in the outcome of the disease. In fact, the genetic component of this disease has gained tremendous significance since its association with HLA alleles encoding the 'shared epitope', however this relationship accounts for only one-third of the RA cases [22]. Cytokines on the other hand, have shown to play a key role in the pathogenesis of RA and other inflammatory disorders, some of which include SLE, alopecia areata, ulcerative colitis and periodontitis. Among all cytokines, focus tends to be on IL-1 which is thought to be central mediator to joint destruction that activates collagenase and stromelysin while inhibiting the synthesis of collagen and proteoglycan [23]. Elevated levels of IL-1" and IL-1\$ have also been measured in RA synovial fluid thus affirming its potent role in disease outcome [24].

Role of IL-1 SNPs in Rheumatoid Arthritis: The polymorphic sequence variations found in IL-1A, IL-1B and IL-1RA have been associated with susceptibility to RA in various studies conducted worldwide where the frequency distributions and genotype carriage rate differences between white Caucasians, Afro-Americans and Japanese are well documented [25]. These polymorphic variations have also been studied as severity markers for RA where an increase in rare allele frequencies are directly linked to destructive arthritis and the number of joints involved.

Saudi population is ethnically diverse since most of the citizens, particularly in Western and Eastern Saudi Arabia, are naturalized Saudis that migrated from other regions. Considering ethnicity can greatly influence the genotype patterns [26], we decided to implement stringent criteria for sample collection, where it was made sure the

Table 2: Genotype carriage rates for the four IL-1 single nucleotide polymorphisms are compared between the RA patient and normal Control groups. Statistically significant differences in genotype carriage rates were observed only for IL-1B +3954

Genotype	RA patients n=100 (%)	Controls n=200 (%)	*P-value
IL-1A +4845			
1/1	49 (49)	104 (52)	0.87
1/2	42 (42)	80 (40)	
2/2	9 (9)	16 (8)	
IL-1B-511			
1/1	47 (47)	90 (45)	0.93
1/2	43 (43)	88 (44)	
2/2	10 (10)	22 (11)	
IL-1B +3954			
1/1	48 (48)	120 (60)	0.022
1/2	39 (39)	70 (35)	
2/2	13 (13)	10 (5)	
IL-1RA +2018			
1/1	51 (51)	108 (54)	0.87
1/2	41 (41)	78 (39)	
2/2	8 (8)	14 (7)	

*P value < 0.05 was taken as significant

Table 3: Allele frequency distribution for the four IL-1 single nucleotide polymorphisms in the RA patient and normal control groups. Statistically significant differences in allele frequencies were observed only for IL-1B +3954

Genotype	RA patients n=100 (%)	Controls n=200 (%)	*P-value
IL-1A +4845			
1	140 (70)	288 (72)	0.61
2	60 (30)	112 (28)	
IL-1B -511			
1	137 (68.5)	268 (67)	0.71
2	63 (31.5)	132 (33)	
IL-1B +3954			
1	135 (67.5)	310 (77.5)	0.010
2	65 (32.5)	90 (22.5)	
IL-1RA +2018			
1	143 (71.5)	294 (73.5)	0.61
2	57 (28.5)	106 (26.5)	

*P value < 0.05 was taken as significant

participants, both RA patients and healthy controls, had homogeneous ethnic tribal lineage. Thus in our study we demonstrated a statistically significant increase in carriage rate of the rare 2/2 (T/T) genotype for IL-1B +3954, furthermore the allelic frequency showed the rare allele +3954T was significantly increased in the RA patient group. These results suggest the IL-1B +3954 polymorphism is associated with susceptibility to RA and could be used as a prognostic marker for RA in ethnically Saudi RA patient.

Apart from RA, the rare allele for IL-1B +3954 has been implicated in the pathogenesis of several autoimmune diseases, however the extent of associations tend to differ from one population to another [27, 28]. This polymorphism has also shown to affect IL-1 expression followed by altered protein processing subsequently causing protein production differences. It is important to mention that these studies are controversial, where some reports hold this polymorphism responsible for increased plasma levels of IL-1B; others tend to contradict them

either by reporting no affect or reduction in IL-1 synthesis [29, 30, 31].

Although our results show IL-1B genotype as a susceptibility (risk) marker and not a severity marker for RA, however the severe condition of our patients, where 65% had > 6 inflammatory or tender joints and 66% had 1hr early morning stiffness, deserve further investigation with respect to IL-1 polymorphisms. It is possible that their genotype made them high risk targets in first place for developing RA however further studies considering the severity aspect, may fully explain the aggressive nature of RA in these patients. Furthermore these ethnic Saudi RA cases could be more suitable candidates for recombinant IL-1 receptor antagonist therapy that may deliver better response rate than reported elsewhere, since it has been demonstrated that RA patients with rare alleles for IL-1A +4845 and IL-1B +3954 respond positively to this treatment with significant reduction in joint swelling [32, 33].

