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ASSOCIATION OF CASPASE-1 GENE POLYMORPHISMS WITH RHEUMATOID ARTHRITIS SUSCEPTIBILITY AND TEMPOROMANDIBULAR JOINT INVOLVEMENT: AN IRAQI COHORT STUDY

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Caspase-1 (*CASP1*) is an integral part of the inflammasome, and rheumatoid arthritis (RA) is a complex, progressive, chronic inflammatory autoimmune disease. The present study aimed to investigate two aspects of RA: i) the association of *CASP1* gene polymorphisms with susceptibility to RA; and ii) the prevalence of temporomandibular joint (TMJ) involvement in patients with RA. This cross-sectional study included 90 participants: 60 patients with RA (20 newly diagnosed and untreated, 40 receiving combination therapy with methotrexate and etanercept) and 30 healthy controls. Three a priori-selected SNPs were screened: rs580253 (C>T), rs501192 (G>A), and rs56244680 (11-bp deletion TTCCATATTTA). Control variant allele frequencies were rs580253 T = 0.10, rs501192 A = 0.10, and rs56244680 del = 0.017. No significant differences in CASP1 genotype frequencies were observed among the treated, newly diagnosed, and control groups. Both components of the Helkimo index revealed significantly greater TMJ involvement in patients than in controls (both $p < 0.001$). Subjective TMJ symptoms (Ai >0) were reported by 28/40 (70.0%) treated and 10/20 (50.0%) newly diagnosed patients, compared with 5/30 (16.7%) controls. Objective TMJ dysfunction (Di >0) was observed in 30/40 (75.0%) of treated and 14/20 (70.0%) of newly diagnosed patients, compared with only 4/30 (13.3%) of controls, all of whom presented with mild dysfunction. Notably, treated patients reported more severe subjective symptoms than newly diagnosed patients ($p = 0.048$), whereas objective dysfunction did not differ significantly between these groups ($p = 0.303$). These findings highlight the importance of clinical TMJ assessment in all patients with RA, regardless of genetic background or treatment status.

Keywords: rheumatoid arthritis, caspase-1, gene polymorphism, temporomandibular joint, inflammasome, single nucleotide polymorphism

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INTRODUCTION

Rheumatoid arthritis (RA) is an inflammatory condition that predominantly impacts the joints of the extremities. One of its most common manifestations is persistent, recurrent joint inflammation (1–3). The disease typically affects multiple joints, characteristically presenting first in the periphery (4,5). While initial involvement of the temporomandibular joint (TMJ) as the first manifestation of RA is rare (6), TMJ involvement has been reported in 5%–86% of patients with RA, with bilateral involvement being the most common presentation (6).

When TMJ involvement is assessed using patient-reported symptoms, clinical examination, or radiographic imaging, the wide range of reported prevalence reflects differences in assessment methods among studies. Imaging studies, in particular, tend to detect higher rates of TMJ involvement than clinical assessment alone (7,8).

Early research has suggested that genetic variables are responsible for 50% to 65% of RA cases (9). Single nucleotide polymorphisms (SNPs) serve as valuable genetic markers for elucidating evolutionary history and identifying common genetic factors that account for heritable risk of prevalent diseases and influence progression of certain diseases (10). Previous studies have found a link between SNPs in multiple genes and the resistance or favorable response to treatment in patients with RA (11–13).

One of the primary features of RA is continuous inflammation (14). Identifying individual molecules and their roles in inflammation is crucial for understanding the RA etiology and creating novel therapeutics. A class of proteolytic enzymes known as caspases transduce signals that ultimately result in cell death (15). Caspase-1 (*CASP1*), in particular, plays a central role in inflammation as the primary activator of pro-inflammatory cytokines IL-1β and IL-18 through its function within the inflammasome complex (16).

The three *CASP1* polymorphisms (rs580253, rs501192, and rs56244680) were selected a priori based on existing evidence linking them to altered inflammatory responses in diverse non-RA cohorts, including those with restenosis and severe infectious diseases (17,18). Biologically, *CASP1* is a strong candidate for RA pathogenesis because it drives synovial inflammation and joint destruction by activating IL-1β and IL-18 within the inflammasome complex (16,19–21).

While previous studies have investigated other *CASP1* variants in rheumatoid arthritis (RA), including rs2409062 in Chinese populations (22) and rs572687 in a Brazilian cohort (23), the specific intronic variants rs580253 (C>T) and rs501192 (G>A), together with the 11-bp deletion variant rs56244680, have not been investigated in RA. Based on a comprehensive literature review and the potential functional significance of the 11-bp deletion, these three *CASP1* variants were selected as novel, hypothesis-driven candidates for assessing genetic susceptibility to RA in an Iraqi population.

The present study aimed to investigate two aspects of RA: i) the association of *CASP1* gene polymorphisms with susceptibility to RA; and ii) the prevalence of temporomandibular joint (TMJ) involvement in patients with RA.

METHODS

This cross-sectional study included 90 participants recruited from the outpatient rheumatology unit of Baghdad Teaching Hospital (Baghdad, Iraq) between mid-March 2023 and mid-September 2023, with the approval of the Scientific and Ethical Unit of Baghdad Teaching Hospital in Baghdad, Iraq (Approval No. 859, issued on 19 February, 2023). In accordance with the Declaration of Helsinki, written informed consent was obtained from all participants prior to their enrollment in the study.

