Available online freely at www.isisn.org

# Bioscience Research

**Print ISSN: 1811-9506 Online ISSN: 2218-3973**

***Journal by Innovative Scientific Information & Services Network***

**RESEARCH ARTICLE** BIOSCIENCE RESEARCH, 2019 16(2): 1046-1052. **OPEN ACCESS**

# The role of IL-5 gene polymorphisms in rheumatoid arthritis

**Ali S. Dawood[1] and Rafid A. Abdulkareem[2]**

[1]Department of Pathology and Forensic Medicine, College of Medicine, Wasit University, Wasit**, Iraq.**
[2]Department of Genetic Engineering, Institute of Genetic Engineering and Biotechnology for Post Graduate Studies, University of Baghdad, Baghdad, **Iraq.**

.
***Correspondence:** alisalim883@yahoo.com **Accepted: 08** Mar. 2019 **Published online: 13** Apr. 2019

Rheumatoid arthritis (RA) well-known as autoimmune inflammatory disease usually related to gradual joint destruction. Interleukin 5 (IL-5) was originally produced by type-2 T helper cells and mast cells to stimulate antibody production from activated B cells. Our objective was to determine the contribution of IL-5 gene polymorphism (SNP: rs# 4526098 A/G) in rheumatoid arthritis development among the Iraqi population. A case-control study involved 240 patients diagnosed with RA and 240 individual unrelated Iraqis matched in ages and sex, free disease and they didn't have family history of (RA) considered as a control group. Laboratory investigations were performed to patients included (RF) test and (ACPAs) test, then collecting blood samples from both patients and control for DNA extraction. The genotyping of (SNP: rs# 4526098 A/G) was obtained for patients and control by using allele-specific technique and thermocycler. The results showed 32 patients with positive family history, while 208 patients negative to family history. Also, the results showed 86 patients were positive to the RF test, but 154 patients were negative to the RF test. The results showed statistical significance in the correlation between family history and genotypes ($P$ = 0.025), otherwise, we didn't find the statistical association regarding RF test compared with genotypes ($P$ = 0.429). The statistical analysis showed a significant difference between the patients and control in genotypes AA and AG ($P$ =0.001 and $P$ = 0.008) respectively, while no statistical association in genotype GG ($P$ = 0.604) among the patients and control. The current results suggested that the IL-5 gene polymorphism (SNP: rs# 4526098 A/G) mostly associated with RA development.

**Keywords: IL-5, Genotype, Rheumatoid arthritis, Genetic susceptibility, SNP**

## INTRODUCTION

Rheumatoid arthritis (RA) is a popular systemic autoimmune disease, described by multiple joints chronic inflammation. This is mainly due to synovial cell proliferation with subsequent T-lymphocyte accumulation that leads to the destruction of both cartilage and bone, RA affects ~1% of the adults worldwide (Choy and Panayi, 2001, Sharma et al., 2004, Costenbader et al., 2008). RA considers a combination of multifactorial disorder, where together environmental and genetic factors contribute to disease development (John and Worthington, 2001, MacGregor et al., 2000). It is worth noting that genetic factors play a crucial role and may contribute to about 60% of disease predisposition and occurrence (Turesson and Matteson, 2006).

Many genes have been found to be associated with RA susceptibility. Of all these genes most loci investigated that HLA-DR and found it consistency about one-third of the genetic prediction to RA (Kurkó et al., 2013). Furthermore, Others loci in many genes noticed it is implicate in predisposition to RA development, such as

peptidyl arginine deiminase 4 (PADI4), Tyrosine-protein phosphatase non-receptor type 22 (PTPN22), cytotoxic T-lymphocyte–associated antigen 4 (CTLA4), Interleukin 1- beta (IL-1β), Tumor necrosis factor-alpha (TNF- α), Interleukin 5 (IL-5) and Signal transducer and activator of transcription 4 (STAT4) (Kurkó et al., 2013, Hinks et al., 2005, Cantagrel et al., 1999, Dawood et al., 2016).

