DOI: http://dx.doi.org/10.24327/ijcmes2018.08060049

Available online at http://journalijcmes.com

INTERNATIONAL JOURNAL OF CURRENT MEDICAL SCIENCES

Research Article

ISSN: 2320- 8147

International Journal of Current Medical Sciences- Vol. 8, Issue, 6(A), pp. 437-439, June, 2018

A STUDY OF IL-18 POLYMORPHISM WITH RHEUMATOID ARTHRITIS IN CENTRAL INDIAN POPULATION

Poorva shrivastava., Rashmi Arnold., Pallavi Mishra., Jitendra Kumar Tripathi., Udita Singh and Arvind Kumar Tripathi

Centre for Biotechnology, APS University Rewa (M.P.) 486003

A R T I C L E I N F O

Article History:

Received 9th March, 2018
Received in revised form 15th April, 2018 Accepted 20th May, 2018
Published online 28th June, 2018

Key words:

Rheumatoid arthritis, cytokines, IL18

A B S T R A C T

Rheumatoid arthritis is complex autoimmune disorder associated with peripheral. As it is associated with immune system, cytokines and their polymorphisms are widely studied. IL-18 is a cytokine associated with many autoimmune disease. This investigation has been done to study effect of IL-18 polymorphism in pathophysiology of Rheumatoid Arthritis. PCR followed by RFLP method has been used to detect genotype and allele frequency. The pattern of genotype and allele distribution in disease and control group suggested a significant strong association of CC genotype carriage and C allele carriage (p value-0.0090*, OR-1.940 95%CI- 1.182 – 3.185 and p value-0.0074, OR-1.704, 95%CI- 1.163 – 2.498 respectively) meanwhile A allele was protective (p value- 0.0074, OR- 0.5867, 95%CI- 0.4003 – 0.8600) in RA susceptibility.

INTRODUCTION

Rheumatoid Arthritis is a chronic, multisystemic, autoimmune disorder, its characteristics feature is chronic, symmetrical and erosive synovitis usually involving peripheral joints. Rheumatoid arthritis (RA) is an inflammatory disease which mediate inflammation on those joint of the body that are lined with synovium (synovial fluid), responsible for maintaining the nutrition and lubrication of the joint. Some patients may experience only a mild articular illness of brief duration while some patients experience progressive polyarthritis with functional impairment and disability. (O.Sangha., 2000). Studies have shown that both innate and adaptive immune response are involved in the pathogenesis of RA. Over expression of pro-inflammatory and anti-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukins and interferon have been observed in serum of RA patients and the altered expression of these cytokines leads to complex immune cascade causing tissue injury and inflammation. Many genes are involved in complex RA pathophysiology but due to multifactorial nature of RA not a single factor could be sufficient to cause Rheumatoid arthritis. Mutations in these candidate genes lead to genetic polymorphism (variation in DNA sequence) among random mating individuals, groups, or populations due to the effects of environment The IL-18 gene is regulated by its promoter region polymorphisms, which lead to differences in transcription factor binding, low promoter activities were observed for A and C alleles at positions -607 and -137, while higher promoter activities were observed for C and G alleles at similar positions.

MATERIAL AND METHODS

Study population

The study population consisted of 260 unrelated subjects and comprised 120 patients and 160 ethnically matched healthy controls of Indo-European ethnicity. Cases included consecutive patients who attended the Department of Medicine at Shyam Shah Medical College, Rewa, India; Ayurveda Medical College, Rewa, India; Ranbaxy Pathology Regional Collection Centre, Rewa, India; and the District Hospital, Satna, India.

DNA isolation

Genomic DNA was extracted from whole blood using a modified version of the salting-out procedure described by Miller *et al*.

PCR RFLP

DNA were amplified with specific primers and 301 bp fragment were amplified. The *Mse I* restriction enzyme introduces change in guanine to adenine. Digestion of the

Corresponding author:* **Poorva shrivastava
Centre for Biotechnology, APS University Rewa (M.P.) 486003

amplified 301 bp PCR product was excisable by *MseI*. Depending on the digestion pattern, CC homozygous individuals when cut into 199 and 73bp fragments, and as AA homozygous individuals when cut into 101, 98 and 73 bp fragments. Thus, the CA heterozygous individuals were identified when showing the expected fragments: 199, 101, 98 and 73 bp.

Association of IL-18 genotypes, alleles and carriage rates with susceptibility to disease in RA cases compared to controls using Fisher exact test.

Il-18 genotype	Case N= 112 N %	Control N=125 N %	P value	Odds ratio & ci
CC CA AA	65 54.2 51 42.5 4 3.3	53 37.8 74 52.8 13 9.3	0.0090** 0.1064 0.0763	1.940 1.182- 3.185 0.6592, 0.4034- 1.077 0.3369, 0.1068- 1.063
Alleles C A	181 5.4 59 4.6	180 64.3 100 35.7	0.0074**	1.704, 1.163- 2.498 0.5867, 0.4003- 0.8600
Carrige Rate C A	116 96.6 55 45.8	127 90.7 87 62.1	0.0902	1.445, 0.9481- 2.202 0.6921, 0.4542- 1.055

N - Number of individuals carrying particular genotype in a study group
% - Genotype frequency, allele frequency and carriage rates in percentage;
*- significant values
χ^2 (*P* Value) - indicates χ^2 *P* Value when HC is compared to RA