A total of 60 patients with RA and 30 healthy controls were enrolled. The patient group consisted of 20 newly diagnosed, untreated RA patients and 40 RA patients receiving combination therapy with methotrexate and etanercept. The control group comprised 30 healthy individuals, matched for age and sex, with no history of systemic diseases or medication use.

The inclusion criteria were as follows:

For the patients with RA: i) age 20–60 years; ii) diagnosis of RA according to the 2010 ACR/EULAR classification criteria (24).

For the newly diagnosed subgroup: confirmed RA diagnosis within the past 3 months and no prior use of disease-modifying antirheumatic drugs (DMARDs) or biological therapy.

For the treated subgroup: established diagnosis of RA with stable methotrexate and etanercept combination therapy for ≥3 months.

For the healthy controls: i) age 20-60 years; ii) no history or current diagnosis of any systemic disease (including RA, other autoimmune diseases, diabetes, cardiovascular disease, or malignancy); iii) no current medication use; iv) age- and sex-matched to the patient group.

The following exclusion criteria were applied:

For the patients with RA: i) age <20 or >60 years;

ii) presence of other concomitant autoimmune diseases (e.g., systemic lupus erythematosus, psoriatic arthritis, or Sjögren's syndrome); iii) major comorbidities influencing systemic inflammation or TMJ status, including diabetes mellitus, chronic kidney or liver disease, malignancy, active infections; iv) history of TMJ trauma/surgery or pre-existing temporomandibular joint disorder; v) bruxism or other parafunctional habits; vi) pregnancy or lactation.

For the newly diagnosed subgroup: any prior DMARD/or corticosteroid exposure.

For the treated subgroup: poor medication adherence or dosage modification within the past 3 months.

For the healthy controls: i) age <20 or >60 years; ii) any known systemic disease (determined by structured interview); iii) any current medication use; iv) clinical symptoms suggestive of an undiagnosed disease (such as joint pain, morning stiffness, or oral dryness); v) a history of TMJ trauma/surgery, or any current TMJ symptoms; vi) bruxism or other parafunctional habits; vii) pregnancy or lactation; viii) a first-degree relative with RA (to minimize genetic confounding); and ix) age- and sex-matched to the patient group at the group level.

The control group's healthy status and absence of systemic disease were confirmed through a structured medical history interview and a brief physical examination, including assessment of vital signs, an oral examination, and bilateral TMJ palpation.

Disease activity assessment

Rheumatologists evaluated disease activity in all patients with RA using the Clinical Disease Activity Index (CDAI). The CDAI is a composite measure calculated as the sum of four components: i) tender joint count based on assessments of 28 joints; ii) swollen joint count based on assessment of 28 joints; iii) patient global assessment of disease activity (on a 0–10 cm visual analog scale); and iv) physician global assessment of disease activity (on a 0–10 cm visual analog scale).

CDAI scores were interpreted according to the following validated thresholds (25,26): remission, ≤2.8; low disease activity, >2.8 to ≤10.0; moderate disease activity, >10.0 to ≤22.0; and high disease activity, >22.0.

TMJ assessment

TMJ involvement was evaluated using the Helkimo index, which comprises two components based on the standardized criteria established by Helkimo (27) and described previously (28,29). The Helkimo anamnestic index (Ai) assesses subjective symptoms: 0, no symptoms; I, mild symptoms; and II, severe symptoms. The Helkimo clinical dysfunction index (Di) evaluates objective clinical findings: 0, no dysfunction; I, mild dysfunction; II, moderate dysfunction; and III, severe dysfunction. The Di score is based on five clinical criteria: mandibular mobility, TMJ function, muscle pain, TMJ pain, and pain during mandibular movement.

CASP1 genotyping

For the genetic association analysis, a post hoc power calculation was performed using the WINPEPI software package (version 11.65; Brixton Health). Assuming a minor allele frequency of approximately 10% for rs580253 and rs501192 (based on preliminary data and public databases for Middle Eastern populations), the study sample provided 80% power to detect large genetic effects [an odds ratio (OR) ≥3.5], 60% power to detect an OR ≥3.0, and <30% power to detect an OR <2.5.

These findings are based on the assumption that the significance threshold was α=0.05. For the deletion variant rs56244680, with an observed frequency of approximately 3% to 10%, power levels are markedly lower, yielding 45% power to detect an OR of 4.0. These calculations indicate that although the study had sufficient power to identify large genetic effects, it may fail to identify moderate or mild effects (OR <3.0). Thus, the negative findings for *CASP1* polymorphisms should be interpreted with caution, as they do not preclude smaller effect sizes that require larger cohorts to detect. Of note, the data on TMJ involvement were highly significant ($p < 0.001$), suggesting that the sample size was sufficient to detect the primary clinical outcome.