The IL-5 cytokine essentially presented as T-cell replacing factor which excreted from T cells to motivate the production of antibody from stimulated B cells (Takatsu et al., 1980). Partially, the role of IL-5 cytokine has been mentioned briefly in the development of rheumatoid arthritis (Finnerty et al., 2008, Cicuttini et al., 1995, Chalan et al., 2016). it's crucial to investigate more loci in many genes to prove the susceptibility to RA particularly some genes that coding cytokines contributing to RA. In the current study, we aimed to assess the contribution of the polymorphic locus (rs# 4526098 A/G) in the IL-5 gene among RA Iraqis patients. Our research considers the first study of its kind among researches related to genetic polymorphisms and RA.

## MATERIALS AND METHODS

### Study of population

The current case-control study was recruited in Baghdad teaching hospital, medical city, Baghdad, Iraq, via enrolled 240 cases (138 male and 102 female) with an established diagnosis of RA according to American Rheumatism Association (ACR) 1987 Criteria (Arnett et al., 1988), and laboratory investigation. And 240 individual (92 male and 184 female) unrelated Iraqis matched in ages and sex, free disease and they didn't have a family history of (RA) considered as a control group. The ethics committees of participating universities and university hospitals approved the study, and informed consent was obtained from all participants.

### Blood samples collection and genomic DNA isolation

The blood samples collected from both cases and control (1.5 ml of venous blood) then kept in EDTA tubes under deep freezing at (-20$^{o}$ C) for later DNA extraction. The DNA extraction performed manually by using the specific method described previously by (Shubeita et al., 1987). The quantity of DNA yield was done by using NanoDrop-1000, Thermo Scientific, USA.

### Genotyping of selected SNP (rs4526098 A/G) in the IL-5 gene

The SNP (rs# 4526098 A/G) in the IL-5 gene was amplified by using allele-specific technique, the primers used in reaction ( A-allele specific primer: F1: 5- CTTTGTGGACTCCAGGTCCC-3, G-allele specific primer: F2: 5- CTTTGTGGGCTCCAGGTCCC-3 and Common reverse primer: 5- GGGACGCTTCTGCTCCTAAG-3) were designed newly by primer blast online programme (http://www.ncbi.nlm.nih.gov/tools/primer-blast/), purchased from Sigma Aldrich, USA. Based on allele-specific technique, two reactions for each sample were prepared to perform PCR genotyping, first reaction composited of 2µl DNA, 1µl A-allele specific primer, 1µl common reverse primer, 12.5 µl Taq master mix, then topped up the volume to 25 µl with nucleases free water, while the second reaction was composited of 2µl DNA, 1µl G-allele specific primer, 1µl common reverse primer, 12.5 µl Taq master mix, then topped up the volume to 25 µl with nucleases free water. The PCR conditions were as follow denaturation at 95$^{o}$ C for 5 min, 30 cycles of denaturation at 94$^{o}$ C for 60 s, annealing at 60$^{o}$ C for 30 s and extension at 72$^{o}$ C for 30 s, then extension at 72$^{o}$ C for 10 min. the amplification is done by using Veriti™ 96-Well Thermal Cycler (Applied Biosystems™). The PCR amplicon was detached by electrophoresis using 2% agarose stained with ethidium bromide, then visualized under UV transilluminator (UVP Visi-Blue™, Fisher Scientific). The AA genotype appeared in lane 1 only at 272 bp, while the GG genotype appeared in lane 2 only at 272 bp, and the AG genotype appeared in both lane 1 and 2 together at 272 bp as shown in figure 1.

