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Haplotype Synergism of MACIR Gene Polymorphisms Reveals a 'Flip-Flop' Phenomenon Driving Rheumatoid Arthritis Susceptibility in an Iraqi Population"

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ABSTRACT:

Background: "Rheumatoid arthritis (RA) is a systemic autoimmune pathology driven by intricate genetic predispositions and chronic synovial inflammation. The macrophage immunometabolism regulator MACIR gene, also known as (C5orf30), has emerged as a key regulator of macrophage-mediated synovial inflammation. This study investigated the association of MACIR gene polymorphisms (rs26232 and rs26233) with RA susceptibility in an Iraqi cohort, focusing on genetic inheritance models and haplotype synergism.

Methods: A case-control study was conducted involving 90 participants (45 newly diagnosed RA patients and 45 healthy controls). Precise genotyping was performed using bidirectional PCR-Sanger sequencing. Genetic associations were evaluated across multiple inheritance models (co-dominant, dominant, and recessive). Linkage disequilibrium (LD) and haplotype frequencies were estimated to determine the combined allelic impact.

Multivariable logistic regression was utilized to calculate adjusted odds ratios (AOR), controlling for potential demographic confounding factors.

Results: High-resolution mapping revealed an exceptionally strong linkage disequilibrium between rs26232 and rs26233 (D' = 0.97, r2 = 0.85), identifying a stable "genetic block" in the Iraqi population. The T allele for both SNPs was identified as the primary risk variant ($p < 0.001$). Under the co-dominant model, the homozygous mutant TT genotype conferred a 6.93-fold increase in RA risk for rs26232 and a 10.74-fold increase for rs26233. Haplotype analysis confirmed that the T-T haplotype significantly amplified disease susceptibility (OR = 4.134, $p < 0.001$), while the C-G haplotype exhibited a robust protective effect (OR = 0.304).

Conclusion: Our findings establish the MACIR gene, TT genotype and T-T haplotype as potent genetic markers for RA in Iraq. The identification of the T allele as a risk factor-contrary to some Western studies-highlights a significant "flip-flop" phenomenon and underscores the necessity of population-specific genetic screening for personalized medicine in the Middle East.

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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disorder characterized by persistent synovial inflammation, which leads to progressive joint destruction and functional disability if not managed early [1]. In the Iraqi context, Rheumatoid Arthritis (RA) is recognized as a major health challenge, with clinical studies emphasizing the impact of specific clinical and immunological profiles on disease severity among Iraqi patients[2]. While the exact etiology remains multifaceted, accumulating evidence highlights that genetic predisposition contributes to approximately 50-60% of the disease susceptibility[3]. In Iraq, epidemiological and clinical observations conducted at the University of Baghdad have suggested that the genetic and clinical landscape of RA patients may exhibit unique characteristics compared to other populations, necessitating the identification of population-specific genetic markers[4].

Historically, the strongest genetic association with RA has been linked to the Major Histocompatibility Complex (MHC) region, specifically the HLA-DRB1 alleles carrying the "shared epitope"[5]. However, genome-wide association studies (GWAS) have identified numerous non-HLA loci that play crucial roles in immune regulation, such as PTPN22, STAT4, and TNFAIP3, which are associated with increased risk across various ethnic groups[6]. Beyond these well-known loci, the macrophage immunometabolism regulator *MACIR* gene, (formerly known as C5orf30), located on chromosome 5q21.1, has recently gained attention as a key regulator of macrophage immunometabolism and inflammatory resolution[7]. While global research has focused on various genetic loci, recent immunogenetic insights from Iraq suggest that the genetic landscape of Middle Eastern patients may harbor unique variations that require population-specific investigation [8].

The *MACIR* gene acts as a protective buffer against excessive tissue damage; experimental studies have demonstrated that its downregulation enhances macrophage activity in producing pro-inflammatory cytokines and promoting cartilage degradation[9]. Among its variants, the single nucleotide polymorphisms (SNPs) rs26232 and rs26233 have emerged as significant candidates influencing gene expression and function[10]. However, global reports indicate a clear ethnic heterogeneity; While the C allele of rs26232 is linked to joint damage in European cohorts, genetic susceptibility profiles often exhibit significant ethnic and regional heterogeneity, including within Middle Eastern and Iraqi populations[11]. Given this established variability, our study represents a novel investigation to explore whether the T allele might conversely act as a primary driver of RA susceptibility in an Iraqi cohort.

Despite the well-documented role of *MACIR* in Western and East Asian cohorts, there is a profound lack of data regarding these polymorphisms in Middle Eastern populations. This study, therefore, aims to investigate the association of *MACIR* gene polymorphisms (rs26232 and rs26233) with RA susceptibility in an Iraqi cohort. By employing bidirectional PCR-Sanger sequencing, we provide the first exploration of these variants and their synergistic haplotype effects within this specific ethnic context, aiming to identify population-specific biomarkers for early diagnosis.

MATERIALS AND METHODS

Study Population and Ethical Considerations

This case-control study was conducted between (August 2024) and (September 2025). A total of 90 participants were enrolled, including 45 Arab Iraqi newly diagnosed RA patients. And 45 age- and sex-matched healthy Arab Iraqi individuals who served as control. RA diagnosis was established according to the 2010 ACR/EULAR classification criteria [12].

