Original Article

# Identifying the Link Between IRAK1 Genetic Variations (rs1059703, G/A and rs7061789, G/A) and Vulnerability to Rheumatoid Arthritis

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## Abstract

**Background:** Rheumatoid arthritis (RA) is a chronic autoimmune disorder characterized by inflammatory processes. **Objectives:** This study focuses on identifying specific genetic variations (gene polymorphisms) in the interleukin-1 receptor-associated kinase (IRAK1) that plays a role in RA. Previous research has produced conflicting outcomes regarding the functional implications of these genetic variations. **Materials and Methods:** The research involved 200 individuals diagnosed with RA based on ACR criteria and 200 healthy controls in a case-control design. Genomic DNA was extracted from frozen blood samples collected in EDTA tubes. For DNA extraction, a spin column method and Add Prep Genomic DNA Extraction Kit (Add Bio, Korea) were used. Two specific variations in IRAK1 (rs1059703 G>A and rs7061789 G>A) were genotyped. The genotypes and allele frequencies of these variations were subjected to a chi-square test to determine their potential contribution to RA susceptibility. **Results:** The study identified a significant association between the rs1059703 A allele and an increased risk of RA. Specifically, individuals with AA and GA genotypes exhibited higher susceptibility (odd ratio [OR] = 2.5, 95% confidence interval [95% CI] = 1.83–3.4, $P = 0.0001$) to the rs1059703 G/A polymorphism. This risk was evident in codominant, dominant, and over-dominant genetic models. The combined GA+AA genotypes had notably higher RA risk compared to the control group (adjusted OR = 3.21, 95% CI = 1.8–5.5, $P = 0.0001$) under the dominant pattern. In the over-dominant model, individuals with the heterozygous genotype (GA) exhibited increased RA risk (OR = 2.43, 95% CI = 1.71–3.9, $P = 0.0001$). ADditionally, the study revealed significant differences in IRAK1 rs1059703 genotypes concerning clinical and laboratory features of patients. No significant association between rs7061789 G>A and the risk of RA ($P = 0.42$). **Conclusion:** The findings highlight that the rs1059703 A allele (considered a risk allele) within the IRAK1 gene contributes to elevated susceptibility to RA, leading to greater disease severity and influencing the onset age of RA among Iraqi patients. While the rs7061789 SNP of IRAK1 did not demonstrate any impact on RA development within the studied Iraqi population.

**Keywords:** Go tag probe, IRAK1, metalloproteinases, primer, rheumatoid arthritis

## Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune condition characterized by persistent inflammation that impacts the diarthrodial joints and affects roughly 1% of the global populace.[1] Synovial inflammation and hyperplasia, the production of autoantibodies such as rheumatoid factor (RF) and anti-citrullinated protein antibody (ACPA), cartilage and bone deformities, as well as systemic symptoms such as heart, lung, psychological, skin, and skeletal disorders, are the main characteristics of RA.[2]

The onset of this condition often occurs gradually and lasts for several weeks or months in individuals. However, in about one-third of patients, the beginning is abrupt and extends across a few days or weeks. In

Access this article online

Quick Response Code:

Website: https://journals.lww.com/mjby

DOI: 10.4103/MJBL.MJBL_1428_23

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**Submission:** 18-Sep-2023 **Accepted:** 16-Dec-2023 **Published:** 23-Jan-2026



**How to cite this article:** Hussein HA, Alsahlawy MMR. Identifying the link between IRAK1 genetic variations (rs1059703, G/A and rs7061789, G/A) and vulnerability to rheumatoid arthritis. Med J Babylon 2025;22:1358-68.

the early stages of the disease progression, the joints in smaller areas such as the hands, wrists, and forefeet are predominantly affected.[3] Individuals with chronic polyarthritis, heightened levels of acute-phase reactants, and positive indicators of RF and/or anti-cyclic citrullinated peptide (CCP) antibodies are at an elevated risk of developing early-stage erosive disease within 1 to 2 years of onset symptoms, as well as early functional impairment.[3]

Both genetic and environmental factors contributed to RA but the exact etiology of this disease remains unclear. The shared epitope of human leukocyte antigen (HLA)-DRB1 is the strongest genetic link identified for the development of RA.[1] Other important genes that contribute to susceptibility include the CD40 gene and the interleukin-1 receptor-associated kinase (IRAK1) gene (non-HLA genes).[4,5]

The duration of the illness and the ability to work exhibit a nearly linear relationship. Over a span of 15 years, approximately 15% of people with RA experience complete incapacitation. While severe seropositive RA is associated with reduced life expectancy, the availability of more aggressive early treatment approaches might potentially help improve this aspect. People diagnosed with RA are at increased risk of developing conditions such as non-Hodgkin lymphoma, infections, and coronary artery disease.[6] Interleukin-1-receptor-associated kinase 1 (IRAK1) is a serine/threonine protein kinase that participates in the signaling pathway of the toll/interleukin-1 receptor (TIR) family.[7] Upon activation of toll-like receptors (TLRs), IRAK1 is drawn into the TLR signaling complex where it undergoes phosphorylation. As a pivotal intracellular signaling molecule, IRAK1 becomes active in response to TLR ligands and can detect diverse types of ligands, including pathogens. This activity contributes to inflammation triggered by pathogens.[8] Different isoforms of this gene, resulting from alternative splicing of transcript variants, have been identified. It is located on the long arm (q arm) of the X chromosome at band 28 and is called "pelle." In the latest update, this gene consists of 15 exons and 14 introns, yielding eight distinct transcripts. The largest transcript comprises 3581 nucleotides, ultimately producing an IRAKI protein composed of 712 amino acids.[9]

