

RESEARCH ARTICLE

Association of Cd40 (rs1883832) SNP in some Iraqi patients with Rheumatoid Arthritis and their correlation with disease activity

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Abstract

Objective: To determine the genotyping of CD40 gene (rs1883832) single nucleotide polymorphism (SNP) among rheumatoid arthritis patients, and to investigate its correlation with disease activity.

Method: The case-control study was conducted at Baghdad Teaching Hospitals, its affiliated Rheumatology units and Al-Yarmouk Teaching Hospital, Baghdad, Iraq, from July 2020 to May 2021, and comprised adult females with rheumatoid arthritis in group A and healthy controls in group B. The disease activity in group A was evaluated using the Disease Activity Score in 28 Joints scale. Samples from both groups were genotyped using TaqMan Assay for cluster of differentiation gene rs1883832 single nucleotide polymorphism. Data was analysed using SPSS 28.

Results: Of the 118 participants, 76(64.4%) were in group A with mean age 45.38±1.23 years, and 42(35.6%) were in control group B with mean age 46.48±2.02 years. At cluster of differentiation gene rs1883832 single nucleotide polymorphism, there was a non-significant difference in the frequency of T and A alleles as well as in TT, AA and TA in group A patients compared to group B controls ($p>0.05$). Rheumatoid factor and C-reactive protein concentrations with TT, TA and AA genotypes in group A were significantly higher in group A compared to group B ($p<0.05$). Within group A, there was a significant increase in the TT genotype and a significant decrease in the TA genotype in severe cases compared to mild cases. ($p<0.05$).

Conclusion: The cluster of differentiation gene rs1883832 single nucleotide polymorphism T allele was found to be a risk factor for rheumatoid arthritis and disease severity. The TT and AA alleles acted as protective factors.

Keywords: Polymorphism, Disease activity, Genotype. **DOI:** <https://doi.org/10.47391/JPMA.IQ-20>

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune illness that affects the articular joints. Around 1% of the world's population has RA, which causes synovial hyperplasia, infiltration of large numbers of inflammatory cells into the joints, production of autoantibodies, systemic inflammation, and cardiovascular issues.^{1,2} The cause of RA is unknown. Certain environmental factors as well as autoimmune initiating agents are thought to induce an autoimmune response in genetically susceptible individuals.^{3,4} More polymorphisms for RA would be useful in better understanding the disease's pathogenesis.^{5,6} The tumour necrosis factor (TNF) receptor superfamily includes cluster of differentiation 40 (CD40), a 48-kDa transmembrane protein that is expressed constitutively and/or indelibly on antigen-presenting cells (APCs), monocytes, and T cells, as well as non-immune cells, such as fibroblasts, endothelial, smooth muscle and epithelial cells. By interacting with its

ligand CD40 (CD154), which is primarily produced by activated CD4+ T cells, CD40 signalling regulates many mechanisms involved in the regulation of cellular and humoral adaptive immune responses.^{7,8} Several organs, including B cells, monocytes, dendritic cells, endothelium and epithelial cells, smooth muscle cells and fibroblasts express this transmembrane glycoprotein.² To better understand the relationship between CD40 gene polymorphism and susceptibility to RA, interesting findings are revealed from the observation that CD40-CD40 ligand interaction, a critical stage in the pathogenesis of autoimmune diseases, which is thought to be involved in atherogenesis and plaque rupture in RA.⁵ When CD40 binds to its ligand on T cells, it acts as a transmembrane signal transducer, activating intracellular kinases and transcription factors and causing inflammatory responses. CD40 signalling has been linked to the pathogenic mechanisms of chronic inflammatory and autoimmune disorders.^{9,10} CD40 is involved in humoral immune responses mediated by T cells. CD154 is found in many different types of cells. Patients with systemic lupus erythematosus (SLE), RA and Sjögren's syndrome have higher levels of soluble CD154 (sCD154), which has been linked with disease activity. CD40 interacts with ligand CD154, which is produced by activated T cells, and this interaction is critical for immune system T cell function.¹¹

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The current study was planned to determine the genotyping of CD40 gene (rs1883832) single nucleotide polymorphism (SNP) among RA patients, and to investigate its correlation with disease activity.

Patients and Methods

The case-control study was conducted at Baghdad Teaching Hospitals, its affiliated Rheumatology units and Al-Yarmouk Teaching Hospital, Baghdad, Iraq, from July 2020 to May 2021 and comprised adult females with RA in group A and healthy controls in group B.