Ethical Approval: The study protocol was strictly performed in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the College of Pharmacy, University of Baghdad (Ref No: RECAUBCP0624116R at 5 July2024). All participants provided written informed consent prior to enrollment.

Inclusion and Exclusion Criteria

RA Group: Patients were recruited from the Rheumatology Unit at Baghdad Teaching Hospital. All patients were therapy-naïve or had not started biological therapy at the time of sampling.

Control Group: Healthy Arab Iraqi adult individuals with no history of autoimmune disorders, chronic inflammatory diseases, or family history of RA.

Exclusion Criteria: RA patients with other autoimmune or rheumatic disease (e.g., SLE, psoriatic arthritis, or ankylosing spondylitis), diabetes mellitus, malignancies, chronic kidney, chronic liver, or concurrent systemic infections were excluded to maintain the specificity of the genetic association.

Sample Collection and DNA Extraction

Three milliliters (3 mL) of venous blood were collected from each participant in EDTA-containing tubes. Genomic DNA was extracted from whole blood using a specialized DNA extraction kit (EasyPure®Blood Genomic DNA kit, TransGen Biotech) according to the manufacturer's instructions. The concentration and purity of the extracted DNA were assessed using a Nanodrop™ Spectrophotometer (Thermo Scientific, USA), ensuring an *A260/A280* ratio between 1.8 and 2.0 ·

PCR Amplification and Sanger Sequencing

The target regions of the *MACIR* gene containing rs26232 and rs26233 were amplified using specific primers according to standard molecular protocols.[13] The PCR reaction was performed in a total volume of 25 µL containing Master Mix, 12.5 µL of 2Xeasy Taq®PCR SuperMix, 1µl of Forward primer (100pmol/µl), (1µl Reverse primer 100pmol/µl), 4µlGenomic template DNA, 6.5 µl Nuclease free water.

Sanger Sequencing was performed on the amplified PCR fragments using an ABI3730XL automated DNA sequencer (Macrogen Corporation, Korea)[14]. For bioinformatics analysis, the resulting sequences were compared with the human reference genome using the NCBI BLAST tool. The sequences showed high similarity (99% identity) with the Homo sapiens chromosome 5 clone CTC-449I11 (GenBank Accession: AC008509.5).

Statistical Analysis

Data analysis was performed using IBM SPSS Statistics version 22.0 Genotype and allele frequencies were calculated by direct counting.

Hardy-Weinberg Equilibrium (HWE): Evaluated in the control group using the Chi-square (χ^2) test to ensure the representative nature of the sample [15].

Association Testing: Odds Ratios (OR) and 95% Confidence Intervals (CI) were calculated using logistic regression models (Co-dominant, Dominant, and Recessive) [16].

Linkage Disequilibrium (LD) and Haplotype Analysis: D' and r^2 values were calculated to evaluate the linkage between the two SNPs. Linkage disequilibrium (LD) analysis, haplotype block construction, and frequency estimations were conducted using the SHEsis online software platform[17]. Furthermore, associations between the genetic polymorphisms and RA susceptibility under various inheritance models (co-dominant, dominant, and recessive), as well as Hardy-Weinberg equilibrium (HWE) testing, were evaluated utilizing SNP Stats and IBM SPSS Statistics (Version 22.0). A p-value of < 0.05 was considered statistically significant.

To account for potential confounding variables, multivariable logistic regression analysis was performed. Adjusted odds ratios (AORs) with their 95% confidence intervals (CIs) and adjusted p-values were calculated to evaluate the independent association between *MACIR* genotypes and RA susceptibility, meticulously adjusting for demographic covariates including age, gender, smoking status, and residency.

RESULTS

Demographic and Baseline Clinical Characteristics

A total of 90 participants were enrolled in this study, divided equally into 45 patients with Rheumatoid Arthritis (RA) and 45 healthy controls. Demographic analysis revealed a high degree of homogeneity between the two groups, with no statistically significant differences in mean age (45.13 ±11.67 years for RA vs. 44.20 ±10.09 years for controls; *p=0.978*) or sex distribution *(p=0.999)*, where females predominated in both cohorts. Furthermore, baseline characteristics including residential area *(p = 0.227)*, smoking status *(p=0.725)*, and alcohol consumption *(p=1.000)* were well-balanced across the groups. Clinical assessment of the RA cohort using the DAS28-ESR score indicated a state of active disease, with 77.78% of patients exhibiting high disease activity and 22.22% showing moderate activity; notably, no patients were in remission or low activity states. In contrast to the healthy controls, RA patients demonstrated markedly elevated levels of inflammatory and

serological markers, including ESR (50.00 vs. 10.00 mm/h), CRP (30.00 vs. 1.00 mg/l), ACPA (161.60 vs. 0.70 ng/ml), and RF (130.00 vs. 1.00 IU/ml), all reaching high statistical significance at *p<0.0001*, as shown as (Table1).