DNA extraction

To isolate DNA for *CASP1* genotyping, 2 mL of venous blood was collected from each participant into EDTA tubes. DNA concentrations ranged from 30 to 150 ng/μL, and the DNA quality was adequate for all 90 samples, with A260/A280 ratios ranging from 1.8 to 2.0. Furthermore, there were no sample processing failures that necessitated repeat collection. Extraction of genomic DNA was performed according to the manufacturer's instructions using the EasyPure® gDNA EE101-01 kit (TransGen Biotech Co., Ltd.). DNA concentration and purity were determined spectrophotometrically using a NanoDrop One spectrophotometer (Thermo Fisher Scientific, USA).

Primer design and optimization

Primers were designed to amplify a *CASP1* region encompassing the three target SNPs (rs580253, rs501192, and rs56244680) and optimized to yield specific results. The primer sequences were as follows: forward, 5'-ACCTCTGACAGCACGTTCCT-3'; and reverse, 5'-GGTGTGGAAGAGCAGAAAGC-3'. To determine the optimal annealing temperature, a gradient PCR was performed across a temperature range of 55–65 ˚C. The annealing temperature yielding the clearest single band with minimal nonspecific amplification was 60˚C, which was subsequently used for all reactions.

PCR amplification conditions

PCR was performed in a total reaction volume of 25 µl containing 100 ng of genomic DNA, 0.4 µM of each primer (forward and reverse), 12.5 µl of 2× GoTaq® Green Master Mix (Promega Corporation) containing Taq DNA polymerase, dNTPs, $MgCl_2$, and reaction buffer, and nuclease-free water to make up the final volume. All PCR reactions were performed using a Veriti® 96-Well Thermal Cycler (Model No. 4375786, Applied Biosystems, Inc.). The thermal cycling protocol consisted of an initial denaturation at 95˚C for 5 min, followed by 35 cycles of denaturation at 95˚C for 30 s, annealing at 60˚C for 30 s, and extension at 72˚C for 45 s. The final extension was performed at 72˚C for 7 min, followed by a hold at 4˚C.

Product verification

PCR products were visualized by electrophoresis on a 2% agarose gel stained with ethidium bromide, alongside a 100-bp DNA ladder (Cat. no. DM003-R500; GeneDireX, Inc.) to confirm the expected product size. Gels were subsequently imaged using a UV transilluminator (BioDoc-It™ Imaging System; UVP).

Sequencing and genotype determination

PCR products were purified using the ExoSAP-IT™ Express PCR Product Cleanup Reagent (cat. No. 78201, Applied Biosystems, Inc.) according to the manufacturer's instructions. The purified products underwent Sanger sequencing on an ABI3730XL automated DNA sequencer (Applied Biosystems, Inc.) using the same primers as for PCR amplification. Both forward and reverse strands were sequenced to ensure accuracy.

Following sequencing, chromatogram files (.ab1) were imported into Geneious Prime software (version 2023.0; Biomatters) for analysis. Using the MUSCLE alignment algorithm, sequences were assembled into contiguous sequences (contigs) and aligned with the CASP1 reference sequence from GenBank (Accession No. NG_029124.2). This alignment was performed to screen for the three *a priori*-selected polymorphisms in the Iraqi population: rs580253 (C>T), rs501192 (G>A), and rs56244680 (11-bp deletion TTCCATATTTA). A visual examination of the electropherograms at the polymorphic sites allowed for genotype determination for each SNP at the following locations:

For rs580253 and rs501192, homozygosity was indicated by a single peak (reference or variant allele), while heterozygosity was indicated by two overlapping peaks of approximately equal height at the same position. For rs56244680 (11-bp deletion TTCCATATTTA), genotype determination was based on chromatogram patterns, as follows: i) homozygous wild-type (ins/ins) – a perfect match with the reference sequence and a clear single peak throughout the deletion region; ii) heterozygous deletion (ins/del), indicated by the presence of overlapping chromatogram peaks beginning immediately downstream of the deletion site; these overlapping peaks demonstrated the presence of two alleles of different lengths, one containing the 11-bp sequence and the other lacking it; iii) homozygous deletion (del/del), characterized by a sequence shortened by 11 base pairs with single, unambiguous peaks throughout the region, confirming that both alleles carry the deletion.

All sequences were independently examined by two investigators who were blinded to participants' clinical status. Any discrepancies were resolved by joint review and, when necessary, repeat sequencing. During the final analysis, only sequences with Phred quality scores >30 (with a base call accuracy of at least 99.9%) were considered.

Statistical analysis

All data were analyzed using IBM SPSS Statistics (version 26; IBM Corp.). Additional analyses were performed using WINPEPI (version 11.65) for power calculations and Hardy-Weinberg equilibrium testing, and GraphPad Prism (version 9; GraphPad Software, San Diego, CA, USA) for figure generation. For descriptive statistics, continuous variables (e.g., age) are presented as means ± standard deviations (SDs). Categorical variables (sex, genotype frequencies, and the Helkimo index categories) are presented as frequencies and percentages.

Demographic matching confirmation

Age: one-way analysis of variance (ANOVA) was used to compare mean age across the three study groups (newly diagnosed RA, treated RA, and control). Sex: the chi-squared (χ^2) test was used to compare sex distribution among the three groups. A p-value >0.05 for both tests was considered to support the comparability of the groups with respect to age and sex.