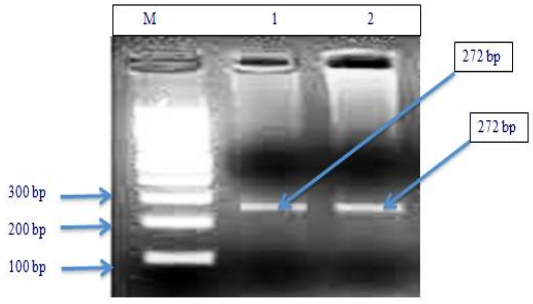


**Figure 1: 2% agarose gel electrophoresis for allele-specific PCR for the IL-5 gene (rs 4526098 A/G)**

**Performance of data statistical analysis**

The performance of data statistical analysis was done by using SPSS, version 22.0, Armonk, New York, 2013, to compare the variables. The alleles frequencies, genotypes in both groups were compared using Fisher's exact test, also the family history and rheumatoid factor were compared with genotypes in cases group using the Chi-squared test ($X^2$). Mean ± SD of ages in both groups was done. 95% confidence interval (95% CI) and Odds ratio were calculated among different studied groups. The significance of the results was taken at the value $P \leq 0.05$.

**RESULTS**

The selection characteristics distributions of the cases and control are listed in Table 1. The results showed the Mean ± SD of ages among cases was (67.20000 ± 11.71674), otherwise, the Mean ± SD of ages among control was (31.65833 ± 11.49167). Also, the results showed that 32 patients have a family history of (RA), while 208 patients didn't have a family history of (RA). The statistical analysis showed that 86 patients were positive to rheumatoid factor (RF) test, while 154 patients were negative to rheumatoid factor (RF) test. Moreover, all patients were positive to Anti-citrullinated protein antibodies (ACPAs) test. Regarding the correlation between family history and genotypes in patients, our results showed high statistical significance (*P* = 0.025) with 95 % CI (0.005248-0.044752), whereas noticed that the frequency of genotypes AA, GG, and AG among patients they didn't have family history of (RA) were as follow 10 (4.2 %), 14 (5.8 %) and 8 (3.3 %) respectively, on the other hand, the frequency of genotypes AA, GG and AG among patients they found with family history of (RA) were as follow 96 (40.0 %), 46 (19.2 %) and 66 (27.5 %) respectively as shown in Table 2. The correlation statistic between rheumatoid factor (RF) test results and genotypes distribution among patients didn't show any significance (*P* = 0.429) with 95 % CI (0.366547-0.491786), furthermore, the frequency of genotypes AA, GG and AG among patients they presented with positive to (RF) were as follow 40 (16.7 %), 24 (10.0 %) and 22 (9.2 %) respectively, while, the frequency of genotypes AA, GG and AG among patients they presented with positive to (RF) were as follow 66 (27.5 %), 36 (15.0 %) and 52 (21.7 %) respectively as shown in Table 3.

Regarding to the distribution of genotypes among cases and their control, our results appeared statistical significance in genotype AA among cases and control (*P* = 0.001) with 95 % CI (1.27 - 2.79) and OR = 1.88, and the frequency of AA genotype was as follow 106 (44.2 %) in cases and 71 (29.6 %) in control. As well as, the results didn't reveal to statistical association in genotype GG among the cases and control (*P* = 0.604) with 95 % CI (0.57 - 1.35) and OR = 0.88 and the frequency of GG genotype was as follow 60 (25.0 %) in cases and 66 (27.5 %) in control. Otherwise, our results exhibited statistical significance linkage in genotype AG among cases and control (P = 0.008) with 95 % CI (0.40 - 0.88) and OR = 0.59 and the frequency of AG genotype was as follow 74 (30.8 %) in cases and 103 (42.9 %) in control as shown in Table 4.

**Table 1: Show the selected characteristic of the patients and healthy individuals**

| Characteristics | Cases | Control |
|---|---|---|
| **Sample size** | 240 | 240 |
| **Sex** | | |
| **Male** | 138 | 92 |
| **Female** | 102 | 148 |
| **Ages** | | |
| **Minimum** | 35 | 13 |
| **Maximum** | 85 | 61 |
| **Mean ± SD** | 67.20000 ± 11.71674 | 31.65833 ± 11.49167 |
| **Family History** | | |
| **Positive** | 32 | |
| **Negative** | 208 | |
| **Rheumatoid Factor (RF) test** | | |
| **Positive** | 86 | |
| **Negative** | 154 | |
| **Anti-citrullinated protein antibodies (ACPAs) test** | | |
| **Positive** | 240 | |
| **Negative** | 0 | |