IRAK1 has been identified as a risk gene for ADs in many populations because it was found to play a significant role in both ADs patients and an autoimmune animal model. Single nucleotide polymorphisms (SNPs), or mutations, can change the expression of a gene and influense a person's susceptibility to certain illnesses.[10] In addition to genetics, there are also lifestyle risk factors that can be modified. Taking these factors into account can not only reduce the severity of the illness, but also reduce the risk of developing RA in the first place.[11]

## Materials and Methods

### Subjects

This case-control study involved 200 healthy (150 female+50 male) participants (control) and 200 patients with RA, comprising 41 male and 159 female individuals, whose ages ranged from 30 to 69 years, with a mean age of 53.68 ± 11.79. Specialists diagnosed the patients and they were selected from the Medical Rehabilitation and Physiotherapy Center in Al-Sadder Medical City, Al City, Iraq. From December 2021 to April 2022, all cases were clinically diagnosed at the center. Blood samples were collected in the clinic's laboratory and the analysis of the studied biomarkers was carried out in the laboratories of the Biochemistry Department, Faculty of Medicine, University of Kufa. A questionnaire gathering details such as name, gender, age, medical background, and family history was filled out for each participant. Moreover, additional clinical and biochemical information was incorporated, encompassing parameters such as ESR, TWBC, PCV, S. Cr, B. urea, and C-RP. Additionally, a seroclinical examination was conducted, including RF and ACCP, for all participants.

The treatment approach consisted of conventional drug-modified anti-rheumatic drugs (DMARDs) taken at least 6 months prior, alongside glucocorticoids and NSAIDs as supplementary therapies.

In the allele discrimination/SNP's real-time PCR, the amplification reactions were set up as follows: GoTaq® Probe qPCR Master Mix = 10 µL, forward primer = 1 µL, reverse primer = 1 µL, wild probe = 1 µL, mutant probe = 1 µL, genomic DNA = 4 µL, nuclease-free water = 7 µL, resulting in a total volume of 25 µL. The real-time PCR process involved 40 cycles. Each cycle included an initial denaturation step at 95°C for 5 min, followed by denaturation at 95°C for 10 s, annealing at 58°C, and extension at 72°C for 30 s.

The experimental technique employed fluorescent probes to identify the composition of DNA samples at the allele level. These probes were labeled with different dyes (Hex and FAM) to distinguish alleles, even if they differed by only one nucleotide. The presence or absence of a specific allele was determined by the Ct value obtained from the allele-specific probe. Amplification plots were used to visualize cycles versus fluorescence for specific data collection points. The data collection point for the analysis was selected using the allele discrimination/SNP's real-time PCR TaqMan method utilizing the Mx3005P QPCR system.[12] Taq polymerase differentiates between different alleles by binding to particular fluorogenic probes on the template DNA. This binding results in the cleavage of these probes, releasing a reporter dye, either "FAM" or "Hex," that was previously hidden. Each assay necessitates two unlabeled PCR primers and two allele-specific probes

for execution. The Mx3005P QPCR Systems generated an allele discrimination plot.

## Results of the Amplification Reaction

To ascertain the genotype of an unknown DNA sample, its fluorescence levels are compared to the background fluorescence levels of both dyes. If the fluorescence is notably higher for the dye corresponding to the wild-type allele, but not for the dye specific to the mutant allele, the sample is identified as wild-type homozygous. Conversely, if fluorescence exceeds the background for the dye associated with the mutant allele, but not for the dye linked to the wild-type allele, the sample is classified as a mutant homozygous. If the sample yields intermediate fluorescence values for both dyes, it is categorized as heterozygous for the two alleles. Selected amplification curves are illustrated in Figures 1 and 2.

## Determination of Anthropometry and Phenotype Characteristics

The height and weight of both patients and control group were recorded in meters and kilograms, respectively, to calculate their BMI.[13] Various biochemical characteristics of all participants, such as erythrocyte sedimentation rate (ESR),[14] serum creatinine (S.Cr),[15] and blood urea (B.urea).[16] The underlying principle of the CRP and RF tests involves latex agglutination test.[17] Anti-CCP antibody was estimated by Elisa KIT.[18] Total WBC determination and packed cell volume (PCV) are analyzed by the Spinreact auto analyzer from complete blood count (CBC).

### IRAK1 genotyping

Blood samples were collected from two groups through venous puncture, and each sample was divided into two tubes. Three milliliters of the blood were placed into a tube containing an anticoagulant, while the remaining sample was placed in an EDTA tube. The first tube was allowed to coagulate at room temperature for 10–15 min and then centrifuged at approximately 2000*g* for about 10 min. The resulting serum was stored at −20°C until it was analyzed for phenotypic determination. The second tube (EDTA tube) was utilized for DNA extraction. The extracted DNA sample was then frozen at −20°C, awaiting analysis for genetic polymorphism.

