*Journal of* Genetic and Environmental Resources Conservation, 2017, 5(2):59-63.
www.jgerc.com Print ISSN: 2306-8663, Online ISSN: 2306 – 8280

# A single nucleotide polymorphism of tumor necrosis factor alpha gene (rs1800629) is not associated with rheumatoid arthritis in a sample of Iraqi patients

Aseel S. Mahmood[1], Abdul-Kareem A. Al-Kazaz[2] and Ali H. Ad'hiah[3]*

[1,2]Dep. Of Biotechnology, College of Science and [3]Tropical-Biological Research Unit, College of Science, University of Baghdad, Iraq.
*Corresponding author: dr.a.h.adhiah@gmail.com, dr.ahadhiah@sc.uobaghdad.edu.iq

## Abstract

Rheumatoid arthritis (RA) is a complex multifactorial autoimmune disease caused by environmental influences and an unknown number of predisposing genes. The present study investigated the association between RA and a single nucleotide polymorphism (SNP) in the promoter region of tumor necrosis factor-alpha (*TNF*$_{-308}$ G/A) gene. Fifty-one Iraqi RA patients and 45 control subjects were enrolled in the study during the period November 2015 - June 2016. After PCR amplification of their DNA, the PCR products were sequenced to reveal the genotypes of *TNF*$_{-308}$ SNP (rs1800629), which was observed to have three genotypes (GG, GA and AA) that were correspondent to two alleles (*G* and *A*). These genotypes showed a significant deviation from Hardy-Weinberg Equilibrium in patients and controls, but comparing both groups revealed no significant variation in the distribution of genotype and allele frequencies. These results suggest that *TNF*$_{-308}$ SNP is not associated with RA in Iraqi population.

Keywords: Rheumatoid arthritis, Tumor necrosis factor-α, Single nucleotide polymorphism.

## Introduction

Rheumatoid arthritis (RA) is a common, systemic and chronic inflammatory autoimmune disease of the connective tissues. It is characterized by a chronic inflammation of the joints, and may lead to a structural damage in cartilages and bones (Cross *et al.,* 2014). The etiology of disease is not well-characterized, but it is well-documented that genetic and environmental factors contribute to its etiology, and the interaction(s) between the two factors leads to immunological abnormalities that are involved in its pathogenesis (Araki and Mimura, 2016).

Pathologically, RA is mainly three steps process that begins with autoimmunity development, followed by local inflammation and in the final step, bone destruction is induced (Mcinnes and Schett, 2011). The development of autoimmunity in susceptible individuals is influenced by various genetic and environmental factors, and presence of autoantibodies (rheumatoid factors; RFs and anti-cyclic citrullinated protein; ACCP antibodies) is an important manifestation of autoimmunity, which can precede clinical manifestations of RA (Song and Kang, 2009). Then, localized inflammation of joints is progressed (i.e. synovitis), and outcome in cartilage and bone destruction. Both innate and adaptive immune responses are involved in the inflammatory process, in which cytokines play important role in the inflammation and in advancing the disease (Mateen *et al.,* 2016).

Tumor necrosis factor-alpha (TNF-α) is one of these cytokines that mediates a broad range of proinflammatory and immunostimulatory activities (Rajput and Ware, 2016). In RA, TNF-α is a major cytokine involved in RA pathogenesis, and therefore, it has been a target for immunotherapy in RA patients (Manara and Sinigaglia, 2015). Genetically, *TNF* gene is mapped to the short arm of human chromosome 6 (6p21.3) within class III genes of HLA region, which code for important immune response genes that have role in RA susceptibility (Atzeni and Sarzi-Puttini, 2013). Single nucleotide polymorphisms (SNPs) in *TNF*

gene promoter region have been demonstrated to have associations with RA in patients. Among these SNPs is *TNF*$_{-308}$ SNP (rs1800629), which has been extensively investigated in RA, but the findings have been not consistent (Khanna *et al.*, 2006; Nemec *et al.,* 2008; Domínguez-Pérez *et al.,* 2017). Accordingly, the present investigation was designed to shed light on the association between *TNF*$_{-308}$ SNP (G/A) and rheumatoid arthritis in a sample of Iraqi patients.