The RA patients fulfilled the American College of Rheumatology 2010 criteria.¹² Most of the patients attending the clinics were females, and there were very few male patients who did not agree to participate in the study. RA patients had been diagnosed by consultant rheumatologists, and the information was documented on the patients' record files. The controls were age-matched healthy people not related to the patients.

After approval from the ethics review committee of the Iraqi Ministry of Health, the sample size was calculated using the equation:¹³

$$n = \frac{NP(1-P)}{(N-1)\left(\frac{d}{Z_{1-\alpha/2}}\right)^2 + P(1-P)} \quad (1)$$

The sample was raised using simple random sampling technique. Those included were females aged 26-60 years diagnosed with RA. RA patients suffering from kidney disease, hypogonadism, metabolic bone disease, primary bone tumour or bone metastasis, osteomyelitis, and patients receiving anti-TNF therapy were excluded.

After obtaining informed consent from all the subjects, 8ml venous blood was collected under aseptic conditions. Three test tubes were used for each sample; 3ml were utilised for serological tests, 3ml were added to the ethylenediaminetetraacetic acid (EDTA) tube and kept at -2°C for TaqMan genotyping assay, and 2ml were used for erythrocyte sedimentation rate (ESR) test. Serum was used to determine the anti-cyclic citrullinated peptide (anti-CCP) antibodies.

Anti-citrullinated protein/peptide antibody (ACPAs) and rheumatoid factor (RF) were assessed (Elabscience Biotech, China). Genomic deoxyribonucleic acid (DNA) was isolated from the blood sample following the protocol outlined by the kit manufacturer (Quick-gDNA Blood MiniPrep, Zymo, United States), and the NanoDrop technology was used to determine DNA concentration and purity.

The samples of all subjects were genotyped using TaqMan

genotyping assay for rs1883832 SNP (its position chr20:46118343 on GRCh38) (Thermo Fisher Scientific, Waltham, MA USA)

RA patient's disease activity was evaluated according to the Disease Activity Score in 28 Joints (DAS28) scale.¹⁴

Data was analysed using SPSS 28. Mean \pm standard error (SE) as well as frequencies and percentages were used to express the data as appropriate. Chi-square and t-test were used, odds ratios (ORs) were calculated with 95% confidence intervals (CIs), and Hardy-Weinberg equilibrium (HWE) calculations were done as appropriate. $P < 0.05$ was considered statistically significant.

Results

Of the 118 participants, 76(64.4%) were in group A with mean age 45.38 ± 1.23 years, and 42(35.6%) were in control group B with mean age 46.48 ± 2.02 years. Serum C-reactive protein and (CRP) and ESR levels were significantly increased in group A patients compared to group B controls (Table 1).

At CD40 rs1883832 SNP, A and T alleles were found, resulting in TT, TA and AA genotypes. There was no significant difference in the frequency of the T and A alleles in RA patients compared to the controls ($p > 0.05$). TT and AA genotypes were non-significantly lower in group A compared to group B, and TA genotype was non-significantly lower in group A compared to group B (Table 2).

There were no significant differences in the genotype within the same group, but intra-group differences were significant (Table 3).

With respect to RF, intra-group differences were not significant, but there was a significant inter-group

Table-1: Clinical and demographic characteristics. Groups (ALL patients and Controls were of the female gender)

Studied parameters	RA Patients (76)	Control (42)	p-value
Age (years)	45.38 \pm 1.23	46.48 \pm 2.02	$P > 0.05$
Age range (years)	26-60	26-60	
CRP (mg/dl)	29.37 \pm 2.0	3.12 \pm 0.19	$P < 0.01$
ESR (mm/h)	42.50 \pm 1.46	7.48 \pm 0.82	$P < 0.1$
Duration of Disease	6.31 \pm 0.48	0.0 \pm 0.0	$P < 0.01$
Females	100%	100%	$P < 0.01$
Family history			
Yes	39 (51.3)	0 (0.0)	$P < 0.01$
No	37 (48.7)	42 (100.0)	
Morning stiffness			
Yes	76 (100.0)	0 (0.0)	$P < 0.01$
No	0 (0.0)	42 (100.0)	
Smoking			
Yes	4 (5.3)	0 (0.0)	$P > 0.05$
No	72 (94.7)	42 (100.0)	

RA: Rheumatoid arthritis, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate.