Table 1: Demographic and clinical characteristics of rheumatoid arthritis (RA) patients and healthy controls

Variable	Controls (n=45)	RA (n=45)	p-value
Age (years)	44.20 ± 10.09	45.13 ± 11.67	0.978
Sex			
Female	35 (77.78%)	34 (75.56%)	0.999
Male	10 (22.22%)	11(24.44%)	
Zone of Residence			
Urban	36 (80%)	32 (71.11%)	0.227
Rural	9 (20%)	13 (28.89%)	
Smoking Status			
Yes	4 (8.89%)	5 (11.11%)	0.725
No	41(91.11%)	40 (88.89%)	
Alcohol consumption			
Yes	1(2.22%)	1(2.22%)	1.000
No	44 (97.78%)	44 (97.78%)	
DAS28-ESR			
High	–	35 (77.78%)	–
Moderate		10 (22.22%)	
ESR (mm/h)	10.00 (5.75–14.00)	50.00 (38.75–65.00)	**<0.0001**
CRP (mg/l)	1.00 (0.80–2.00)	30.00 (25.75–35.25)	**<0.0001**
ACPA (ng/ml)	0.70 (0.500–0.825)	161.60 (100.00–300.00)	**<0.0001**
RF (IU/ml)	1.00 (0.60–4.00)	130.00 (109.83–160.00)	**<0.0001**

Where: CRP= C-reactive protein; ESR= Erythrocytes sedimentation rate; RF= Rheumatoid factor; RA = Rheumatoid arthritis; DAS28 = Disease Activity Score using 28 joints.

Data of continuous variables are presented as median (IQR) for non-normally distributed variables and mean ± SD for normally distributed variables. Group comparisons were performed using the Mann–Whitney U test or independent t-test, as appropriate. Categorical variables are presented as number (percent) and were compared using the Chi-square (χ^2) or Fisher exact test.

High-Resolution Genotyping and Allelic Architecture

The genetic landscape of the *MACIR* gene (C5orf30) was meticulously mapped using bidirectional Sanger sequencing. The analysis revealed a profound association between two key polymorphisms, rs26232 and rs26233, and Rheumatoid Arthritis (RA) susceptibility within the Iraqi population.

For both loci, the T allele emerged as the primary genetic risk factor. In rs26232, the T allele frequency was significantly higher in RA patients (42.2%) compared to controls (17.8%; $p < 0.001$). Similarly, for rs26233, the T allele showed a marked enrichment in the patient cohort (42.2%) versus only 14.4% in the control group ($p<0.001$). The control group's genotype distribution was in equilibrium with the Hardy-Weinberg law ($p>0.05$), validating the structural integrity of the genetic data, as shown as (Table 2).

Table 2: Genotype and Allele Frequencies of *MACIR* rs26232 and rs26233

SNP	Genotype/ Allele	RA (n=45)	Controls (n=45)	p-value	HWE p-value*
rs26232 (C/T)	CC (wild)	20 (44.4)	32 (71.1)	0.01	0.108
	CT	12 (26.7)	10 (22.2)		
	TT	13 (28.9)	3 (6.7)		
	C	52 (57.8)	74 (82.2)	<0.001	
	T	38 (42.2)	16 (17.8)		
rs26233 (G/T)	GG (wild)	19 (42.2)	34 (75.6)	0.002	0.201
	GT	14 (31.1)	9 (20)		
	TT	12 (26.7)	2 (4.4)		
	G	52 (57.8)	77 (85.6)	<0.001	
	T	38 (42.2)	13 (14.4)		

**HWE tested in controls only.*

Where: RA = Rheumatoid arthritis; SNP = Single nucleotide polymorphism; HWE = Hardy–Weinberg equilibrium; CC, CT, TT = genotypes of rs26232; GG, GT, TT = genotypes of rs26233.

The data were presented as number (percentage). The difference in genotype and allele frequency between RA patients and controls was assessed using Chi-square (χ^2) or Fisher exact test.

Association Between *MACIR* rs26232 Variants and RA Risk

Table (3) summarizes the association between the *MACIR* rs26232 polymorphism and the risk of RA under different genetic models. In the co-dominant model, individuals carrying the CT genotype showed no significant increase in RA risk compared with the CC genotype (OR = 1.92; 95% CI: 0.70–5.26; p = 0.205). However, carriers of the TT genotype demonstrated a markedly higher risk of RA relative to the CC genotype, with an odds ratio of 6.93 (95% CI: 1.75–27.40; $p = 0.006$). After adjustment for age, sex, smoking status, alcohol consumption, and residence, the TT genotype remained significantly associated with RA (adjusted p = 0.004), as shown in (Figure 1).

In the dominant model (CT+TT vs CC), the presence of at least one T allele was significantly associated with increased RA susceptibility (OR = 3.08; 95% CI: 1.29–7.36; *p=0.012*). This association persisted following multivariable adjustment (adjusted p = 0.01).

Similarly, the recessive model (TT vs CC+CT) revealed that homozygosity for the T allele was significantly associated with RA risk (OR = 5.69; 95% CI: 1.49–21.66; *p = 0.011*), with the association remaining significant after adjustment (adjusted *p = 0.008*).

The additive allelic model further supported a risk-enhancing effect of the T allele. RA patients exhibited a significantly higher frequency of the T allele compared with controls (42.2% vs. 17.8%), corresponding to an OR of 2.43 (95% CI: 1.33–4.45; *p = 0.004*). The additive association also remained significant after adjustment (adjusted *p = 0.003).*

Overall, the rs26232 T allele, particularly in homozygous form, was consistently associated with an increased risk of RA across multiple genetic models, even after adjustment for potential confounding variables.