Hardy-Weinberg equilibrium (HWE)

For each SNP (rs580253, rs501192, and rs56244680), HWE was tested in the control group using the chi-square goodness-of-fit test (WINPEPI software). A p-value >0.05 indicated that the genotype frequencies did not significantly deviate from HWE.

Genetic association analysis

Genotype frequencies were compared among the three groups (newly diagnosed RA, treated RA, and control) using the chi-squared test or Fisher's exact test (when expected cell counts were <5), while allele frequencies were compared across groups using the chi-squared test. ORs and 95% confidence intervals (CIs) were computed using binary logistic regression (SPSS).

To assess the risk associated with carrying a variant allele (T for rs580253; A for rs501192), a dominant genetic model was applied by comparing variant allele carriers (heterozygotes and homozygous variants) to non-carriers (homozygous reference), with the control group serving as the baseline reference category. The OR was interpreted as follows: an OR >1.0 indicates an increased risk, an OR <1.0 indicates a decreased risk, and an OR =1.0 indicates no association. Statistical significance was established based on whether the 95% CI excluded 1.0.

TMJ involvement analysis

For the Helkimo Ai, the chi-squared test was used to analyze the distribution of Ai categories (0, I, and II) across the three groups. Similarly, the chi-squared test was applied to evaluate the distribution of the Helkimo Di categories (0, I, II, and III) among the groups. For pairwise comparisons, post hoc testing between specific groups (control vs. newly diagnosed, control vs. treated, and newly diagnosed vs. treated) was performed using the chi-squared test with a Bonferroni correction applied for multiple comparisons.

Multiple testing considerations

The genetic association analysis included three different SNPs that were independent of one another. To account for multiple comparisons, statistical significance was set at $p < 0.05$, unless specified otherwise for post hoc corrections.

RESULTS

Demographic characteristics

The demographic characteristics of patients with newly diagnosed RA, patients treated for RA, and healthy controls are presented in Table 1.

Age matching: the mean age ± SD was 40.65±10.21 years in the newly diagnosed group, 44.45±10.30 years in the treated group, and 39.90±9.99 years in the control group. One-way ANOVA revealed no statistically significant differences in mean age among the three groups ($F = 2.65$, $p = 0.08$), indicating successful age matching.

Sex matching: the newly diagnosed group included 18 females (90%) and 2 males (10%); the treated group consisted of 28 females (70%) and 12 males (30%); and the control group comprised 24 females (80%) and 6 males (20%). The chi-squared test confirmed no significant differences in sex distribution among the groups ($\chi^2 = 3.23$, $p = 0.20$), confirming successful sex matching.

Disease activity: the mean CDAI score was 24.6 ± 6.8 in the newly diagnosed group (indicating high disease activity) and 9.8 ± 4.2 in the treated group (indicating low disease activity).

Involvement of TMJ

Helkimo Ai: the distribution of the Helkimo Ai categories across the three study groups is presented in Table 2, part A. Statistically significant differences were observed among the groups ($p < 0.001$). Overall, subjective TMJ symptoms (Ai >0) were reported in 28/40 (70.0%) treated patients and 10/20 (50.0%) newly diagnosed patients, compared with 5/30 (16.7%) controls. The mean Ai score ± SD was 1.18 ± 0.87 in the treated group, 0.65 ± 0.75 in the newly diagnosed group, and 0.17 ± 0.38 in the controls.

Helkimo Di: breakdown of the categories comprising the Helkimo Di is presented in Table 2, part B, for the three different study groups, showing that statistically significant differences were observed among the groups ($p < 0.001$). Overall, objective TMJ dysfunction (Di >0) was observed in 30/40 (75.0%) treated patients and 14/20 (70.0%) newly diagnosed patients, compared with 4/30 (13.3%) controls, all

Table 1. *Demographic characteristics of the study groups*

Variable	Treated (n = 40)	Newly diagnosed (n = 20)	Control (n = 30)	Test statistic	df	P-value
Age (years), mean ± SD	44.45 ± 10.30	40.65 ± 10.21	39.90 ± 9.99	F = 2.65	2,117	0.08 (NS)
Sex, n (%)				$\chi 2 = 3.23$	2	0.20 (NS)
Male	12 (30%)	2 (10%)	6 (20%)	–	–	–
Female	28 (70%)	18 (90%)	24 (80%)	–	–	–
Mean CDAI score	9.8 ± 4.2	24.6 ± 6.8				

Data are presented as the mean ± standard deviation (SD) for continuous variables and as frequencies (percentages) for categorical variables. Age was compared across groups using a one-way analysis of variance (F = 2.65; df = 2, 117; p = .080), and sex distribution was analyzed using the chi-squared test ($\chi^2 = 3.23$; df = 2; P = 0.20); NS, not significant.