Table-2: Allele frequencies and genotype of CD40 (rs1883832) SNP in RA and contro1 groups.

Genotyping and alleles frequencies	Patients group		OR	95% CI	Fisher's exact p-value
	n (%)				
T	84 (55.0)	44 (52.0)	1.12	0.66-1.91	0.684
A	68 (45.0)	40 (48.0)	0.89	0.57-1.52	0.684
TT	25 (32.9)	16 (38.1)	0.80	0.37-1.73	0.687
TA	34 (44.7)	12 (28.6)	2.02	0.91-4.50	0.115
AA	17 (22.4)	14 (33.3)	0.58	0.25-1.32	0.274
Tota1	76 (100.0)	42 (100.0)			
P-HWE	0.4064	0.0056			

RA: Rheumatoid arthritis, CD40: Cluster of differentiation 40, SNP: Single nucleotide polymorphism, OR: Odds ratio, CI: Confidence interval, P-HWE: Probability of Hardy-Weinberg equilibrium.

Table-3: The relationship between SNP (rs1883832) genotype frequencies and level of anti-CCP in RA and control subjects.

Genotyping	Anti-CCP mean \pm SE of mean (U/1)		p-value
	Patients	Contro1	
TT	65.44 \pm 4.28A	14.19 \pm 0.52B	0.00001
TA	58.94 \pm 4.38A	14.60 \pm 0.51A	0.00098
AA	67.71 \pm 6.63A	13.43 \pm 0.53A	0.000007

SNP: Single nucleotide polymorphism, Anti-CCP: Anti-cyclic citrullinated peptide, RA: Rheumatoid arthritis, SE: Standard error. *Duncan test: Similar letters denote non-significant differences between the genotypes of the same group.

Table-4: The relationship between SNP (rs1883832) genotype frequencies and the level of RF in RA patients and control.

Genotyping	RF mean \pm SE of mean (IU/ml)		p-value
	Patients	Contro1	
TT	36.13 \pm 4.22A	5.38 \pm 0.96A	0.003
TA	38.07 \pm 3.66A	4.80 \pm 1.25A	0.009
AA	37.45 \pm 5.99A	4.86 \pm 0.74A	0.006

SNP: Single nucleotide polymorphism, RF: Rheumatoid factor, RA: Rheumatoid arthritis, SE: Standard error. *Duncan test: Similar letters denote non-significant differences between the genotypes of the same group.

Table-5: The relationship between SNP (rs1883832) genotype frequencies and level of CRP in RA patients and controls.

Genotyping	CRP mean \pm SE of mean (mg/dl)		p-value
	Patients	Contro1	
TT	25.24 \pm 2.65A	3.49 \pm 0.25A	0.011
TA	29.21 \pm 2.79A	2.38 \pm 0.33A	0.007
AA	35.79 \pm 5.70A	3.37 \pm 0.31B	0.0001

SNP: Single nucleotide polymorphism, CRP: C-reactive protein, RA: Rheumatoid arthritis, SE: Standard error. *Duncan test: Similar letters denote non-significant differences between the genotypes of the same group.

Table-6: Comparison of mean DAS-28 scores of SNP (rs1883832) genotype frequencies between severe and mild RA cases.

Genotyping	DAS28 mean \pm SE of mean of patients group		p-value
	Severe	Mild	
TT	23.88 \pm 1.19A	17.58 \pm 8.0C	0.013
TA	22.84 \pm 1.17A	32.67 \pm 7.17A	0.027
AA	22.47 \pm 1.97A	15.0 \pm 3.0B	0.204

DAS28: Disease Activity Score in 28 Joints scale, SNP: Single nucleotide polymorphism, RA: Rheumatoid arthritis, SE: Standard error. *Duncan test: Similar letters denote non-significant differences between the genotypes of the same group.

difference in individuals with TT, TA and AA genotypes (Tab1e 4).

In terms of CRP, intra-group differences were not significant, but inter-group differences were significant in patients with TT, TA and AA genotypes (Tab1e 5).

Within group A, there was a significant increase in the TT genotype and a significant decrease in the TA genotype in severe cases compared to mild ones (Tab1e 6).