Table 3: Association between *MACIR* rs26232 Variants and Risk of RA

Genetic Model	Genotype / Allele	RA n (%)	Controls n (%)	OR (95% CI)	p-value
Co-dominant	CT	12 (26.7)	10 (22.2)	1.92 (0.70–5.26)	0.205
	TT	13 (28.9)	3 (6.7)	6.93 (1.75–27.40)	0.006*
	CC *(Reference)*	20 (44.4)	32 (71.1)	–	–
Dominant	CT+TT	25 (55.6)	13 (28.9)	3.08 (1.29–7.36)	0.012*
	CC *(Reference)*	20 (44.4)	32 (71.1)	–	–
Recessive	TT	13 (28.9)	3 (6.7)	5.69 (1.49–21.66)	0.011*
	CC+CT *(Reference)*	32 (71.1)	42 (93.3)	–	–
Allelic (Additive)	T allele	38 (42.2)	16 (17.8)	2.43 (1.33–4.45)	0.004*
	C allele *(Reference)*	52 (57.8)	74 (82.2)	–	–

**p-value < 0.05 after adjustment*

Adjusted p-values were calculated using multivariable logistic regression controlling for age, sex, smoking, alcohol consumption and residence. Adjusted p-values: CT vs CC = 0.2097; TT vs CC = 0.004; Dominant model = 0.01; Recessive model = 0.008; Additive model = 0.003.

Where: RA = Rheumatoid arthritis; OR = Odds ratio; CI = Confidence interval; CC, CT, TT = genotypes of rs26232.

Association Between *MACIR* rs26233 Variants and RA Risk

Table (4) presents the association between the *MACIR* rs26233 polymorphism and susceptibility to RA across multiple genetic models. In the co-dominant model, individuals carrying the GT genotype had a higher, statistically significant risk of RA compared with the GG genotype (OR = 2.78; 95% CI: 1.02–7.63; *p = 0.047*). A much stronger association was observed among TT homozygotes, who exhibited a markedly increased risk relative to the GG genotype (OR = 10.74; 95% CI: 2.17–

53.12; p = 0.004). After adjustment for age, sex, smoking, alcohol consumption, and residence, both comparisons remained statistically significant (adjusted p = 0.042 and 0.002, respectively), as shown as (Figure 2).

Under the dominant model (GT+TT vs. GG), carriers of at least one T allele showed a significantly elevated risk of RA (OR = 4.23; 95% CI: 1.72–10.42; *p = 0.002*). This association remained robust following multivariable adjustment (adjusted p = 0.001).

The recessive model (TT vs. GG+GT) similarly demonstrated a strong association between homozygosity for the T allele and RA risk (OR = 7.82; 95% CI: 1.64–37.36; *p = 0.01*), with the adjusted p-value also remaining significant (0.007).

The allelic (additive) analysis further supported a significant contribution of the T allele to RA susceptibility. The T allele frequency was more than twice as high in RA patients compared with controls (42.2% vs. 14.4%), corresponding to an OR of 3.10 (95% CI: 1.59–6.05; *p = 0.001*). This association persisted after adjustment (adjusted *p = 0.001*).

Collectively, the rs26233 T allele, particularly in homozygous form, demonstrated a consistent and significant association with increased risk of RA across all genetic models, even after accounting for relevant demographic and lifestyle confounders.

Table 4: Association between *MACIR* rs26233 Variants and Risk of RA

Genetic Model	Genotype/ Allele	RA n (%)	Controls n (%)	OR (95% CI)	p-value
Co-dominant	GT	14 (31.1)	9 (20)	2.78 (1.02–7.63)	0.047*
	TT	12 (26.7)	2 (4.4)	10.74 (2.17–53.12)	0.004*
	GG *(Reference)*	19 (42.2)	34 (75.6)	–	–
Dominant	GT+TT	26 (57.8)	11 (24.4)	4.23 (1.72–10.42)	0.002*
	GG *(Reference)*	19 (42.2)	34 (75.6)	–	–
Recessive	TT	12 (26.7)	2 (4.4)	7.82 (1.64–37.36)	0.01*
	GG+GT *(Reference)*	33 (73.3)	43 (95.6)	–	–
Allelic (Additive)	T allele	38 (42.2)	13 (14.4)	3.10 (1.59–6.05)	0.001*
	G allele *(Reference)*	52 (57.8)	77 (85.6)	–	–

**p-value < 0.05 after adjustment.*

Adjusted p-values were calculated using multivariable logistic regression controlling for age, sex, smoking, alcohol consumption, and residence: GT vs GG = 0.042; TT vs GG = 0.002; Dominant model = 0.001; Recessive model = 0.007; Additive model = 0.001.

Where: RA = Rheumatoid arthritis; OR = Odds ratio; CI = Confidence interval; GG, GT, TT = genotypes of rs26233.