**Table 2: Shown the relation between the family history and genotype distribution among patients**

| Family history | | Genotype | | | Total | 95% Confidence Interval | | Pearson's chi-squared test |
|---|---|---|---|---|---|---|---|---|
| | | AA | GG | AG | | Lower Bound | Upper Bound | |
| Positive | Count | 10 | 14 | 8 | 32 | (0.005248-0.044752) | | *P* = 0.025** |
| | Total % | 4.2 % | 5.8 % | 3.3 % | 13.3% | | | |
| Negative | Count | 96 | 46 | 66 | 208 | | | |
| | Total % | 40.0% | 19.2% | 27.5% | 86.7% | | | |
| Total | Count | 106 | 60 | 74 | 240 | | | |
| | Total % | 44.2% | 25.0% | 30.8% | 100.0% | | | |

**Table 3: Shown the relation between the rheumatoid factor test and genotype distribution among patients**

| Rheumatoid factor test | | Genotype | | | Total | 95% Confidence Interval | | Pearson's chi-squared test |
|---|---|---|---|---|---|---|---|---|
| | | AA | GG | AG | | Lower Bound | Upper Bound | |
| Positive | Count | 40 | 24 | 22 | 86 | (0.366547-0.491786) | | *P* = 0.429 |
| | Total % | 16.7% | 10.0% | 9.2% | 35.8% | | | |
| Negative | Count | 66 | 36 | 52 | 154 | | | |
| | Total % | 27.5% | 15.0% | 21.7% | 64.2% | | | |
| Total | Count | 106 | 60 | 74 | 240 | | | |
| | Total % | 44.2% | 25.0% | 30.8% | 100.0% | | | |

**Table 4: IL-5 gene polymorphism and allele frequencies among rheumatoid arthritis patients and their control**

| `Gene polymorphism IL-5 gene (rs # 4526098 A/G) | Cases | | Control | | OR | (95% CI) | Fisher's exact test |
|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | | | |
| AA | 106 | 44.2 | 71 | 29.6 | 1.88 | (1.27 - 2.79) | *P* = 0.001** |
| GG | 60 | 25.0 | 66 | 27.5 | 0.88 | (0.57 - 1.35) | *P* = 0.604 |
| AG | 74 | 30.8 | 103 | 42.9 | 0.59 | (0.40 - 0.88) | *P* = 0.008** |
| Total | 240 | 100 | 240 | 100 | | | |

## DISCUSSION

RA is a disorder causes swelling of joints and may cause pain, stiffness and gradual loss of function. The rigorous reasons of RA are unclear, researches have been discovered that there are many possible causes, including sex, age, genetics, hormones, cytokines, infectious agents (viruses or bacteria), obesity, smoking, etc., also the family history palpable contributed in the GWA studies (Hopper et al., 2005, Burton et al., 2005, Hemminki et al., 2008). Family history has been shown to be a risk factor for developing of RA in previous researches (Koumantaki et al., 1997, Frisell et al., 2013, Kwoh et al., 1996).

Rojas-Villarraga et al. suggested that patients found with family history in first-degree family relatives are associated with the early appearance of joint damage (Rojas-Villarraga et al., 2009). While other study conducted in (Saint Margaret Memorial Hospital Rheumatoid Arthritis Registry, Pittsburgh, Pennsylvania) suggested that the

patients presented with first degree-relatives family history are often falsely recorded as positive (Kwoh et al., 1996). Koumantaki, Y., et al., proved in the research that the familial clustering mothers are more likely than fathers to develop susceptibility to RA in their offspring (Koumantaki et al., 1997). In our study, we proved that the family history is not always associated with RA patients. Otherwise, RA patients had a sensitivity of 60% ~ 90% for rheumatoid factor test and a specificity of 85% (Nishimura et al., 2007, Nell et al., 2005). Regarding our finding, we suggested that the RF test didn't consider a specific test for RA disease.

The relation between genotypes and family history had investigated in our research, we suggesting there is association between them due to statistical significance that found in data analysis between positive or negative to family history and genotypes, moreover we supposed that the patients reported with negative to family history who carrying genotype (AA) mostly candidate to be negative to family history more than other patients who carrying (GG) or (AG) genotype among our study.