Discussion

RA is a common chronic autoimmune disease characterised by its diversity and unknown causes. CD40 plays an important role in the regulation of adaptive cellular and humoral immune responses and has therefore been associated with several autoimmune diseases and inflammatory diseases, including RA.¹³ Many studies have shown that these polymorphisms influence CD40 expression at messenger ribonucleic acid (mRNA) and protein levels.¹⁴

The current study is the first to look at the relationship between CD40 functional characteristics and RA susceptibility in an Iraqi population, as, to our knowledge, there have been no such studies.

Interestingly, CD40 T>C, T>G (rs1883832) SNP is present in several populations studied previously.^{10,15} To our knowledge, there have been no studies to date on these genetic variants of CD40 T>A (rs1883832) SNP and their relationship to RA in Iraqi patients compared to another population.

The T allele was found to be more sensitive to RA. When stratified by genotype among RA patients with the control group, there were no significant differences in allele frequencies for rs1883832. The current results agree with studies in which this polymorphism was identified as a genetic risk factor in many European groups.^{16,18} The biological pathways underlying susceptibility and severity are likely distinct concerning the triggering of CD40. This

would explain the finding that the minor T allele had a protective effect in susceptibility analysis, but was associated with a more severe disease course. It is essential to perform further studies on the mechanisms by which CD40 polymorphisms associate with erosive outcomes in RA.

The current study observed that the TA genotype was a risk factor for RA. CD40 T>A (rs1883832) is a functional SNP at position-1 from the CD40 gene's start codon. The rs1883832T mutation reduced CD40 mRNA translational efficiency, resulting in lower CD40 protein levels.

Novel results from the current investigation demonstrated that the CD40 rs1883832 SNP (TA) genotype was responsible for the risk genotype for RA among Iraqis. The findings indicated that the heterogeneity of the Iraqi race differs from that of Asian or European races, and is essential to understand RA onset. The rationale is that different population groups have different genetic and environmental components that influence the development of RA disease.

Additionally, rs1883832 was related to RA risk in a Tunisian study.¹⁹ According to rs4810485, this polymorphism was confirmed to be related to SLE and RA risk in a European sample,¹¹ but not in South Korean and Tunisian populations.^{19,20}

In a study comprising southern Chinese Han population, rs1883832 and rs4810485 were related to RA risk compared to the current findings, with another study noting that the differences may correlate with several reasons, like sample size variations, genotyping of the CD40 rs1883832 polymorphism using different molecular techniques, and different ethnicities.²¹

The current study found a non-significant result regarding TT genotype versus TA and AA in females, suggesting that whether or not this polymorphism may relate to RA risk in RA patients regardless of gender needs to be further discussed with a larger sample size, especially with larger female patients, and using the same genotyping method.

The current findings suggested that AA, TT and TA genotypes were effective in increasing the serum level of anti-CCP and can be regarded as a risk factor for RA susceptibility. CD40 is overexpressed in CD4+ and CD8+ T cells, and monocytes from the synovial fluid of RA patients.²² CD40 signals are required to induce immunoglobulin M (IgM) anti-CCP Ab secretion by B cells from either healthy controls or RA patients, but only B cells secrete anti-CCP Ab in seropositive patients, suggesting that these cells already received CD40 signals within the synovial compartment.²³

With the function of CD40 in promoting Ig class switching, and stimulating the production of several pro-

inflammatory cytokines, it is logical to suppose that the functional polymorphisms studied in the current study may be associated with autoantibody production and RA clinical activity. The current results showed an association between CD40 SNP and anti-CCP Abs present. These results are not in agreement with a 2010 study which found no association between the two.¹⁸ Intriguingly, for many of these genetic risk factors, the associations are confined to RA patients positive for ACPA. It remains unknown whether genetic factors also affect the severity of joint destruction in ACPA positive. In this the current results agreed with Orozco et al.¹⁸ There was a significant relationship between rs1883832 and the presence of anti-RF and anti-CCP antibodies in a study done in the United Kingdom.¹⁹

CD40 gene rs1883832 SNP was not associated with severe RA disease activity in the current study. However, this was not mentioned in any previous study. This may be due to stratification, the influence of genetic and environmental risk factors, and RA being different among various populations.

There was no significant association between TT, TA and AA genotypes of rs1883832 and severe DAS28 score. In addition, no correlation between these genetic polymorphisms and clinical disease activity or the presence of antibodies was found in the current study after stratification according to rs1883832 genotypes, which is in line with literature.^{24,11}

Conclusion

CD40 gene rs1883832 SNP T allele was found to be a risk factor for RA and disease severity. The TT and AA alleles acted as protective factors.

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