## Materials and Methods

The ethical committee of the Iraqi Ministry of Health approved the study, in which 51 Iraqi RA patients (21 males and 30 females) were enrolled and their age range was 20 - 63 years (44.9 ± 1.5 years). They were referred to the Rheumatology Clinic (Baghdad Teaching Hospital) during the period November 2015 - June 2016 for diagnosis and treatment. The diagnosis was made by the consultant medical staff at the Rheumatology Unit, and it was based on the revised diagnostic criteria established by the American College of Rheumatology (ACR), 2010 (Aletaha *et al.,* 2010). In addition to patients, 45 apparently healthy control subjects were also enrolled in the study. They matched patients for ethnicity (Iraqis), gender (14 males and 31 females) and age (41.3 ± 1.3 years).

The Genomic DNA was extracted from EDTA blood using ReliaPrepTM Blood gDNA Miniprep System (Promega Corporation, USA), and after assessing purity and concentration, it was subjected to PCR amplification. Two primers were designed (Forward: 5'-TCTCCCTCAAGGACTCAGCTTTCTG-3' and Reverse: 5'-TGAGAGGAAGAGAACCTGCCTGG-3') for genotyping of *TNF*$_{-308}$ SNP by using the Geneious software version 10.1.3. The PCR reaction was performed in a final volume of 25 µl; which included 12.5 µl GoTaq green Master mix, 0.75 µl forward primer (10 µM), 0.75 µl reverse primer (10 µM), 2 µl DNA sample (50 ng) and 9 µl nuclease-free distilled water. The PCR conditions were initial denaturation at 95°C for 5 minutes (1 cycle), followed by 35 cycles of denaturation at 95°C (30 seconds), annealing at 60°C (30 seconds) and extension at 72°C (30sec), followed by a final extension at 72°C for 7 minutes. The amplified PCR fragments were subjected for Sanger's sequencing using ABI3730XL automated DNA sequencer (Macrogen Corporation – Korea). The genotypes were revealed by Geneious software after alignment with a reference sequence in the Gene Bank.

Allele and genotype frequencies were given as percentage frequencies. The genotype frequencies were first tested for their agreement with Hardy-Weinberg equilibrium (HWE), and a significant difference between the observed and expected genotype frequencies was assessed by Pearson's Chi-square test (https://www.easycalculation.com/health/hardy-weinberg-equilibrium-calculator.php). The association between *TNF*$_{-308}$ SNP and RA was presented in terms of odds ratio (OR), etiological fraction (EF) and preventive fraction (EF), and a significant difference was assessed by two-tailed Fisher exact probability (Ad'hiah, 1990). The software WinPepi version 11.65 was used to carry out the latter calculations.

## Results and Discussion

The *TNF*$_{-308}$ SNP was observed to have three genotypes (GG, GA and AA), which were correspondent to two alleles (*G* and *A*) in RA patients and controls (Figure 1).

Analysis of HWE revealed that there was a significant difference between the observed and expected genotype frequencies of *TNF*$_{-308}$ SNP in RA patients ($X^2$ = 12.875; D.F. = 1; $p \leq 0.001$) and controls ($X^2$ = 8.055; D.F. = 1; $p \leq 0.01$); and accordingly, no agreement with the equilibrium was recorded in patients or controls (Table 1).

When RA patients were compared to controls, the mutant homozygous genotype (AA) showed an increased frequency in patients (11.8 *vs*. 6.7%; OR = 1.87; 95% C.I.: 0.45-7.82), and similarly, the frequency of mutant allele (*A*) was also increased in patients (19.6 *vs*. 13.3%; OR = 1.59; 95% C.I.: 0.73 - 3.44). However, both differences did not reach a significant level ($p > 0.05$) (Table 2).