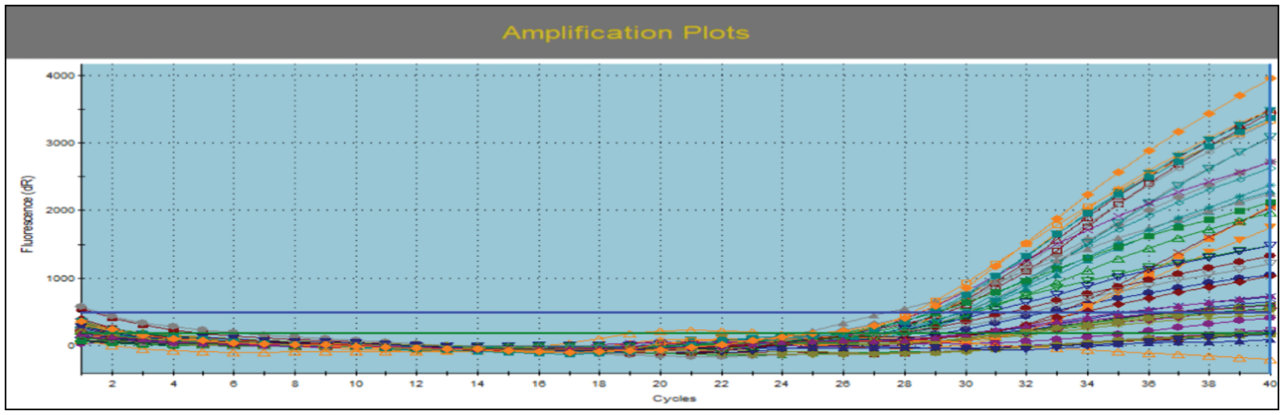


**Figure 1:** Amplification plot for rs1059703 G> A of IRAK1 gene for the patient group. Changes in fluorescence are plotted against the cycle of amplification

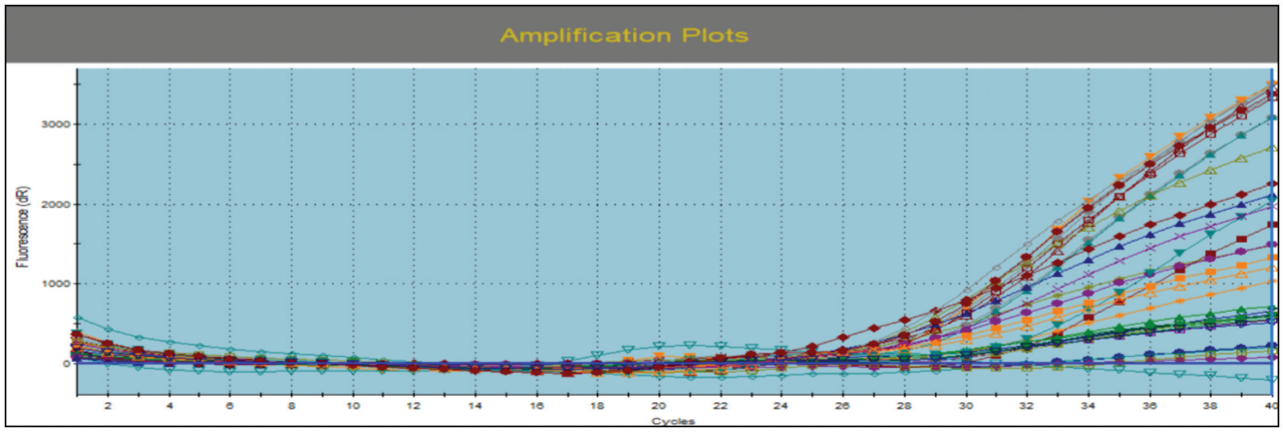


**Figure 2:** Amplification plot for rs7061789 G>A for IRAK1 gene for the patient group. Changes in fluorescence are plotted against the cycle of amplification

The genomic DNA (gDNA) was isolated from whole blood using purification kits (Add Prep Genomic Product Code: 10023, Korea). The quality of the DNA was assessed using a bio-drop instrument, and its structural integrity was investigated through agarose gel electrophoresis. To evaluate these characteristics, measurements were taken based on the absorption of ultraviolet light. The amount of UV light absorbed by DNA changes with the wavelength used. In this study, a nano-drop device (Bio drops, UK) was used and readings were recorded at wavelengths of 260 and 280 nm.

In this study, specific primers and probes were utilized for each SNP under investigation. For rs1059703 G>A SNP, the forward primer (F) was AAGCTGCAGGCAGTGGTG, and the reverse primer (R) was TGCTGGACACGTAGGAGTTCT. The wild-type probe was labeled with FAM and sequence FAM-CCGGGCATTCGGAGG-BHQ, while the mutant probe was labeled with HEX and had the sequence HEX-CGGGCATTTGGAGGC-BHG.

For the rs7061789 (G/A) SNP, the forward primer was F-CCTGGTCTGTCTGATTTCAGAACT, and the reverse primer was R-GCCCCACAGACAAAGAGGTA. The wild-type probe was labeled with FAM and had the sequence FAM-ATGGCCTCCGGTCCT-BHQ, while the mutant probe was labeled with HEX and had the sequence HEX-AGATGGCCTCTGGTCCT-BHQ.

### Statistical analysis

In this study, the comparison between the healthy and patient (RA) groups involved utilizing multi-nominal logistic regression through IBM-SPSS version 25. The aim was to assess the differences in median and interquartile range (IQR) standard deviation under various inheritance models, such as codominant, dominant, overdominant, recessive, and additive models. The analysis of categorical data, including alleles and genotypes, was carried out using the chi-square test. For all statistical evaluations, a significance level below 0.05 was adopted as the threshold to identify significant distinctions.