Table 2. *Anamnestic and dysfunction components of the Helkimo index across study groups*

A, Helkimo Ai						
Ai category (Ai)	Treated (n = 40)	Newly diagnosed (n = 20)	Control (n = 30)	$\chi 2$	df	p-value
No symptoms (Ai 0), n (%)	12 (30%)	10 (50%)	25 (83.3%)	–	–	–
Mild symptoms (Ai I), n (%)	9 (22.5%)	7 (35%)	5 (16.7%)	–	–	–
Severe symptoms (Ai II), n (%)	19 (47.5%)	3 (15%)	0 (0%)	–	–	–
Mean score ± SD	1.18 ± 0.87	0.65 ± 0.75	0.17 ± 0.38			
Overall Ai comparison	–	–	–	45.6	4	**<0.001**
B, Helkimo Di						
Dysfunction index (Di)	Treated (n = 40)	Newly diagnosed (n = 20)	Control (n = 30)	$\chi 2$	df	p-value
No dysfunction (Di0), n (%)	10 (25%)	6 (30%)	26 (86.6%)	–	–	–
Mild dysfunction (DiI), n (%)	11 (27.5%)	9 (45%)	4 (13.3%)	–	–	–
Moderate dysfunction (DiII), n (%)	15 (37.5%)	3 (15%)	0 (0%)	–	–	–
Severe dysfunction (DiIII), n (%)	4 (10%)	2 (10%)	0 (0%)	–	–	–
Mean score ± SD	1.33 ± 0.97	1.05 ± 0.94	0.13 ± 0.35			
Overall Di comparison	–	–	–	72.3	6	**<0.001**

Chi-squared test was used to analyze Ai and Di across groups, yielding a) for anamnestic index (Ai): $\chi^2 = 45.6$, df = 4, p <0.001; b) for dysfunction index (Di): $\chi^2 = 72.3$, df = 6, p <0.001. Fisher's exact test was applied where expected cell counts were <5, confirming significance.

Table 3. *Pairwise post hoc comparisons of the Helkimo anamnestic component among the study groups*

Groups	$\chi 2$ (Ai)	df (Ai)	p-value (Ai)	$\chi 2$ (Di)	df (Di)	p-value (Di)
Control vs. treated	28.4	2	<0.001	41.7	3	<0.001
Control vs. newly diagnosed	12.1	2	0.002	29.5	3	<0.001
Newly diagnosed vs. treated	6	2	0.048	3.6	3	0.303 (NS)

Pairwise post hoc comparisons were performed using the chi-squared test. Statistical significance was established at p <0.05, and non-significant differences are denoted as NS (p >0.05). Fisher's exact test was applied to confirm the results when expected cell counts were <5.

4 of whom had mild dysfunction (DiI). The mean Di score ± SD was 1.33 ± 0.97 in the treated group, 1.05 ± 0.94 in the newly diagnosed group, and 0.13 ± 0.35 in the controls.

Pairwise comparisons revealed distinct patterns for subjective versus objective TMJ measures (Table 3). For the Ai (subjective symptoms), a statistically significant difference was observed between newly diagnosed and treated patients (p = 0.048), with treated patients reporting more severe symptoms (47.5%) compared to newly diagnosed patients (15%), as presented in Table 2. In contrast, for the Di (objective findings), no significant difference was observed between these groups (p = 0.303), as shown in Table 3. Detectable TMJ dysfunction was highly prevalent in both the treated and newly diagnosed groups, indicating that TMJ pathology is a common finding regardless of current treatment status.

CASP1 gene sequencing

CASP1 gene sequencing revealed the presence of three single nucleotide polymorphisms, rs580253, rs501192, and rs56244680 in both the control group and patients with RA, as shown in Figure 1. Among these variants, the polymorphism rs580253 resulted from a cytosine-to-thymine (C>T) substitution, while rs501192 resulted from a guanine-to-adenine (G>A) substitution. Lastly, rs56244680 displayed the TTCCATATTTA sequence deletion.

CASP1 C/T (*rs580253)*: no statistically significant differences were observed among the three groups for genotype distribution (χ^2 = 2.01, p = 0.742) or allele frequencies (χ^2 = 0.76, p = 0.683), as presented in Table 4.

CASP1 G/A (*rs501192*): the observed genotype frequencies (proportion) were as follows: the AA genotype was found in 2 (10.0%) newly diagnosed patients, 0 (0.0%) treated patients, and 1 (3.3%) control; the GA genotype was found in 4 (20.0%) newly diagnosed patients, 6 (15.0%) treated patients, and 1 (3.3%) of the controls; the GG genotype was found in 70.0% of the newly diagnosed patients, 85.0% of the treated patients, and 83.3% of the controls (Table 5).

The A allele frequency was 8 (20%) in the newly diagnosed group, 14 in the treated group, and 6 (10%) in the control group. Statistical analysis revealed no significant differences in genotype distribution (χ^2 = 5.78, p = 0.218) or allele frequencies (χ^2 = 3.71, p = 0.156) among the three groups.

HWE testing for rs501192 yielded the following results: i) control group – observed frequency (proportion): GG=25 (83.3%), GA=4 (13.3%), AA=1 (3.3%) which was in HWE (χ^2 = 0.18, df = 1, p = 0.671); ii) newly diagnosed group – observed frequency (proportion): GG=14 (70.0%), GA=4 (20%), AA=2 (10%) revealing no significant deviation (χ^2 = 0.02, df = 1, p = 0.888); iii) treated group – observed frequency (proportion): GG=34 (85%), GA=6 (15%), AA=0 (0%), which was consistent with HWE (χ^2 = 0.26, df = 1, p = 0.610). These results confirm that genotype distributions for rs501192 were in HWE across all groups (all p >0.05), as presented in Table 5.