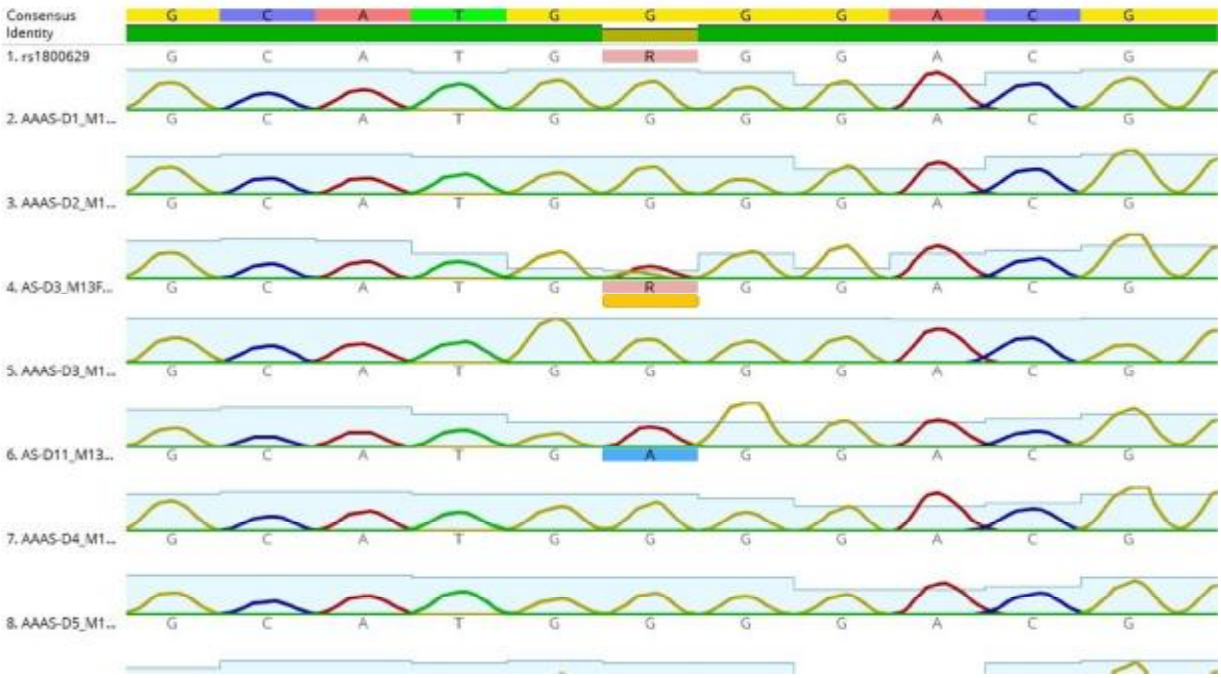


Figure (1): DNA sequence chromatogram of *TNF*$_{-308}$ SNP showing three genotypes: GG (samples 2, 3, 5, 7 and 8), GA (sample 4; R) and AA (sample 6). In addition, the reference sequence is also given (rs1800629).

Table (1): Numbers and percentage frequencies (observed and expected) of *TNF*$_{-308}$ SNP (rs1800629) genotypes and their Hardy-Weinberg equilibrium in rheumatoid arthritis patients and controls.

| Genotype | Rheumatoid Arthritis Patients (No. = 51) | | | | Controls (No. = 45) | | | |
|---|---|---|---|---|---|---|---|---|
| | Observed | | Expected | | Observed | | Expected | |
| | No. | % | No. | % | No. | % | No. | % |
| GG | 37 | 72.5 | 33.0 | 64.7 | 36 | 80.0 | 33.8 | 75.1 |
| GA | 8 | 15.7 | 16.0 | 31.4 | 6 | 13.3 | 10.4 | 23.1 |
| AA | 6 | 11.8 | 2.0 | 3.9 | 3 | 6.7 | 0.8 | 1.8 |
| HWE | $X^2$ = 12.875; D.F. = 1; $p \leq 0.001$ | | | | $X^2$ = 8.055; D.F. = 1; $p \leq 0.01$ | | | |

HWE: Hardy-Weinberg equilibrium

Table (2): Statistical analysis of association between genotypes and alleles of *TNF*$_{-308}$ SNP (rs1800629 dbSNP) and rheumatoid arthritis.