The results were displayed through *P* value, odds ratio (OR), and a 95% confidence interval (95% CI). Additionally, the data were adjusted for variables such as gender, age, and BMI 95% CI, OR. Subsequently, the *P* value was recalculated. The Hardy–Weinberg calculator was accessed from the following link: https://scienceprimer.com/hardy-weinberg-equilibrium-calculator. Haplotype analysis was performed using (Haplotype Viewer) version 9.

### Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with the patient's verbal and analytical approval before the sample was taken. The study protocol, the subject information, and the consent form were reviewed and approved by a local ethics committee according to document number 22 (including the number and the date of August 11, 2021) to get this approval.

## Results

The data obtained from this study showed considerable deviations from normality. Consequently, the nonparametric Mann–Whitney *U* test was employed to compare the distribution between patients with rheumatoid disease (RA) and healthy control subjects.[19] 200 participants with RA and 200 control participants are included in the study. There are significant differences between the patient group and the control group for a number of anthropometric and biochemical measurements that are shown in Table 1. These measurements revealed that all anthropometric and biochemical measurements, with the exception of age, have a significant association with RA.

Based on the information presented in Table 1, it is evident that all parameters except for age (urea, BMI, ESR, PCV, T WBC, S.cr, ACCP) exhibit noteworthy variations between the patient and control groups(*P* value < 0.05). These differences suggest an elevated probability of RA occurrence. However, the age parameter suggests that age does not play a role in augmenting the likelihood of developing RA, *P* value of 0.84.

### Biochemical parameters and IRAK1 polymorphism relationship

#### *rs 1059703*

RF+ and CRP+ve are more than RF–ve, CRP–ve for rs1059703 of IRAK1 gene under both codominance and dominance model of inheritance [Tables 2 and 3].

Other biochemical parameters were as follows: The findings from the analysis of rs 1059703 indicated a significant association between Age, BMI, ESR, PCV, and the risk of RA, and an insignificant relationship for WBC, B. Urea, S. Cr, ACCP, and the risk of RA under the codominant model. In the dominant model, all parameters showed an insignificant relationship with the risk of RA, as shown in Tables 4 and 5.

### Hardy–Weinberg equilibrium

During the real-time PCR analysis, two specific SNPs (rs1059703 G>A, rs7061789 G>A) for IRAK1 gene were examined, and their outcomes were monitored.

Hardy–Weinberg equilibrium of the IRAK1 gene for the control suggests that the rs1059703 variant is in agreement,

**Table 1: Clinical and biochemical data of RA patients and control individual**

| Parameter | Group | IQR | Median | *P* value |
|---|---|---|---|---|
| Urea (mg/dL) | Control | 33.40–41.00 | 35.80 | |
| | Patients | 24.00–34.31 | 28.40 | 0.0001 |
| Age(years) | Control | 36.00–50.00 | 45.00 | |
| | Patients | 33.00–55.00 | 44.00 | 0.84 |
| BMI (kg) | Control | 33.90–43.00 | 35.00 | |
| | Patients | 25.82–37.13 | 32.58 | 0.0001 |
| ESR (mm/h) | Control | 11.00–16.00 | 14.00 | |
| | Patients | 31.50–60.00 | 45.00 | 0.0001 |
| PCV | Control | 38.00–42.00 | 40.00 | |
| | Patients | 32.00–40.00 | 37.00 | 0.0001 |
| T WBC ($10^3$/μL) | Control | 5.40–8.40 | 7.19 | |
| | Patients | 6.23–9.30 | 8.10 | 0.0001 |
| S.Cr(mg/dL) | Control | 0.81–0.95 | 0.88 | |
| | Patients | 0.65–1.10 | 0.84 | 0.03 |
| ACCP unit/mL | Control | 2.20–5.00 | 3.60 | |
| | Patients | 17.28–26.00 | 19.85 | 0.0001 |

BMI, body mass index; ESR; erythrocyte sedimentation rate, PCV; packed cell volume, TWBC; total white blood cells, S. Cr Serum creatinine, ACCP; anti-citrulinated C-peptide antibody; $P < 0.05$ significant ($P = 0.000$ indicates less than 0.0001)

**Table 2: rs 1059703 for +ve and –ve RF under codominant and dominant model**

| | | Codominant | | |
|---|---|---|---|---|
| **Genotype** | **GG** | **GA** | **AA** | **Total** |
| RF +ve | 56 | 100 | 23 | 179 |
| RF–ve | 4 | 10 | 7 | 21 |
| Total | 60 | 110 | 30 | 200 |
| **Dominant** | | | | |
| Genotype | GG | GA+AA | Total | |
| RF +ve | 56 | 123 | 179 | |
| RF–ve | 4 | 17 | 21 | |
| Total | 60 | 140 | 200 | |

**Table 3: rs 1059703 for +ve and –ve CRP under codominant and dominant model**

| | | Codominant | | |
|---|---|---|---|---|
| **Genotype** | **GG** | **GA** | **AA** | **Total** |
| CRP+ve | 54 | 95 | 29 | 178 |
| CRP–ve | 6 | 15 | 1 | 22 |
| Total | 60 | 110 | 30 | 200 |
| **Dominant** | | | | |
| Genotype | GG | GA+AA | Total | |
| CRP+ve | 54 | 124 | 178 | |
| RF−ve | 6 | 16 | 22 | |
| Total | 60 | 140 | 200 | |

whereas the rs7061789 variant is not in agreement, as shown in Table 6.