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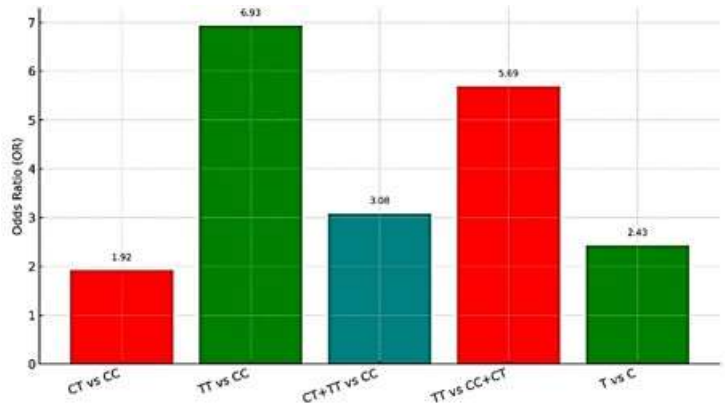

Figure 1 : Odds ratios (OR) for rs26232 under multiple genetic models. The TT genotype and T allele demonstrate higher risk estimates, suggesting that rs26232 contributes to increased susceptibility to rheumatoid arthritis in the studied population.

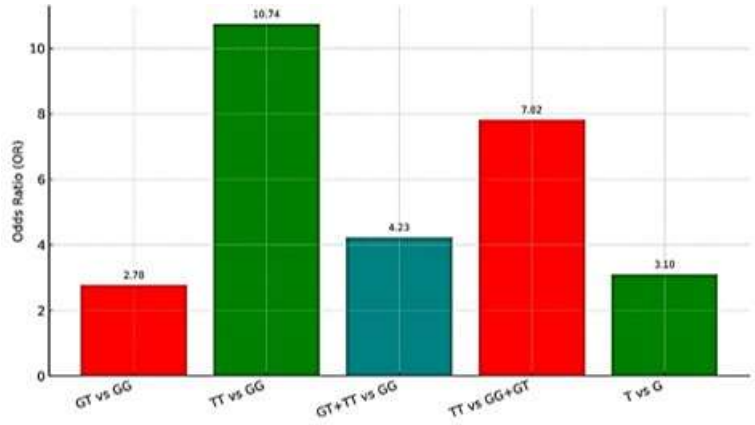

Figure 2 : Odds ratios (OR) for rs26233 under different genetic models (co-dominant, dominant, recessive, and allelic). The T allele and TT genotype show markedly increased risk of rheumatoid arthritis across all models, indicating a strong association of rs26233 with disease susceptibility.

Linkage Disequilibrium Between *MACIR* rs26232 and rs26233

Linkage disequilibrium (LD) analysis was performed to assess the degree of non-random association between the two *MACIR* polymorphisms, rs26232 and rs26233, in the studied population. The analysis demonstrated a strong LD between the two SNPs, with a D' value of 0.97 and an r^2 value of 0.85; Table (5).

These findings indicate that rs26232 and rs26233 are highly co-inherited and exhibit a substantial correlation, suggesting that the two variants do not segregate independently in the Iraqi population. The high r^2 value further implies that a large proportion of the genetic information conveyed by one SNP is shared with the other, reflecting a strong allelic correlation between these loci, as shown in (Figure 3).

Table 5: Linkage disequilibrium between *MACIR* rs26232 and rs26233 polymorphisms

SNP pair	D'	r^2	Interpretation
rs26232 – rs26233	0.97	0.85	Strong LD

Where: LD= Linkage disequilibrium; SNP: Single nucleotide polymorphism; D': Lewontin's standardized measure of linkage disequilibrium; r^2: Squared correlation coefficient between alleles at two loci.

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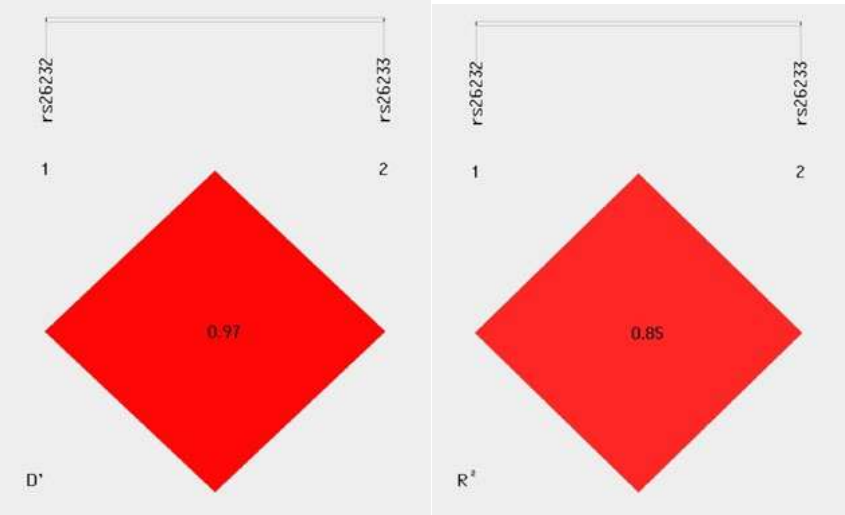

Figure 3: Linkage disequilibrium (LD) plot of the *MACIR* gene polymorphisms (rs26232 and rs26233). The dark red color and the numerical value indicate perfect LD, reflecting a complete genetic correlation between the two loci.

Haplotype Analysis of *MACIR* rs26232 and rs26233

Haplotype analysis was conducted for the two *MACIR* polymorphisms, rs26232 and rs26233, to evaluate the combined effect of allelic variants on RA susceptibility. Haplotypes with a frequency below 0.03 were excluded from the analysis.

Two major haplotypes were identified. The C-G haplotype was significantly more frequent among controls than RA patients (0.811 vs. 0.566, respectively), showing a strong inverse association with RA risk ($\chi^2 = 12.546$, $p < 0.001$). This haplotype was associated with a significantly reduced odds of RA (OR = 0.304, 95% CI: 0.155–0.596).