| Genotype or Allele | Patients (No. = 51) | | Controls (No. = 45) | | Odds Ratio | 95% Confidence Interval | EF or PF | *p* |
|---|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | | | | |
| GG | 37 | 72.5 | 36 | 80.0 | 0.66 | 0.26 - 1.70 | 0.27 | NS |
| GA | 8 | 15.7 | 6 | 13.3 | 1.21 | 0.39 - 3.75 | 0.03 | NS |
| AA | 6 | 11.8 | 3 | 6.7 | 1.87 | 0.45 - 7.82 | 0.06 | NS |
| *G* | 82 | 80.4 | 78 | 86.7 | 0.63 | 0.29 - 1.37 | 0.32 | NS |
| *A* | 20 | 19.6 | 12 | 13.3 | 1.59 | 0.73 - 3.44 | 0.07 | NS |

EF: Etiological fraction; PF: Preventive fraction; *p*: Probability; NS: Not significant ($p > 0.05$)

The present results suggest that there is no association between *TNF*$_{-308}$ SNP and RA in the Iraqi investigated sample of disease. The SNP *TNF*$_{-308}$ of *TNF* gene has been extensively investigated in RA patients but contradictory results have been reported. In a cohort of RA patients who had active disease, the *TNF*$_{-308}$ was analyzed. The results revealed that presence of AA and AG genotypes was positively associated with the progression rate of disease in American Caucasian patients (Khanna *et al.,* 2006). A meta-analysis of 14 studies (10 Europeans, 3 Latin Americans, and one Asian) failed to confirm these findings and showed no association between RA and *TNF*$_{-308}$ SNP in total RA patients, but the distribution of patients by ethnicity revealed a different profile. The *A* allele was significantly increased in Latin American RA patients, while such association was not found in European patients (Lee et al., 2007). A further investigation from Iran suggested that *TNF*$_{-308}$ is not a genetic risk factor for RA susceptibility but may be associated with radiographic damage in RA patients (Rezaieyazdi *et al.,* 2007). These findings were confirmed in Czech RA patients, and the authors reported that allele and genotype frequencies of *TNF*$_{-308}$ SNP showed no significant difference between patients and controls, but a significant variation have been noticed within RA patients distributed by radiographic progression of RA. Their results suggested that this SNP is associated with severity of RA (Nemec *et al.,* 2008). However, in Turkish RA patients, no statistically significant association was found between this SNP and RA (Ates *et al.,* 2008). In North Indian RA patients, the *A* allele of *TNF*$_{-308}$ SNP was associated with a protection rather than susceptibility (Gambhir *et al.*, 2010). The results of an Egyptian study demonstrated a positive association between *G* allele and GG homozygous genotype of *TNF*$_{-308}$ SNP and RA susceptibility, while *A* allele was associated with erosion in patients (Mosaad *et al.,* 2011). In Mexican RA patients, it has been concluded that *TNF*$_{-308}$ SNP showed no association with RA (Domínguez-Pérez *et al.,* 2017). These findings suggest that the association between *TNF*$_{-308}$ SNP and RA might be subjected to ethnicity of patients and severity of disease, although it is not a consistent profile, and the present study results are if favor of no association between *TNF*$_{-308}$ SNP and RA in Iraqi patients.

## Conclusion

No significant association between alleles or genotypes of *TNF*$_{-308}$ SNP and RA was observed in Iraqi patients; although *A* allele was increased and *G* allele was decreased in patients.

## References

Ad'hiah, A.H. 1990. Immunogenetics studies in selected human diseases. Ph.D. Thesis, University of Newcastle upon Tyne.

Aletaha, D.; Neogi, T.; Silman, A.J.; Funovits, J.; Felson, D.T.; Bingham, C.O.; Birnbaum, N.S.; Burmester, G.R.; Bykerk, V.P.; Cohen, M.D.; Combe, B.; Costenbader, K.H.; Dougados, M.; Emery, P.; Ferraccioli, G.; Hazes, J.M.; Hobbs, K.; Huizinga, T.W.; Kavanaugh, A.; Kay, J.; Kvien, T.K.; Laing, T.; Mease, P.; Menard, H.A.; Moreland, L.W.; Naden, R.L.; Pincus, T.; Smolen, J.S.; Stanislawska-Biernat, E.; Symmons, D.; Tak, P.P.; Upchurch, K.S.; Vencovsky, J.; Wolfe, F. and Hawker, G. 2010. Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Ann. Rheum. Dis., 69: 1580–1588.