## Analysis of the IRAK1 gene variant rs1059703 G/A

The analysis revealed noteworthy associations in various genetic models with RA susceptibility. In the context of the codominant model, RA patients with the heterozygous genotype (GA) displayed a markedly elevated risk of developing RA in comparison to the control group. This risk was quantified with an adjusted OR of 3.4 (95% CI = 2.2–5.4, $P = 0.0001$). Similarly, RA patients with the homozygous genotype (AA) exhibited a significantly increased RA risk, indicated by an adjusted OR of 3.5 (95% CI = 1.5–8.1, $P = 0.0001$).

**Table 4: rs 1059703 under the co-dominant model**

| Parameter | rs1059703 G>A | IQR | Median | *P* value |
|---|---|---|---|---|
| Age | GG | 39.00–55.75 | 45.00 | 0.02 |
| | GA | 33.00–50.00 | 42.50 | |
| | AA | 32.50–60.25 | 50.00 | |
| BMI | GG | 28.66–38.08 | 32.30 | 0.01 |
| | GA | 26.43–36.92 | 33.35 | |
| | AA | 22.62–34.13 | 25.03 | |
| ESR | GG | 25.00–59.25 | 41.00 | 0.02 |
| | GA | 32.00–55.00 | 45.00 | |
| | AA | 40.00–70.00 | 56.00 | |
| PCV | GG | 31.00–39.00 | 36.00 | 0.0001 |
| | GA | 32.00–40.00 | 36.00 | |
| | AA | 38.00–42.25 | 40.00 | |
| WBC | GG | 6.04–9.95 | 8.20 | 0.7 |
| | GA | 6.14–9.11 | 8.10 | |
| | AA | 6.78–9.88 | 8.15 | |
| B.Urea | GG | 24.36–35.00 | 30.00 | 0.8 |
| | GA | 23.25–34.75 | 27.53 | |
| | AA | 23.75–31.50 | 28.50 | |
| S.Cr | GG | 0.65–1.10 | 0.88 | 0.9 |
| | GA | 0.63–1.00 | 0.81 | |
| | AA | 0.70–1.20 | 0.85 | |
| ACCP | GG | 17.35–25.58 | 19.90 | 0.63 |
| | GA | 17.00–27.23 | 19.50 | |
| | AA | 17.58–25.45 | 21.00 | |

**Table 5: rs 1059703 allelic distribution wit under dominant model**

| Parameter | rs1059703 G>A | IQR | Median | *P* value |
|---|---|---|---|---|
| Age | GG | 39.00–55.75 | 45.00 | 0.07 |
| | GA+AA | 33.00–53.25 | 44.00 | |
| BMI | GG | 28.66–38.08 | 32.30 | 0.8 |
| | GA+AA | 24.66–36.50 | 32.68 | |
| ESR | GG | 25.00–59.25 | 41.00 | 0.93 |
| | GA+AA | 35.00–60.00 | 45.00 | |
| PCV | GG | 31.00–39.00 | 36.00 | 0.1 |
| | GA+AA | 32.75–40.00 | 38.00 | |
| WBC | GG | 6.04–9.95 | 8.20 | 0.6 |
| | GA+AA | 6.33–9.14 | 8.10 | |
| Urea | GG | 24.36–35.00 | 30.00 | 0.6 |
| | GA+AA | 23.26–33.00 | 28.00 | |
| Cr | GG | 0.65–1.10 | 0.88 | 0.8 |
| | GA+AA | 0.66–1.10 | 0.81 | |
| ACCP | GG | 17.35–25.58 | 19.90 | 0.87 |
| | GA+AA | 17.15–26.28 | 19.75 | |

Under the dominant pattern, the collective GA+AA genotypes demonstrated a substantially higher RA risk relative to the control group, as shown by the adjusted OR of 3.21 (95% CI = 1.8–5.5, $P = 0.0001$). Employing the over-dominant model, RA patients with the GA genotype demonstrated heightened RA susceptibility, with an adjusted OR of 2.43 (95% CI = 1.71–3.9, $P = 0.0001$).

In the additive model, RA patients carrying two copies of the minor allele (AA+GA) exhibited an increased RA risk, quantified by an odds ratio of 2.5 (95% CI = 1.83–3.4, $P = 0.0001$) compared to the control group.

These associations remained consistent across all models even after accounting for adjustments related to age, sex, and BMI, as indicated by the adjusted *P* value. However, the recessive model demonstrated a notable deviation.

**Table 6: Hardy–Weinberg equilibrium of rs 1059703, rs 7061789 of IRAK1 gene in the study**

| rs1059703 G>A | | | | |
|---|---|---|---|---|
| **Genotype of control** | **Observed** | **Expected** | ***P* value** | **Consistency** |
| GG | 120 | 119 | 0.57 | Yes |
| GA | 68 | 71 | | |
| AA | 12 | 10 | | |
| G % | 77 | | | |
| A % | 23 | | | |
| **rs7061789 G>A** | | | | |
| Genotype of control | Observed | Expected | *P* value | Consistency |
| GG | 110 | 85 | 0.01 | No |
| GA | 40 | 91 | | |
| AA | 50 | 24 | | |
| G % | 65 | | | |
| A % | 35 | | | |