Otherwise, the linkage between RF factor test and genotypes also tested, regarding our finding we assumed there is no relationship between a specific genotype and positive or negative to RF test among our study.

The cytokines and other soluble factors considered important diagnostic and biomarkers for most autoimmune diseases and several studies investigated their expression. Cytokines divulged in joints did not differ in 12- month disease when compared to most aggressive RA (Canete et al., 1997). Th2 cells generally produce IL-5 and other cytokines (Hoy et al., 1997). Moreover, the IL-5 gene polymorphisms were evaluated in many diseases like decreased pulmonary function in Korean children with atopic asthma (Hong et al., 2005), inhibitor development in severe hemophilia A patients (Fidanci et al., 2014), and the interaction of variants in the 5 genes are associated with the development of GD and Graves' ophthalmopathy (GO) (Zhu et al., 2010) et al.. Otherwise in other study analyzed the mRNA expression of Th1 (IFNγ, IL2) and Th2 (IL4, IL5, and IL10) cytokines in the synovium of RA and SpA (predominantly USpA) patients (Canete et al., 2000). Our study considers the first trail to use the IL-5 gene polymorphism (rs # 4526098 A/G) as predictor in RA development, we found strong association between patients and control via statistical analysis in genotypes (AA) and (AG), moreover we propose that genotype (AA) consider a risk factor and genotype (AG) consider a protective factor among Iraqi population.

## CONCLUSION

In conclusion, we revealed to many facts. The family history doesn't often associate with RA disease in the current study. Moreover, we believe that the persons who carried the genotype (AA) usually will present without a family history of RA. Otherwise, the RF test doesn't consider a specific test for RA occurrence. Furthermore, the genotypes among patients and control don't contribute to RF test determination. On the other hand, we suggest there is strong evidence between IL-5 gene polymorphism and RA among Iraqis ethnic since the presence (AA) genotype in cases considers a risk factor Iraqi peoples to RA development. while the presence (AG) genotype consider a protective factor against RA development.

## CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

## ACKNOWLEGEMENT

The authors are grateful to patients and control volunteers for the donation of blood and their agreement to use their clinical data in this study.

## AUTHOR CONTRIBUTIONS

Both authors contributed together in samples collections, practical work, data analysis and manuscript writing.



## REFERENCES

ARNETT, F. C., EDWORTHY, S. M., BLOCH, D. A., MCSHANE, D. J., FRIES, J. F., COOPER, N. S., HEALEY, L. A., KAPLAN,

S. R., LIANG, M. H., LUTHRA, H. S. & ET AL. 1988. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum,* 31**,** 315-24.

BURTON, P. R., TOBIN, M. D. & HOPPER, J. L. 2005. Key concepts in genetic epidemiology. *Lancet,* 366**,** 941-51.

CANETE, J. D., LLENA, J., COLLADO, A., SANMARTI, R., GAYA, A., GRATACOS, J., BLAY, M. & MUNOZ-GOMEZ, J. 1997. Comparative cytokine gene expression in synovial tissue of early rheumatoid arthritis and seronegative spondyloarthropathies. *Br J Rheumatol,* 36**,** 38-42.

CANETE, J. D., MARTINEZ, S. E., FARRES, J., SANMARTI, R., BLAY, M., GOMEZ, A., SALVADOR, G. & MUNOZ-GOMEZ, J. 2000. Differential Th1/Th2 cytokine patterns in chronic arthritis: interferon gamma is highly expressed in synovium of rheumatoid arthritis compared with seronegative spondyloarthropathies. *Ann Rheum Dis,* 59**,** 263-8.

CANTAGREL, A., NAVAUX, F., LOUBET-LESCOULIÉ, P., NOURHASHEMI, F., ENAULT, G., ABBAL, M., CONSTANTIN, A., LAROCHE, M. & MAZIÈRES, B. 1999. Interleukin-1β, interleukin-1 receptor antagonist, interleukin-4, and interleukin-10 gene polymorphisms: relationship to occurrence and severity of rheumatoid arthritis. *Arthritis & Rheumatism,* 42**,** 1093-1100.