CASP1 (*rs56244680*): the rs56244680 11-bp deletion (TTCCATATTTA) was observed in both heterozygous and homozygous states across the study groups: i) newly diagnosed group, (n = 20) – two patients, 0.1 (10.0%) carried the deletion, one heterozygous (ins/del) and one homozygous (del/del); ii) treated group, (n = 40) – one patient, 0.025 (2.5%) carried the deletion in heterozygous form (ins/del); and iii) control group, (n = 30) – one individual, 0.033 (3.3%) carried the deletion in the heterozygous form (ins/del).

The overall frequency of deletion carriers (combining heterozygous and homozygous) did not differ significantly among the three groups (χ^2 = 2.15, p = 0.341), as illustrated in Figure 2. Due to the low number of these carriers, statistical comparison of heterozygous versus homozygous genotypes was not feasible.

HWE testing for rs56244680 (11-bp deletion) was performed, yielding the following results: i) control group – observed frequencies of ins/ins = 29, ins/del = 1, del/del = 0; due to the low expected frequency of the del/del genotype, Fisher's exact test was used, which confirmed no significant deviation from HWE (p = 1.000); and ii) newly diagnosed group – observed frequencies of ins/ins =18, ins/del = 1, and del/del = 1.

Fisher's exact test revealed consistency with HWE (p = 0.289); and iii) treated group – observed frequencies of ins/ins = 39, ins/del = 1, and del/del = 0, with Fisher's exact test confirming HWE (p = 1.000). These results confirm that genotype distributions for rs56244680 were in HWE across all groups (all p >0.05).

DISCUSSION

In the present study, both components of the Helkimo index confirmed substantially greater TMJ involvement in patients with RA than in controls (Table 2). Objective dysfunction was detectable in approximately three-quarters of the patient cohort, while the controls were almost uniformly asymptomatic, with only mild findings in a small minority. Dysfunction was comparably frequent in newly diagnosed and treated patients, indicating that TMJ pathology is established early in the disease course and persists despite systemic combination therapy.

Importantly, a clear discrepancy was observed between patient-reported symptoms and objective clinical findings. This divergence occurred despite the treated group exhibiting lower peripheral disease activity (CDAI: 9.8 ± 4.2 in the treated group vs. 24.6 ± 6.8 in the newly diagnosed group). This clinical dissociation has significant implications for routine practice.

The discovery that treated patients report more severe subjective symptoms despite having equal objective dysfunction ratings to newly diagnosed patients is surprising. Potential explanations include a longer overall disease duration leading to cumulative joint deterioration, or altered symptom awareness in patients undergoing long-term therapy. However, in the absence of imaging and temporal data, these remain purely speculative hypotheses.

Table 4. *Genotype and allele distributions of the CASP1 C/T (rs580253) polymorphism and associated odds ratios across the study groups*

A, Genotype											
Genotype	Control	Newly diagnosed	χ2	df	p-value	OR (95% CI)a	Treated group	χ^2	df	p-value	OR (95% CI)a
CC	25 (83.3%)	13 (65%)	3.2	2	—	1.00 (Ref)	33 (82.5%)	0.1	2	—	1.00 (Ref)
CT	4 (13.3%)	5 (25%)	1.6	2	0.20 (NS)	2.4 (0.54-10.51)	7 (17.5%)	0.3	2	0.60 (NS)	1.3 (0.34- 5.03)
TT	1 (3.3%)	2 (10%)	1.6	2	0.20 (NS)	3.8 (0.31-46.49)	0	0.3	2	0.20 (NS)	0.4 (0.0099-6.49)
B, Allele											
Allele	Control	Newly diagnosed	χ2	df	p-value	OR (95% CI)	Treated group	χ^2	df	p-value	OR (95% CI)
C alleleb	54 (90%)	31 (80%)	—	1	—	1.00 (Ref)	73 (90%)	—	1	—	1.00 (Ref)
T alleleb	6 (10%)	9 (20%)	2.9	1	0.09 (NS)	2.6 (0.84-8.03)	7 (10%)	0.07	1	0.80 (NS)	0.9 (0.27–2.71)

Data are presented as number of participants with percentage in parentheses for genotypes, and as allele frequency (proportion) with raw counts (variant alleles/total alleles) in parentheses for alleles. CI: confidence interval; df: degrees of freedom; NS: not significant (p >0.05); OR: odds ratio.

a: Odds ratios represent the risk of RA associated with each genotype or allele compared with the reference category (CC genotype or C allele) in the control group. OR >1.0 indicates increased risk; OR <1.0 indicates decreased risk.

b: The frequencies of alleles are presented as proportions, with rates ranging from 0 to 1.0. Raw counts are the number of variant alleles divided by the total number of chromosomes.

Genotype frequencies (CC, CT, TT) and allele frequencies were compared using chi-squared tests. In cases where the expected cell counts were less than five, such as in the case of the TT genotype in the treated group, Fisher's exact test was utilized.