**Table 7: Results of the allelic distribution for the rs1059703 G>A**

| rs1059703 G>A | Control 200 | Patients 200 | Adjusted OR (CI 95%) | *P* value |
|---|---|---|---|---|
| **Codominant** | | | | |
| GG | 120 | 60 | | |
| GA | 68 | 110 | 3.4 (2.2–5.4) | 0.0001 |
| AA | 12 | 30 | 3.5 (1.5–8.1) | |
| Dominant | | | | |
| GA+AA | 80 | 140 | 3.21 (1.8–5.5) | 0.0001 |
| **Over dominant** | | | | |
| GG+AA | 132 | 90 | | |
| GA | 68 | 110 | 2.43 (1.71–3.9) | 0.0001 |
| **Recessive** | | | | |
| GG+GA | 190 | 170 | | |
| AA | 10 | 30 | 1.04(0.0911.4) | 0.97 |
| **Additive** | | | | |
| 2GG+GA | 308 | 230 | | |
| 2AA+GA | 92 | 170 | 2.5(1.83–3.4) | 0.0001 |
| MAF%(A) | 23 | 42.5 | 2.5(1.83–3.4) | 0.0001 |

In this model, RA patients with the homozygous AA genotype showed to be insignificant after accounting for age, sex, and BMI adjustments. The adjusted OR = 1.04, 95% CI = 0.9–11.4, $P = 0.97$ suggesting that the impact of these adjustments on outcomes was inconsistent in the recessive model.

Furthermore, the minor allele frequency (A) was significantly increased ($P < 0.0001$) in RA patients (42.5%) compared to the control group (23%), as reported in [Table 7]

It is worth noting that the results for rs 1059703 G>A of IRAK1 gene are consistent 706 with Hardy–Weinberg equilibrium for control [Table 6].

## Biochemical parameters and IRAK1 polymorphism relationship rs 7061789 G/A

RF+ve and CRP+ve patients were more than RF−ve, CRP−ve for rs7061789 of IRAK1 gene under both codominance and dominance model of inheritance [Tables 8 and 9].

Additional biochemical and anthropometric measurements included the following: BMI, PCV, and S. Cr exhibited a significant link according to the rs7061789 study findings, but age, ESR, T.WBC, B. urea, and ACCP showed no significant correlation under the codominant model. All parameters (age, TWBC, ESR, urea, ACCP, BMI, PCV) showed an insignificant connection in the dominant model, with the exception of and S. Cr, which showed a significant relationship, as shown in Tables 10 and 11.

## Analysis of the IRAK1 gene variant rs7061789 G/A

In the codominant model, individuals with RA who possessed the heterozygous genotype (GA) demonstrated a markedly elevated RA risk in comparison to the control group (adjusted OR = 2.9, 95% CI = 1.7–4.8, $P = 0.0001$).

**Table 8: rs7061789 for +ve and –ve RF under codominant and dominant model**

| | | Codominant | | |
|---|---|---|---|---|
| **Genotype** | **GG** | **GA** | **AA** | **Total** |
| RF+ve | 71 | 81 | 27 | 179 |
| RF−ve | 8 | 10 | 3 | 21 |
| Total | 79 | 91 | 30 | 200 |
| **Dominant** | | | | |
| Genotype | GG | GA+AA | Total | |
| RF+ve | 71 | 108 | 179 | |
| RF−ve | 8 | 13 | 21 | |
| Total | 79 | 121 | 200 | |

**Table 9: rs 1059703 for +ve and –ve CRP under codominant and dominant model**

| | | Codominant | | |
|---|---|---|---|---|
| **Genotype** | **GG** | **GA** | **AA** | **Total** |
| RF+ve | 14 | 5 | 3 | 179 |
| RF−ve | 65 | 86 | 27 | 21 |
| Total | 79 | 91 | 30 | 200 |
| **Dominant** | | | | |
| Genotype | GG | GA+AA | Total | |
| RF+ve | 14 | 8 | 22 | |
| RF−ve | 65 | 113 | 178 | |
| Total | 79 | 121 | 200 | |

Conversely, RA patients with the homozygous genotype (AA) exhibited no significant risk of developing RA (adjusted OR = 0.54, 95% CI = 0.29–1.0, $P = 0.05$).

In accordance with the dominant pattern, the collective genotypes of GA + AA showed a substantially higher risk of RA when compared to the control group (adjusted OR = 1.5, 95% CI = 1.0–2.4, $P = 0.04$).

In the context of the over-dominant model, individuals with RA who had the heterozygous genotype (GA) exhibited an increased risk of developing RA (adjusted OR = 3.31, 95% CI = 2.16–5.29, $P = 0.0001$).

Within the recessive model, individuals with RA who carried the homozygous genotype (AA) demonstrated a protective effect after adjusting for age, sex, and BMI. The adjusted OR was 0.38, with a 95% CI of 0.22–0.67 and a $P$ value of 0.001.

Under the additive model, individuals with RA who possessed two copies of the minor allele (AA+GA) did not exhibit a significant risk for RA, as the odds ratio was 1.12, with a 95% CI of 0.84–1.5 and a $P = 0.42$, indicating an insignificant association. The minor allele frequency (A) displayed a non-significant increase ($P$ value = 0.42) in RA patients (37.75%) in comparison to the control group (35%), as detailed in Table 12.