CHALAN, P., BIJZET, J., VAN DEN BERG, A., KLUIVER, J., KROESEN, B.-J., BOOTS, A. M. & BROUWER, E. 2016. Analysis of serum immune markers in seropositive and seronegative rheumatoid arthritis and in high-risk seropositive arthralgia patients. *Scientific reports,* 6**,** 26021.

CHOY, E. H. & PANAYI, G. S. 2001. Cytokine pathways and joint inflammation in rheumatoid arthritis. *N Engl J Med,* 344**,** 907-16.

CICUTTINI, F., BYRON, K., MAHER, D., WOOTTON, A., MUIRDEN, K. & HAMILTON, J. 1995. Serum IL-4, IL-10 and IL-6 levels in inflammatory arthritis. *Rheumatology international,* 14**,** 201-206.

COSTENBADER, K. H., CHANG, S. C., LADEN, F., PUETT, R. & KARLSON, E. W. 2008. Geographic variation in rheumatoid arthritis incidence among women in the United States. *Arch Intern Med,* 168**,** 1664-70.

DAWOOD, A., HAMAD, O., BASHEER, A., KHUFLA, U. & NEAMA, A. 2016. Lack Evidence Between CTLA-4 Gene Polymorphisms Among Rheumatoid Arthritis Patients. *Journal of Natural Sciences Research,* 6**,** 90-93.

FIDANCI, I. D., ZULFIKAR, B., KAVAKLI, K., AR, M. C., KILINC, Y., BASLAR, Z. & CAGLAYAN, S. H. 2014. A Polymorphism in the IL-5 Gene is Associated with Inhibitor Development in Severe Hemophilia A Patients. *Turk J Haematol,* 31**,** 17-24.

FINNERTY, C. C., JESCHKE, M. G., HERNDON, D. N., GAMELLI, R., GIBRAN, N., KLEIN, M., SILVER, G., ARNOLDO, B., REMICK, D. & TOMPKINS, R. G. 2008. Temporal cytokine profiles in severely burned patients: a comparison of adults and children. *Molecular medicine,* 14**,** 553.

FRISELL, T., HOLMQVIST, M., KÄLLBERG, H., KLARESKOG, L., ALFREDSSON, L. & ASKLING, J. 2013. Familial risks and heritability of rheumatoid arthritis: role of rheumatoid factor/anti–citrullinated protein antibody status, number and type of affected relatives, sex, and age. *Arthritis & Rheumatology,* 65**,** 2773-2782.

HEMMINKI, K., LI, X., SUNDQUIST, K. & SUNDQUIST, J. 2008. Familial risks for common diseases: etiologic clues and guidance to gene identification. *Mutat Res,* 658**,** 247-58.

HINKS, A., BARTON, A., JOHN, S., BRUCE, I., HAWKINS, C., GRIFFITHS, C. E., DONN, R., THOMSON, W., SILMAN, A. & WORTHINGTON, J. 2005. Association between the PTPN22 gene and rheumatoid arthritis and juvenile idiopathic arthritis in a UK population: further support that PTPN22 is an autoimmunity gene. *Arthritis & Rheumatology,* 52**,** 1694-1699.

HONG, S. J., LEE, S. Y., KIM, H. B., KIM, J. H., KIM, B. S., CHOI, S. O., LEE, S. G., SHIN, E. S. & HONG, T. J. 2005. IL-5 and thromboxane A2 receptor gene polymorphisms are associated with decreased pulmonary function in Korean children with atopic asthma. *J Allergy Clin Immunol,* 115**,** 758-63.

HOPPER, J. L., BISHOP, D. T. & EASTON, D. F. 2005. Population-based family studies in genetic epidemiology. *Lancet,* 366**,** 1397-406.

HOY, M. D., WILLIAMS, J. L. & KIRKHAM, B. W. 1997. Symmetrical synovial fluid cell cytokine

messenger RNA expression in rheumatoid arthritis: analysis by reverse transcription/polymerase chain reaction. *Br J Rheumatol,* 36**,** 170-3.