In contrast, the T-T haplotype was significantly more prevalent in RA patients compared with controls (0.411 vs. 0.144, respectively) and was strongly associated with increased susceptibility to RA ($\chi^2 = 15.95$, $p < 0.001$). Individuals carrying the T-T haplotype exhibited a more than four-fold increased risk of RA (OR = 4.134, 95% CI: 2.008–8.514); Table (6).

The overall haplotype distribution differed significantly between RA patients and controls, as indicated by a global chi-square value of 15.402, with both Fisher's exact test ($p = 1.01 \times 10^{-4}$) and Pearson's chi-square test ($p=8.69\times10^{-5}$) confirming a statistically significant global association.

Table 6: Haplotype distribution of *MACIR* rs26232 and rs26233 in participants

Haplotype	RA Cases n (Frequency)	Controls n (Frequency)	χ^2	p-value	OR (95% CI)
C-G	51(0.566)	73(0.811)	12.546	**<0.001**	0.304 (0.155-0.596)
T-T	37(0.411)	13(0.144)	15.95	**<0.001**	4.134 (2.008-8.514)

Where: CI: Confidence interval; n=number; OR= Odd ratio; RA= Rheumatoid arthritis; χ^2: Chi-square test.

Only haplotypes with a frequency ≥ 0.03 are shown; rare and non-significant haplotypes were excluded from the table.

Sanger Sequencing and Chromatogram Quality Control

The accuracy of the genotyping was confirmed via Sanger Sequencing (Macrogen, Korea). The Chromatograms provided clear, high-resolution peaks for all three patterns: homozygous wild-type, heterozygous, and homozygous mutant. This visual evidence confirms the precision of the allele calls and the integrity of the genetic data, as shown in (Figure 4) and (Figure 5).

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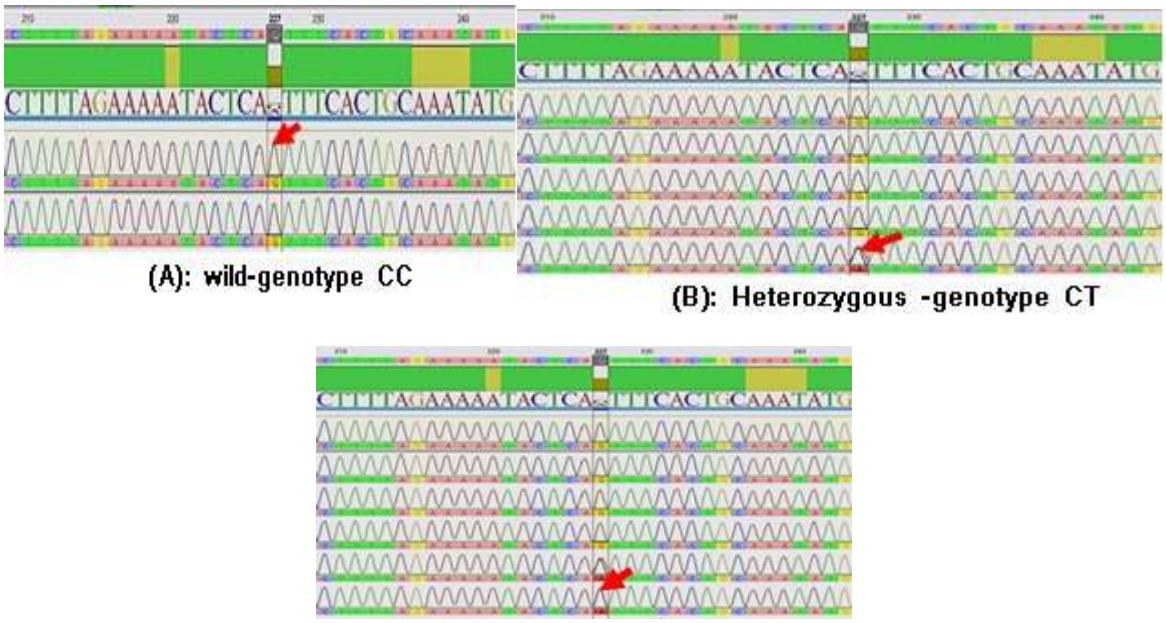

Figure 4: Sanger sequencing chromatograms illustrating the *MACIR* gene rs26232 polymorphism. (A) Wild-type genotype showing the homozygous CC allele. (B) Heterozygous genotype displaying overlapping peaks of C and T alleles. (C) Homozygous mutant genotype showing the variant TT allele. Red arrows indicate the exact position of the single nucleotide polymorphism (SNP).