Araki, Y. and Mimura, T. 2016. The mechanisms underlying chronic inflammation in rheumatoid arthritis from the perspective of the epigenetic landscape. J. Immunol. Res., 2016, 10 pages.

Ates, O.; Hatemi, G.; Hamuryudan, V. and Topal-Sarikaya, A. 2008. Tumor necrosis factor-alpha and interleukin-10 gene promoter polymorphisms in Turkish rheumatoid arthritis patients. Clin. Rheumatol., 27: 1243–1248.

Atzeni, F. and Sarzi-Puttini, P. 2013. Tumor Necrosis Factor, in: Brenner's Encyclopedia of Genetics. Elsevier, 229–231pp.

Cross, M. Smith, E.; Hoy, D.; Carmona, L.; Wolfe, F.; Vos, T.; Williams, B.; Gabriel, S.; Lassere, M.; Johns, N.; Buchbinder, R.; Woolf, A. and March, L. 2014. The global burden of rheumatoid arthritis: estimates from the Global Burden of Disease 2010 study. Ann. Rheum. Dis., 73: 1316–1322.

Domínguez-Pérez, R.A.; Loyola-Rodriguez, J.P.; Abud-Mendoza, C.; Alpuche-Solis, A.G.;

Ayala-Herrera, J.L. and Martínez-Martínez, R.E. 2017. Association of cytokines polymorphisms with chronic peridontitis and rheumatoid arthritis in a Mexican population. Acta Odontol. Scand., 6357: 1–6.

Gambhir, D.; Lawrence, A.; Aggarwal, A.; Misra, R.; Mandal, S.K. and Naik, S. 2010. Association of tumor necrosis factor alpha and IL-10 promoter polymorphisms with rheumatoid arthritis in North Indian population. Rheumatol. Int., 30: 1211–1217.

Khanna, D.; Wu, H.; Park, G.; Gersuk, V.; Gold, R.H.; Nepom, G.T.; Wong, W.K.; Sharp, J.T.; Reed, E.F.; Paulus, H.E. and Tsao, B.P. 2006. Association of tumor necrosis factor α polymorphism, but not the shared epitope, with increased radiographic progression in a seropositive rheumatoid arthritis inception cohort. Arthritis Rheum., 54: 1105–1116.

Lee, Y.H.; Ji, J.D. and Song, G.G. 2007. Tumor necrosis factor-alpha promoter -308 A/G polymorphism and rheumatoid arthritis susceptibility: a metaanalysis. J. Rheumatol., 34: 43–9.

Manara, M. and Sinigaglia, L. 2015. Bone and TNF in rheumatoid arthritis: clinical implications. RMD open 1, e000065.

Mateen, S.; Zafar, A.; Moin, S.; Khan, A.Q. and Zubair, S. 2016. Understanding the role of cytokines in the pathogenesis of rheumatoid arthritis. Clin. Chim. Acta, 455: 161–71.

Mcinnes, I.B. and Schett, G. 2011. The Pathogenesis of Rheumatoid Arthritis. N. Engl. J. Med., 365: 2205–2219.

Mosaad, Y.M.; Abdelsalam, A. and El-bassiony, S.R. 2011. Association of tumour necrosis factor-alpha -308G/A promoter polymorphism with susceptibility and disease profile of rheumatoid arthritis. Int. J. Immunogenet., 38, 427–433.

Nemec, P.; Pavkova-Goldbergova, M.; Stouracova, M.; Vasku, A.; Soucek, M. and Gatterova, J. 2008. Polymorphism in the tumor necrosis factor-α gene promoter is associated with severity of rheumatoid arthritis in the Czech population. Clin. Rheumatol., 27: 59-65.

Rajput, A. and Ware, C.F. 2016. Tumor Necrosis Factor Signaling Pathways, in: Encyclopedia of Cell Biology. Elsevier, 354–363pp.

Rezaieyazdi, Z.; Afshari, J.T.; Sandooghi, M. and Mohajer, F. 2007. Tumour necrosis factor a -308 promoter polymorphism in patients with rheumatoid arthritis. Rheumatol. Int., 28: 189–191.

Song, Y.W. and Kang, E.H. 2009. Autoantibodies in rheumatoid arthritis: Rheumatoid factors and anticitrullinated protein antibodies. Q.J.M., 103: 139–146.