JOHN, S. & WORTHINGTON, J. 2001. Genetic epidemiology. Approaches to the genetic analysis of rheumatoid arthritis. *Arthritis Res,* 3**,** 216-20.

KOUMANTAKI, Y., GIZIAKI, E., LINOS, A., KONTOMERKOS, A., KAKLAMANIS, P., VAIOPOULOS, G., MANDAS, J. & KAKLAMANI, E. 1997. Family history as a risk factor for rheumatoid arthritis: a case-control study. *The Journal of rheumatology,* 24**,** 1522-1526.

KURKÓ, J., BESENYEI, T., LAKI, J., GLANT, T. T., MIKECZ, K. & SZEKANECZ, Z. 2013. Genetics of rheumatoid arthritis—a comprehensive review. *Clinical reviews in allergy & immunology,* 45**,** 170-179.

KWOH, C. K., VENGLISH, C., LYNN, A. H., WHITLEY, D. M., YOUNG, E. & CHAKRAVARTI, A. 1996. Age, sex, and the familial risk of rheumatoid arthritis. *American journal of epidemiology,* 144**,** 15-24.

MACGREGOR, A. J., SNIEDER, H., RIGBY, A. S., KOSKENVUO, M., KAPRIO, J., AHO, K. & SILMAN, A. J. 2000. Characterizing the quantitative genetic contribution to rheumatoid arthritis using data from twins. *Arthritis Rheum,* 43**,** 30-7.

NELL, V. P., MACHOLD, K. P., STAMM, T. A., EBERL, G., HEINZL, H., UFFMANN, M., SMOLEN, J. S. & STEINER, G. 2005. Autoantibody profiling as early diagnostic and prognostic tool for rheumatoid arthritis. *Ann Rheum Dis,* 64**,** 1731-6.

NISHIMURA, K., SUGIYAMA, D., KOGATA, Y., TSUJI, G., NAKAZAWA, T., KAWANO, S., SAIGO, K., MORINOBU, A., KOSHIBA, M., KUNTZ, K. M., KAMAE, I. & KUMAGAI, S. 2007. Meta-analysis: diagnostic accuracy of anti-cyclic citrullinated peptide antibody and rheumatoid factor for rheumatoid arthritis. *Ann Intern Med,* 146**,** 797-808.

ROJAS-VILLARRAGA, A., DIAZ, F. J., CALVO-PARAMO, E., SALAZAR, J. C., IGLESIAS-GAMARRA, A., MANTILLA, R. D. & ANAYA, J. M. 2009. Familial disease, the HLA-DRB1 shared epitope and anti-CCP antibodies influence time at appearance of substantial joint damage in rheumatoid arthritis. *J Autoimmun,* 32**,** 64-9.

SHARMA, P. K., HOTA, D. & PANDHI, P. 2004. Biologics in rheumatoid arthritis. *J Assoc Physicians India,* 52**,** 231-6.

SHUBEITA, H. E., SAMBROOK, J. F. & MCCORMICK, A. M. 1987. Molecular cloning and analysis of functional cDNA and genomic clones encoding bovine cellular retinoic acid-binding protein. *Proc Natl Acad Sci U S A,* 84**,** 5645-9.

TAKATSU, K., TOMINAGA, A. & HAMAOKA, T. 1980. Antigen-induced T cell-replacing factor (TRF). I. Functional characterization of a TRF-producing helper T cell subset and genetic studies on TRF production. *J Immunol,* 124**,** 2414-22.

TURESSON, C. & MATTESON, E. L. Genetics of rheumatoid arthritis. Mayo Clinic Proceedings, 2006. Elsevier, 94-101.

ZHU, W., LIU, N., ZHAO, Y., JIA, H., CUI, B. & NING, G. 2010. Association analysis of polymorphisms in IL-3, IL-4, IL-5, IL-9, and IL-13 with Graves' disease. *J Endocrinol Invest,* 33**,** 751-5.