RESULTS

IL-18 is a proinflammatory cytokine belongs to IL-1 superfamily. Higher level of IL-18 is found in the synovial fluid of RA patients. Patients would have higher frequencies of C alleles at position -607 and higher frequencies of G alleles at position -137 of the IL-18 promoter gene while higher frequencies of A allele at position -607 and higher frequencies of C allele at position -137 would confer protective effects against the development of RA. Analysis of the distribution of genotype frequencies, allele frequencies and carriage rates for IL-18 -607 promoter polymorphism are depicted here. Genotype distribution of HC group (37.8%) showed decrease in 'CC' genotype as compared to RA group (54.2%) means (37.8% vs 54.2.%). Similarly, 'AA' genotype was present in significantly higher frequency in HC (9.3%) as compared to RA group (3.3%) that is (9.3% vs. 3.3%). An odds ratio of RA group for 'CC' genotype (1.940) indicated a harmful and strong positive effect of CC genotype with rheumatoid arthritis disease whereas an odds ratio of AA genotype (0.3369) group indicated a protective effect and AA genotype with the disease. The heterozygous genotype 'CA' was nonsignificantly distributed in HC group as compared to RA group (42.5% in case vs 52.8 % in control). An odds ratio of 0.6592 in RA group respectively was consistent with weak protective effect of 'CA' genotype in RA susceptibility. Although the overall distribution of genotype was not significantly different but CC genotype was significantly higher in case population as compare to control and indicates the strong association of CC genotype with RA susceptibility. Overall allele 'C' was found to be in significantly high frequency in rheumatoid arthritis disease group as compared to HC group in vindhya region whereas allele 'A' was present in significantly low frequency in the disease group ($\chi 2$ = 7.543, P=0.0060*). The pattern of genotype and allele distribution in disease and control group suggested a significant strong association of CC genotype carriage and C allele carriage (p value-0.0090*, OR-1.940 95%CI- 1.182 – 3.185 and p value-0.0074, OR-1.704, 95%CI- 1.163 – 2.498 respectively) meanwhile A allele was protective (p value-0.0074, OR- 0.5867, 95%CI- 0.4003 – 0.8600) in RA susceptibility. Carriage rate of allele 'C' was lower in HC group as compare to RA patients. (90.7% vs. 96.6%) whereas carriage rate of allele 'A' was lower in disease group as compare to control (45.8% Vs 62.1%). Overall strong positive association was found with rheumatoid arthritis disease.

DISCUSSION

IL-18 is a cytokine that belongs to the IL-1 superfamily and is produced by macrophages and other cells. IL-18 works by binding to the interleukin-18 receptor, and together with IL-12 it induces cell-mediated immunity following infection with microbial products like lipopolysaccharide (LPS). The pattern of genotype and allele distribution in disease and control group suggested a strong association of IL-18 -607 in RA susceptibility. Previous findings of Rueda *et al*., and Pawlik *et al*. reported that, IL-18-137 and -607 promoter polymorphisms were not significant with respect to RA courses and severities. Pan *et al*., reported that IL-18 gene promoter -607 A/C polymorphism was not associated with development of autoimmune diseases. Ying *et al*., reported that the genotype and allele frequency of IL-18-607 were not associated with IL-18 serum levels. Huang *et al*., reported that IL-18-607 polymorphism was associated with RA, but not with IL-18-137 polymorphisms. Gracie *et al*., reported that both SNPs found at positions -137 and -607 were involved in pathogenesis of RA. When other auto immune diseases were considered, the results were found to be very conflicting. Takada *et al*., reported that C allele, at position -607, was a risk factor for sarcoidosis in the Japanese population.

Reference

1. Rueda' B, Gonzalez-Gay MA, Mataran L, Lopez-Nevot MA, Martin J. Interleukin-18-Promoter polymorphisms are not relevant in rheumatoid arthritis. *Tissue Antigens*. 2005;65(6):544–48. Rueda, *et al*., (2005) [32] [PubMed]
2. Pawlik A, Kurzawski M, Czerny B, Gawronska-Szklarz B, Drozdzik M, Herczynska M. Interleukin-18 promoter polymorphism in patients with rheumatoid arthritis. *Tissue Antigens.* 2006;67(5):415–18.Pawlik, *et al*., (2006)[33] [PubMed]
3. Pan HF, Leng RX, Ye DQ. Lack of association of interleukin-18 gene promoter-607 A/C polymorphism with susceptibility to autoimmune

diseases: a meta-analysis. Lupus. 2011;20(9):945-51.Pan *et al*., [PubMed]
4. Ying B, Shi Y, Pan X, Song X, Huang Z, Niu Q, *et al*. Association of polymorphisms in the human IL-10 and IL-18 genes with rheumatoid arthritis. *Mol Biol Rep*. 2011;38(1):379-85. [PubMed]
5. Huang XZ, Zhuang JH, Ren YG, Zhou LJ, Zhou Q. Association of interleukin-6 and interleukin-18 gene polymorphism with rheumatoid arthritis in Guangdong Han population. Nan Fang Yi Ke Da Xue Xue Bao. 2007;27(11):1661–64. [PubMed]
6. Gracie JA, Koyama N, Murdoch J, Field M, McGarry F, Crilly A, *et al*. Disease association of two distinct interleukin-18 promoter polymorphisms in Caucasian rheumatoid arthritis patients. *Genes Immun.* 2005;6(3):211–16. [PubMed]
7. Takada T, Suzuki E, Morohashi K, Gejyo F. Association of single nucleotide polymorphisms in the IL-18 gene with sarcoidosis in a Japanese population. *Tissue Antigens*. 2002;60(1):36–42. [PubMed]
8. Zhou Y, Yamaguchi E, Hizawa N, Nishimura M. Sarcoidosis. Roles of functional polymorphisms in the interleukin-18 gene promoter in sarcoidosis. *Vasc Diffuse Lung* Dis. 2005;22(2):105–13. [PubMed]
9. O. Sangha Epidemiology of rheumatic diseases Rheumatology, Volume 39, Issue suppl_2, 1 December 2000, Pages 3–12

****