CONCLUSION

In this study, we addressed the underlying role of IL-1 where the rare allele for IL-1B SNP could be used as a prognostic marker for RA in ethnic Saudi cohort. It further highlights the need for conducting further studies considering the severity aspect that may eventually serve as a more effective genetic marker.

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REFERENCES

1. Choy, E.H. and G.S. Panayi, 2001. Cytokine pathways and joint inflammation in rheumatoid arthritis. *N. Engl. J. Med.*, 344: 907-916.
2. Sharma, P.K., D. Hota and P. Pandhi, 2004. Biologics in rheumatoid arthritis. *J. Assoc. Physicians India*, 52: 231-236.
3. John, S. and J. Worthington, 2001. Genetic epidemiology. Approaches to the genetic analysis of rheumatoid arthritis. *Arthritis Res.*, 3(4): 216-220.

4. MacGregor, A.J., H. Snieder and A.S. Rigby *et al.*, 2000. Characterizing the quantitative genetic contribution to rheumatoid arthritis using data from twins. *Arthritis Rheumatism*, 43: 30-7.
5. Deighton, C.M., P.J. Kelly and D.J. Walker, 1993. Linkage of rheumatoid arthritis with HLA. *Ann. Rheum. Dis.*, 52(9): 638-642.
6. Miossec, P., 1997. Cytokines and the pathophysiology of bone erosions in rheumatoid arthritis. *J. Clin. Rheumatol.*, 3: S81-S83.
7. Duff, G.W., 2006. Evidence for genetic variation as a factor in maintaining health. *Am. J. Clin. Nutr.*, 83(2): 431S-435.
8. Nicklin, M.J., A. Weith and G.W. Duff, 1994. A physical map of the region encompassing the human interleukin-1 alpha, interleukin-1 beta and interleukin-1 receptor antagonist genes. *Genomics*, 19(2): 382-384.
9. Arend, W., 1991. Interleukin-1 receptor antagonist: A new member of the interleukin 1 family. *J. Clin. Invest.*, 5: 1445-1451.
10. Muller, K., E.B. Herner, A. Stagg, K. Bendtzen and P. Woo, 1998. Inflammatory cytokines and cytokine antagonists in whole blood cultures of patients with systemic juvenile chronic arthritis. *Br. J. Rheumatol.*, 37(5): 562-569.
11. Cox, A., N.J. Camp, C. Cannings, F.S. di Giovine, M. Dale, J. Worthington, S. John, W.E. Ollier, A.J. Silman and G.W. Duff, 1999. Combined sib-TDT and TDT provide evidence for linkage of the interleukin-1 gene cluster to erosive rheumatoid arthritis. *Hum. Mol. Genet.*, 8(9): 1707-1713.
12. McDowell, T.L., J.A. Symons, R. Ploski, O. Forre and G.W. Duff, 1995. A genetic association between juvenile rheumatoid arthritis and a novel interleukin-1 alpha polymorphism. *Arthritis Rheum*, 38: 221.
13. Jouvenne, P., A. Chaudhary, N. Buchs, F.S. Di Giovine, G.W. Duff and P. Miossec, 1999. Possible genetic association between interleukin-1" gene polymorphism and the severity of chronic polyarthritis. *Eur. Cytokine Netw.*, 10: 33-36.
14. di Giovine, F.S., E. Takhsh, A.I. Blakemore and G.W. Duff, 1992. Single base polymorphism at -511 in the human interleukin-1 beta gene (IL1 beta). *Hum. Mol. Genet.*, 1(6): 450.
15. Gore, E.A., J.J. Sanders, J.P. Pandey, Y. Palesch and G.M. Galbraith, 1998. Interleukin-1beta +3953 allele 2: Association with disease status in adult periodontitis. *J. Clin. Periodontol.*, 25(10): 781-785.