No statistically significant differences were detected among the three groups for genotype distribution (χ^2 = 2.01, P = 0.742) or allele frequencies (χ^2 = 0.76, P = 0.683).

Table 5. *Genotype and allele distributions, χ^2, df, and odds ratios for CASP1 G/A (rs501192) across the study groups*

A, Genotype											
Genotype	Control	Newly diagnosed RA	χ2	df	p-value	OR (95% CI)a	Treated (n = 40)	χ2	df	P-value	OR (95% CI)a
GG	25 (83.3%)	14 (70%)	—	—	—	1.00 (Ref)	34 (85%)	—	—	—	1.00 (Ref)
GA	4 (13.3%)	4 (20%)	—	—	0.40 (NS)	1.7 (0.38-8.26)	6 (15%)	—	—	0.80 (NS)	1.1 (0.28–4.32)
AA	1 (3.3%)	2 (10%)	—	—	0.30 (NS)	3.5 (0.29-2.99)	0	—	—	0.30 (NS)	0.2 (0.00– 6.29)
Overall genotype χ2	—	—	2.14	2	0.34 (NS)	—	—	1.03	2	0.59 (NS)	—
B, Allele											
Allele	Control	Newly diagnosed RA	χ2	df	P-value	OR (95% CI)	Treated group	χ2	df	P-value	OR (95% CI)
G alleleb	54	32	—	—	—	1.00 (Ref)	66	—	—	—	1.00 (Ref)
A alleleb	6	8	2.63	1	0.10NS	2.2 (0.71–7.07)	14	1.64	1	0.20NS	1.9 (0.68–5.30)

Genotype frequencies (GG, GA, AA) were compared using chi-squared tests (df = 2). Allele frequencies (G vs A) were compared using chi-squared tests (df = 1). Fisher's exact test was applied where expected cell counts were <5 (e.g., AA genotype in the treated group), confirming the reported P-values.

[a]Odds ratios represent the risk of RA associated with each genotype or allele compared with the reference category (GG genotype or G allele) in the control group. OR >1 indicates increased risk; OR <1 indicates decreased risk. [b]Allele frequencies are presented as proportions (range 0-1). Raw counts show the number of variant alleles/total chromosomes. CI, confidence interval; HWE, Hardy-Weinberg equilibrium; OR, odds ratio; NS, not significant.