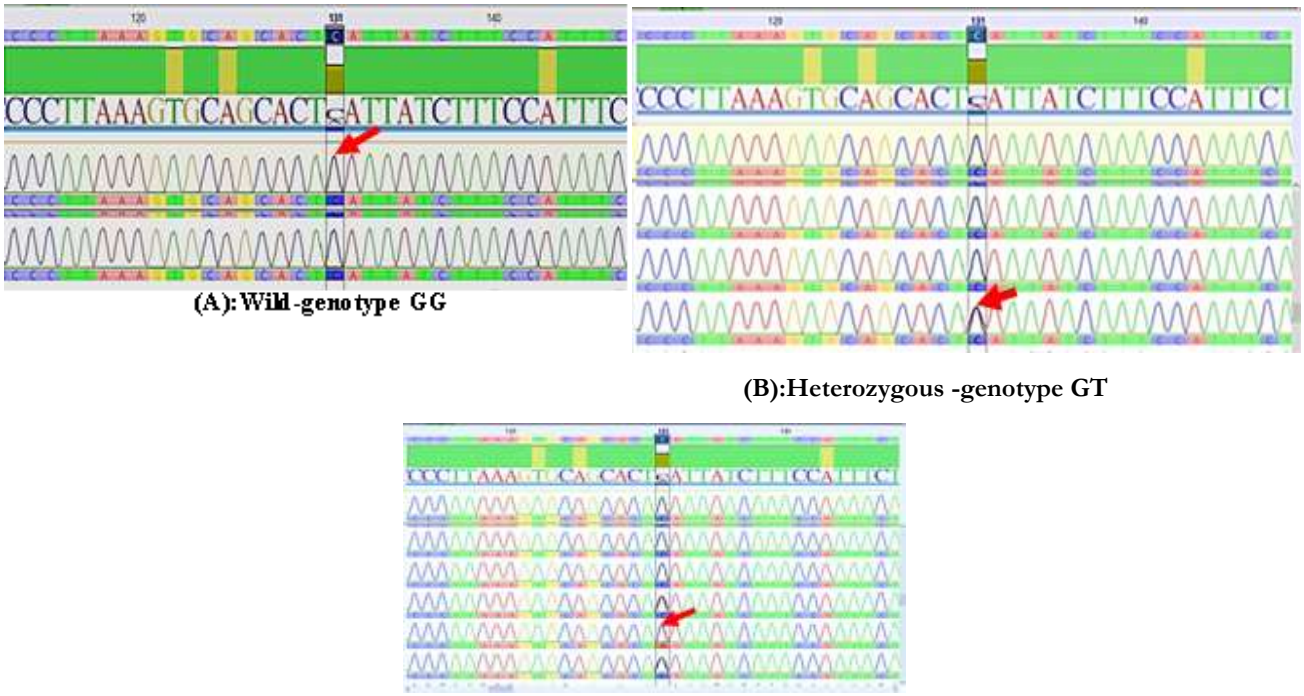

Figure 5: Sanger sequencing chromatograms illustrating the *MACIR* gene rs26233 polymorphism. (A) Wild-type genotype showing the homozygous GG allele. (B) Heterozygous genotype displaying overlapping peaks of G and T alleles. (C) Homozygous mutant genotype showing the variant TT allele. Red arrows indicate the exact position of the single nucleotide polymorphism (SNP).

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DISCUSSION

The genetic architecture of Rheumatoid Arthritis (RA) is characterized by high ethnic heterogeneity, necessitating population-specific investigations to identify robust biomarkers [11]. This study represents the first exploration of the *MACIR* (formerly C5orf30) gene polymorphisms in an Iraqi cohort, providing strong molecular evidence that genetic variation at this locus significantly drives disease susceptibility.

Ethnic Heterogeneity and the "Flip-Flop" Phenomenon

A paramount finding in our study is the identification of the T allele as the primary genetic risk factor for RA susceptibility in the Iraqi population, revealing a striking "flip-flop" phenomenon where the allelic direction of risk is reversed compared to Western cohorts[18]. While European studies frequently highlight the C allele of rs26232 as the variant associated with joint destruction and disease progression [7,10], our data robustly demonstrates that the T allele drives the risk in Mesopotamia. The high frequency of these risk alleles aligns with other Iraqi genetic reports indicating a distinct susceptibility profiles in the Middle Eastern populations that differs significantly from East Asian and European lineages[19]. This divergence is likely attributed to unique ancestral recombination patterns and variations in the surrounding genomic landscape across ethnic groups [18]. Physiologically, the *MACIR* protein functions as a critical negative regulator of the inflammatory and tissue-destructive processes in RA [7,9]. It is predominantly expressed in synovial fibroblasts and macrophages, acting as a molecular buffer that suppresses excessive inflammatory responses[9]. Biologically, this risk allele is hypothesized to lead to reduced *MACIR* expression or impaired protein function, which subsequently promotes a pro-inflammatory microenvironment. Such a deficiency enhances the invasiveness of synovial fibroblasts and shifts macrophage polarization toward the destructive M1 phenotype, thereby accelerating joint damage[20]. This biological plausibility supports our observation of the 'flip-flop' phenomenon, suggesting that the T allele acts as a potent genetic driver of synovial inflammation within this specific ethnic background.

The *MACIR* "Genetic Block" and Haplotype Synergy

The detection of an exceptionally strong Linkage Disequilibrium ($D' = 0.97$, $r^2 = 0.85$) confirms that rs26232 and rs26233 are co- inherited as a highly stable, conserved "Genetic Block" in Iraqis [17]. From a genomic perspective, this high co-segregation suggests that these SNPs may modulate *MACIR* expression through tight linkage with functional regulatory elements [7]. Furthermore, our findings align with clinical observations in Iraq that suggest a strong correlation between molecular markers and the clinical progression of RA, supporting the integration of genetic screening in local diagnostic protocols [4]. Our single-locus findings demonstrated that homozygous mutant TT genotypes confer a massive 7-to 10-fold increase in RA risk reflecting a profound genetic predisposition toward an aggressive inflammatory environment [20].