The findings related to rs7061789 of the IRAK1 gene indicated departures from the Hardy–Weinberg

**Table 10: rs 7061789 of IRAKI gene with corresponding biochemical and clinical parameters under codominant model**

| Parameter | rs7061789G/A | IQR | Median | *P* value |
|---|---|---|---|---|
| Age | GG | 33.00–50.00 | 44.00 | 0.5 |
| | GA | 33.00–55.00 | 44.00 | |
| | AA | 31.75–60.25 | 49.50 | |
| BMI | GG | 30.05–37.81 | 33.90 | 0.0001 |
| | GA | 24.66–38.00 | 32.90 | |
| | AA | 24.03–30.62 | 27.76 | |
| ESR | GG | 28.00–55.00 | 40.00 | 0.05 |
| | GA | 35.00–60.00 | 48.00 | |
| | AA | 40.00–71.25 | 50.00 | |
| PCV | GG | 31.00–39.00 | 36.00 | 0.02 |
| | GA | 32.00–40.00 | 37.00 | |
| | AA | 36.00–42.00 | 39.00 | |
| WBC | GG | 6.10–9.49 | 8.10 | 0.7 |
| | GA | 6.07–9.10 | 8.10 | |
| | AA | 7.07–10.10 | 8.60 | |
| B.Urea | GG | 23.91–32.78 | 26.57 | 0.16 |
| | GA | 23.23–35.00 | 30.00 | |
| | AA | 25.00–32.07 | 27.03 | |
| S.Cr | GG | 0.60–0.90 | 0.80 | 0.03 |
| | GA | 0.67–1.10 | 0.83 | |
| | AA | 0.70–1.20 | 0.90 | |
| ACCP | GG | 17.27–25.42 | 19.50 | 0.5 |
| | GA | 17.00–27.55 | 19.70 | |
| | AA | 18.00–26.13 | 22.65 | |

**Table 11: rs 7061789 of IRAKI gene with corresponding biochemical and clinical parameters under dominant model**

| Parameter | rs7061789G/A | IQR | Median | *P* value |
|---|---|---|---|---|
| Age | GG | 33.00–50.00 | 44.00 | 0.48 |
| | GA+AA | 33.00–55.00 | 45.00 | |
| BMI | GG | 30.05–37.81 | 33.90 | 0.05 |
| | GA+AA | 24.59–36.47 | 31.00 | |
| ESR | GG | 28.00–55.00 | 40.00 | 0.03 |
| | GA+AA | 35.00–60.00 | 50.00 | |
| PCV | GG | 31.00–39.00 | 36.00 | 0.2 |
| | GA+AA | 33.00–40.00 | 38.00 | |
| WBC | GG | 6.10–9.49 | 8.10 | 0.9 |
| | GA+AA | 6.29–9.17 | 8.10 | |
| B.Urea | GG | 23.91–32.78 | 26.57 | 0.3 |
| | GA+AA | 24.00–35.00 | 30.00 | |
| S.Cr | GG | 0.60–0.90 | 0.80 | 0.08 |
| | GA+AA | 0.70–1.10 | 0.90 | |
| ACCP | GG | 17.27–25.42 | 19.50 | 0.7 |
| | GA+AA | 17.08–26.88 | 21.00 | |

equilibrium, as highlighted in Table 6. These deviations were not consistent within the control group, Various factors, including mutation, natural selection, non-random mating, genetic drift, and gene flow, can contribute to such deviations.[20] In this specific instance, the discrepancy observed in this study's adherence to the Hardy–Weinberg equilibrium is likely attributed to the sample size. It's noteworthy that this kind of departure becomes noticeable when there exists a slight disparity in the minor allele frequency (MAF) between the patient and control groups. To mitigate the potential for this anomaly, it becomes imperative to have a substantial sample size.[21]

## Discussion

Within the ongoing research initiative, a comprehensive investigation was conducted on two single nucleotide polymorphisms (SNPs) within the IRAK1 gene, namely rs1059703 and rs7061789, encompassing both patient and control groups. This endeavor is poised to yield valuable insights for identifying individuals in our society who may harbor an elevated susceptibility to the disease, as well as those who might exhibit a protective inclination against it.

The analysis of the SNP results with respect to the Hardy–Weinberg equilibrium unveiled a significant observation: while one SNP within the IRAK1 gene (rs1059703) exhibited compatibility, the other SNP (rs7061789) did not align with the equilibrium. This disparity indicates that the specific SNP, rs1059703, is intrinsically associated with RA in the sampled Iraqi population, while there is a doubt about in association of the RA with the rs 7061789 since it is not in agreement with Hardy–Weinberg equilibrium.

The data garnered from this study regarding the distribution of the minor allele frequency (MAF) between the two groups in relation to the rs1059703 SNP unveiled an odds ratio of 2.5, coupled with a *P* value of 0.0001. These outcomes signify a substantial correlation between the SNP and the incidence of the disease. This implies that individuals possessing the rs1059703 SNP have approximately 2.5 times higher likelihood of developing RA in contrast to those without the SNP. Furthermore, the consistency observed in the rs1059703 SNP aligns harmoniously with prior research outcomes derived from studies carried out across diverse populations, affirming its link with RA.[10,22-26] Since this variant is linked to an elevated risk of RA, the findings of this study suggest that the rs1059703 variant within the IRAKI gene results in a reduction in serine phosphorylation and a diminished likelihood of synthesizing polyproline 2 at the IL1R type II binding site where the mutation occurs.[27] Consequently, the binding affinity to IL1 type II and its signaling activity is decreased. Given that these receptors serve as decoys for IL1 and suppress its activity.[28] The reduced binding capacity of IL1 to IL1RII might potentially elevate the risk of developing RA by intensifying inflammation through heightened activity targeting IL1R1. This observation is important because there exists a well-established correlation between elevated levels of IL-1 in both the blood and synovial fluid, and the extent of disease activity in RA, as well as the deterioration of joint health.[29] However, it is important to note that the exact cause of RA remains unknown.

Nevertheless, it's important to acknowledge that certain studies[30,31] have indicated no association with the disease or have contradicted the findings of this study.