16. Guzman, S., M. Karima, H.Y. Wang and T.E. Van Dyke, 2003. Association between interleukin-1 genotype and periodontal disease in a diabetic population. *J. Periodontol.*, 74(8): 1183-1190.
17. Blakemore, A.I., J.K. Tarlow, M.J. Cork, C. Gordon, P. Emery and G.W. Duff, 1994. Interleukin-1 receptor antagonist gene polymorphism as a disease severity factor in systemic lupus erythematosus. *Arthritis Rheum.*, 37: 1380-1385.
18. Tazi-Ahmini, R., A. Cox, A.J. McDonagh, M.J. Nicklin, F.S. di Giovine, J.M. Timms, A.G. Messenger, P. Dimitropoulou, G.W. Duff and M.J. Cork, 2002. Genetic analysis of the interleukin-1 receptor antagonist and its homologue IL-1L1 in alopecia areata: strong severity association and possible gene interaction. *Eur. J. Immunogenet.*, 29(1): 25-30.
19. Carter, M.J., F.S. Di Giovine, A. Cox, P. Goodfellow, S. Jones, A.J. Shorthouse, G.W. Duff, A.J. Lobo, 2001. The interleukin 1 receptor antagonist gene allele 2 as a predictor of pouchitis following colectomy and IPAA in ulcerative colitis. *Gastroenterology*, 121(4): 805-811.
20. Arnett, F.C., S.M. Edworthy and D.A. Bloch *et al.*, 1988. The American Rheumatism association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum*, 31: 315-324.
21. Manchanda, P.K., H.K. Bid and R.D. Mittal, 2005. Ethnicity greatly influences the interleukin-1 gene cluster (IL-1b promoter, exon-5 and IL-1Ra) polymorphisms: a pilot study of a north Indian population. *Asian Pac. J. Cancer Prev.*, 6(4): 541-546.
22. Wagner, U., S. Kaltenhauser and H. Sauer *et al.*, 1997. HLA markers and prediction of clinical course and outcome in rheumatoid arthritis. *Arthritis Rheum*, 40: 341-351.
23. Stove, J., K. Huch, K.P. Gunther and H.P. Scharf, 2000. Interleukin-1beta induces different gene expression of stromelysin, aggrecan and tumor-necrosis-factor-stimulated gene 6 in human osteoarthritic chondrocytes *in vitro*. *Pathobiology*, 68(3): 144-149.
24. Buchs, N., F.S. di Giovine, T. Silvestri, E. Vannier, G.W. Duff and P. Miossec, 2001. IL-1B and IL-1Ra gene polymorphisms and disease severity in rheumatoid arthritis: interaction with their plasma levels. *Genes. Immun.*, 2(4): 222-228.
25. Tai, H., M. Endo, Y. Shimada, E. Gou, K. Orima, T. Kobayashi, K. Yamazaki and H. Yoshie, 2002. Association of interleukin-1 receptor antagonist gene polymorphisms with early onset periodontitis in Japanese. *J. Clin. Periodontol.*, 29(10): 882-888.
26. Hoffmann, S.C., E.M. Stanley, E.D. Cox, B.S. Di Mercurio, D.E. Koziol, D.M. Harlan, A.D. Kirk and P.J. Blair, 2002. Ethnicity greatly influences cytokine gene polymorphism distribution. *Am. J. Transplant*, 2(6): 560-567.
27. Cox, E.D., S.C. Hoffmann, B.S. Di Mercurio, R.A. Wesley, D.M. Harlan, A.D. Kirk and P.J. Blair, 2001. Cytokine polymorphic analyses indicate ethnic differences in the allelic distribution of interleukin-2 and interleukin-6. *Transplantation*, 72(4): 720-726.
28. Pociot, F., J. Molvig, L. Wogensen, H. Worsaae and J. Nerup, 1992. A Taq I polymorphism in the human interleukin-1 β (IL-1B) gene correlates with IL-1 β secretion *in vitro*. *Eur. J. Clin. Invest.*, 22: 396-402.
29. Hall, S.K., D.G. Perregaux, C.A. Gabel, T. Woodworth, L.K. Durham, T.W. Huizinga, F.C. Breedveld and A.B. Seymour, 2004. Correlation of polymorphic variation in the promoter region of the interleukin-1 beta gene with secretion of interleukin-1 beta protein. *Arthritis Rheum*, 50(6): 1976-1983.
30. Dominici, R., G. Malferrari, C. Mariani, L. Grimaldi and I. Biunno, 2002. The Interleukin 1-beta exonic (+3953) polymorphism does not alter *in vitro* protein secretion. *Exp. Mol. Pathol.*, 73(2): 139-141.
31. Camargo, J.F., P.A. Correa, J. Castiblanco and J.M. Anaya, 2004. Interleukin-1beta polymorphisms in Colombian patients with autoimmune rheumatic diseases. *Genes Immun.*, 5(8): 609-614.
32. Camp, N.J., A. Cox, F.S. di Giovine, D. McCabe, W. Rich and G.W. Duff, 2005. Evidence of a pharmacogenomic response to interleukin-1 receptor antagonist in rheumatoid arthritis. *Genes Immun.*, 6(6): 467-471.
33. Bresnihan, B., J.M. Alvaro-Gracia, M. Cobby, M. Doherty, Z. Domljan, P. Emery, G. Nuki, K. Pavelka, R. Rau, B. Rozman, I. Watt, B. Williams, R. Aitchison, D. McCabe and P. Musikic, 1998. Treatment of rheumatoid arthritis with recombinant human interleukin-1 receptor antagonist. *Arthritis Rheum*, 41(12): 2196-204.