Crucially, our multi-locus haplotype analysis revealed a profound synergistic effect that underscores the clinical importance of multi-locus profiling over single SNP screening [4]. The T-T risk haplotype exhibited a striking association with RA susceptibility, significantly amplifying the odds of developing the disease (OR=4.134, $p< 0.001$), confirming that these two loci reside within a highly stable block [17]. In contrast, the C-G haplotype emerged as a robust protective factor (OR=0.304, $p< 0.001$), significantly lowering the odds of disease development. By utilizing Sanger sequencing-the gold standard for molecular verification-we have ensured the absolute accuracy of these genetic findings[14]. This synergistic interaction indicates that the cumulative effect of these alleles provides a substantially more powerful predictive marker for RA than individual SNP analysis alone [11]. These findings closely align with local clinical observations in Iraq and strongly advocate for the inclusion of *MACIR* haplotype screening in personalized diagnostic protocols for RA in the Middle East[2,4].

Importantly, the structural resolution and absolute accuracy of these genetic configurations were visually verified through high-quality Sanger sequencing chromatograms (Figures 4 and 5)[14]. The presence of clear, well-defined single peaks for homozygotes and clean overlapping peaks for heterozygotes provides an empirical quality control checkpoint, confirming that our calculated haplotype synergistic risks are derived from high-fidelity call rates rather than sequencing artifacts or genotyping errors. This cumulative interaction indicates that multi-locus profiling serves as a substantially more powerful predictive tool for Middle Eastern cohorts than individual variant testing[11].

Regarding clinical and demographic parameters, our study cohort was characterized by high disease activity, reflected by elevated ESR, CRP, and DAS28-ESR scores, alongside high seropositivity for RF and ACPA[2]. To ensure the independent

association of *MACIR* polymorphisms with RA, we employed a multivariable logistic regression analysis to adjust for potential confounders, including age, gender, smoking status, and residency. Even after this rigorous adjustment, the risk alleles remained significantly associated with the disease (Adjusted $p<0.05$), suggesting that the genetic influence of the *MACIR* locus is a robust risk factor independent of environmental and demographic variables[20]. The observed correlation between these risk variants and high inflammatory markers in the patient group further underscores the role of *MACIR* in modulating systemic inflammation and the clinical severity of RA in the Iraqi population[7].

Limitations and Future Perspectives

Despite the robust and high-resolution molecular findings established in this study, certain inherent limitations should be acknowledged. First, while bidirectional Sanger sequencing provided absolute accuracy for mapping the genomic architecture (DNA variants and stable haplotypes), this investigation focused strictly on structural genetic profiling and did not evaluate functional downstream molecular impacts, such as *MACIR* mRNA expression levels via qRT-PCR or localized synovial protein quantification. Consequently, while the clinical-genetic association is highly evident, the exact transcriptional and functional mechanisms by which these specific Middle Eastern risk alleles alter gene expression remain to be fully characterized *in vivo*.

Second, the study cohort represents a single-center design with a sample size of 90 participants. Although this sample was meticulously selected, strictly age- and sex-matched, and comprehensively limited to treatment-naïve patients to eliminate pharmacological and treatment-induced confounding factors on biological profiles, a larger cohort would further strengthen the statistical power. Therefore, future functional multi-center studies that integrate longitudinal clinical monitoring with downstream gene expression analysis (mRNA and protein) are highly recommended to expand upon these findings and fully validate these genetic insights across broader Middle Eastern and Arab populations.

CONCLUSION:

This study provides the first molecular evidence of the significant association between *MACIR* (rs26232 and rs26233) gene polymorphisms and rheumatoid arthritis (RA) susceptibility within an Iraqi cohort. The identification of the "T" allele and homozygous genotypes as a high-risk factor reveals a striking "flip-flop" phenomenon compared to European populations, emphasizing the critical role of ethnic heterogeneity in autoimmune genetics.

The major strength and novelty of this research lie in the integration of high-precision bidirectional Sanger sequencing with a focus on treatment-naïve patients, which eliminated confounding pharmacological effects and provided a clear clinical-genetic correlation. Furthermore, the discovery of a powerful T-T haplotype synergism and strong linkage disequilibrium (LD) suggests that the *MACIR* locus functions as a coordinated regulatory unit rather than through independent variants.

These findings underscoress the clinical potential of *MACIR* genotyping as a robust, population-specific biomarker for early RA diagnosis and risk stratification in the Middle East. Future functional studies are warranted to elucidate the precise molecular mechanisms by which these Iraqi-specific risk alleles modulate *MACIR* expression and inflammatory resolution.

Research Highlights

First study to investigate the *MACIR* (C5orf30) gene haplotypes and linkage disequilibrium in the Iraqi population.

Identification of a unique "Flip-Flop" genetic pattern, where the T-allele serves as a risk factor, contrasting with some Western cohorts.

Confirmation of a strong Genetic Block between rs26232 and rs26233 ($D' = 0.97$).

Utilization of Gold-Standard Sanger Sequencing (Macrogen, Korea) to ensure maximum genotyping accuracy.

Establishment of the T-T haplotype as a robust predictive marker for RA susceptibility ($p < 0.001$).

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