The results for rs7061789 of the IRAK1 gene showed deviations from the Hardy–Weinberg equilibrium. These deviations can be influenced by several factors, such as mutation, natural selection, non-random mating, genetic

**Table 12: Explains the allelic distribution for the rs7061789 G/A of IRAK1 gene**

| Rs7061789 G>A | Control 200 | Patients 200 | Adjusted OR (CI 95%) | *P* value |
|---|---|---|---|---|
| **Codominant** | | | | |
| GG | 110 | 79 | | |
| GA | 40 | 91 | 2.9 (1.7–4.8) | 0.0001 |
| AA | 50 | 30 | 0.54(0.29–1.0) | 0.05 |
| **Dominant** | | | | |
| GA+AA | 90 | 121 | 1.5 (1.0–2.4) | 0.04 |
| **Over dominant** | | | | |
| GG+AA | 160 | 109 | | |
| GA | 40 | 91 | 3.31(2.16–5.29) | 0.0001 |
| **Recessive** | | | | |
| GG+GA | 150 | 169 | | |
| AA | 50 | 31 | 0.38 (0.22–0.67) | 0.001 |
| **Additive** | | | | |
| 2GG+GA | 260 | 249 | | |
| 2AA+GA | 140 | 151 | 1.12(0.84–1.5) | 0.42 |
| MAF%(A) | 35 | 37.75 | 1.12(0.84–1.5) | 0.42 |

drift, and gene flow.[20] In this case, the study's deviation from the Hardy–Weinberg equilibrium is likely attributed to the sample size. It's important to note that such deviation becomes evident when there is a small difference in the minor allele frequency (MAF) between the patient and control groups. To mitigate the possibility of this aberration, a large sample size is crucial.[21]

The congruence between the data of the two SNPs and the expectations set by the Hardy–Weinberg equilibrium appears to be closely linked to the outcomes obtained from the genetic power analysis. The investigation unveiled that the two SNPs, specifically rs7061789, which displayed deviations from the Hardy–Weinberg equilibrium, exhibited low genetic power, quantified at 9%. Conversely, the SNPs that maintained concordance with the Hardy–Weinberg equilibrium showcased notably higher genetic powers. For instance, rs1059703 displayed a genetic power of 98.4%. Consequently, the pivotal determinant impacting the study's findings is the sample size – it proves to be adequate for one of the scrutinized SNPs, while falling short for the other SNPs that demonstrated irregular behavior.

The results concerning the rs7061789 variant within the IRAKI gene unveil significant associations between this genetic variation and the disease when individuals carry it in a heterozygous genotype, as observed across various genetic models including codominant, dominant, overdominant, and recessive. The odds ratios and respective *P* value are as follows: (OR=2.9, *P* = 0.0001) for the codominant model, (OR=1.5, *P* = 0.04) for the dominant model, (OR=3.31, *P* = 0.0001) for the over dominant model, and (OR=0.38, *P* = 0.001) for the recessive model. These findings indicate that the recessive model appears to confer protection against RA disease, whereas the other models suggest an increased risk factor for the disease. Clinical implications tied to this specific SNP brought forth the following insights: Under the codominant model, notable links existed between BMI, PCV, S. Cr, and the likelihood of developing RA. Corresponding *P* value were observed as 0.0001, 0.02, and 0.03, respectively. In the context of the dominant model, only ESR values exhibited a significant correlation with the disease, indicated by a *P* value of 0.02. This suggests that individuals carrying this SNP have an elevated predisposition to contracting the disease, connected to elevated values of these parameters. This, in turn, implies escalated inflammation due to the disease and a possible decrease in bone mineral density, suggesting a propensity for osteoporosis. This assumption finds support in the positive correlation between serum creatinine and lumbar bone mineral density. Consequently, monitoring serum creatinine could serve as a valuable biomarker for timely detection and management of osteoporosis in these individuals.[32] It's worth noting that only one prior study has explored the relationship of this SNP with the autoimmune disease systemic lupus erythematosus.[33] This study is the inaugural attempt to investigate its link with RA.

However, a point of concern is the inconsistency in the genotype distribution of the rs7061789 SNP with the Hardy–Weinberg equilibrium within our population. Data pertaining to the distribution of minor allele frequency (MAF) among the two groups for the rs7061789 SNP revealed an odds ratio of 1.12, with a *P* value of 0.42, indicating a doubt of insignificant correlation between the SNP and the occurrence of the disease because of the inconsistency with the Hardy–Weinberg equilibrium. This suggests that patients with the rs7061789 SNP may have susceptibility to the disease if the sample size increases, and it is advisable to conduct future research with a larger sample size to attain a more conclusive understanding.

There are some limitations in this study:

1. The difficulty in collecting more samples to increase the sample size which is necessary especially for the rs 7061789 to be in agreement with Hardy–Weinberg equilibrium for more dependable results.
2. Patient follow-up is a very challenging matter.
3. Cost of DNA sequencing test to confirm the result of the study.

## Conclusions

1. rs1059703 G>A polymorphism of IRAK1 associated with RA in a studied sample of the Iraqi population with a risk factor of 2.5 [Table 4].
2- rs7061789 G>A polymorphism has no impact on the development of RA in the studied sample of the Iraqi population [Table 7].

### Author contributions

The research was formulated by Mahdi Mohammed Ridha Alsahlawy. Hazim Ali Hussein conducted the research and created the initial draft of the manuscript.

### Acknowledgements

We appreciate all the patients who participated in this study.

### Financial support and sponsorship

Nil.

### Conflicts of interest

There are no conflicts of interest.

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