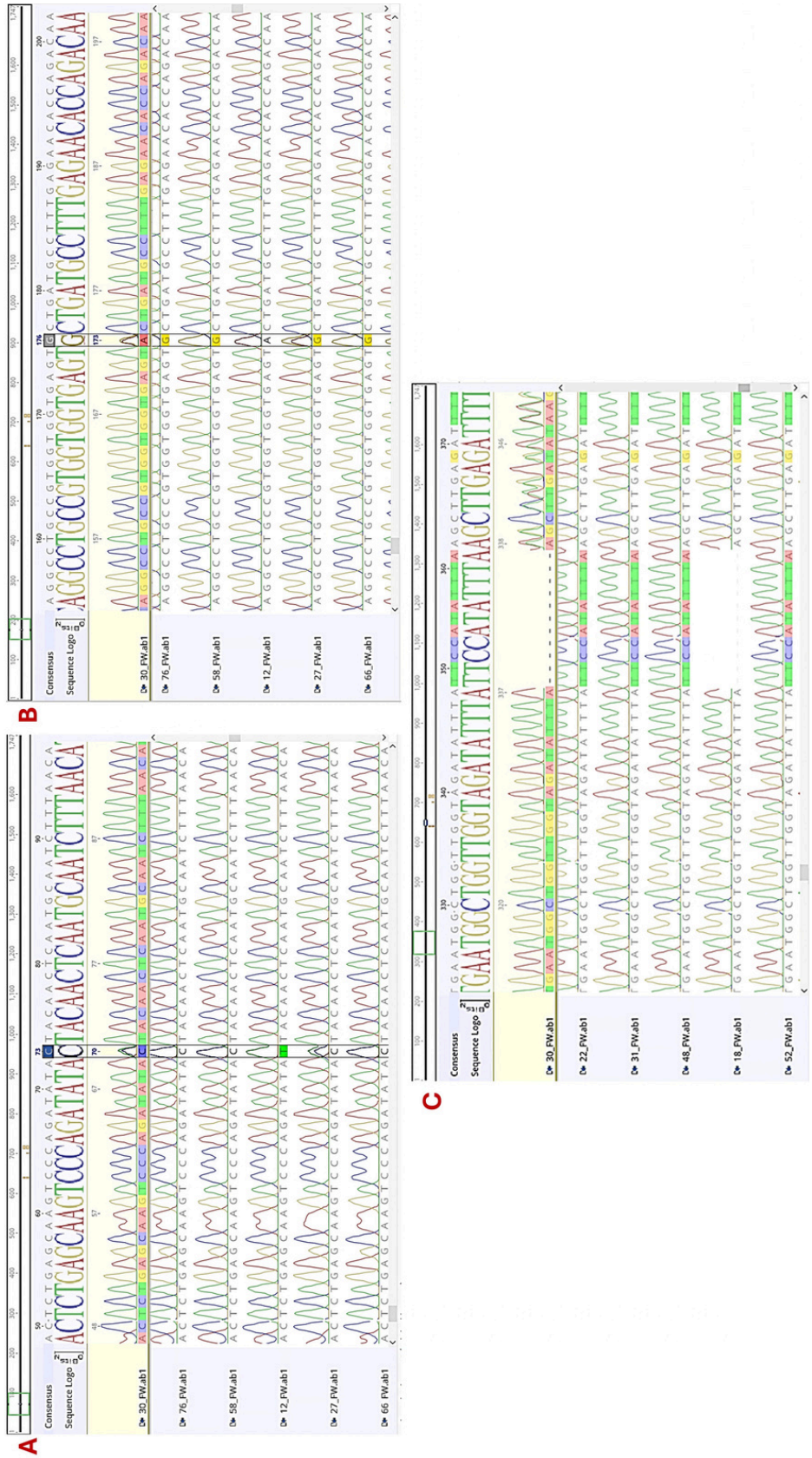


Figure 1. *Representative Sanger sequencing chromatograms of three CASP1 SNP regions from patients with rheumatoid arthritis and controls. (A) rs580253 (C>T): Homozygous CC shows a single blue peak, heterozygous CT shows overlapping blue and red peaks, and homozygous TT shows a single red peak. (B) (B) rs501192 (G>A): Homozygous GG and AA genotypes exhibit distinct single-peak patterns, while heterozygosity (GA) is indicated by overlapping black and green peaks. (C) rs56244680 (11-bp deletion, TTCCATATTTA): The deletion allele (Del) is distinguished from the normal allele (Norm) by a shift in peak alignment and a truncated consensus sequence. These panels validate the precise nucleotide alterations for the genotypes presented in Tables 4 and 5.*

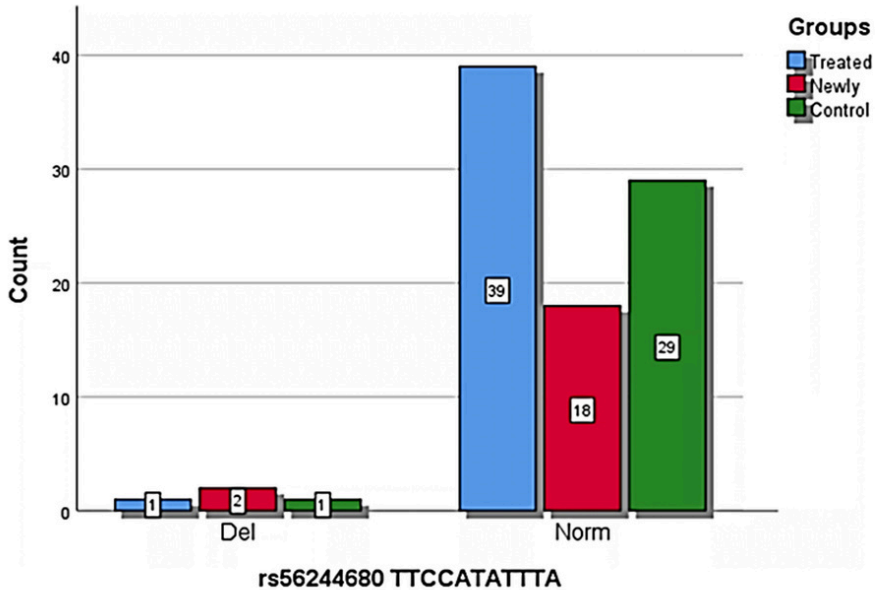


Figure 2. *Distribution of the CASP1 rs56244680 (TTCCATATTTA) variant across study groups. The count of individuals carrying the deletion (Del) and normal (Norm) variants is displayed for treated rheumatoid arthritis patients, newly diagnosed patients, and healthy controls. The Norm variant was predominant across all groups (39 treated, 18 newly diagnosed, 29 controls), while the Del variant was rare, occurring in 1 treated patient, 2 newly diagnosed patients, and 1 control. The figure shows the low frequency of the deletion variant and that its distribution is comparable and consistent across all study groups.*

The present study found no statistically significant associations between the investigated *CASP1* polymorphisms (rs580253, rs501192, and rs56244680) and RA susceptibility in the Iraqi cohort. Globally, genetic variation in *CASP1* has been explored across diverse populations, yielding inconsistent results. For instance, Addobbati et al. (23) reported no significant association for *CASP1* rs572687 and RA risk in a Brazilian cohort. This is in contrast to the findings of Liu et al. (22), who identified a significant correlation between *CASP1* rs2409062 and RA susceptibility in a Chinese population. These disparities between previous and the current study are likely attributable to population-specific genetic architecture.

The allele frequencies of the SNPs investigated in the Iraqi controls in this study were approximately 10.0% for both the rs580253 T allele and the rs501192 A allele, both of which were found in human subjects. When compared with patterns in Middle Eastern and Arab populations documented in public databases (for instance, the gnomAD Middle Eastern ancestry group) and limited regional research, these frequency rates appear to be compatible with such patterns (30).

In East Asian cohorts, however, specific *CASP1* polymorphisms show stronger effects on inflammasome-related features (22,31). These findings are in contrast to the current findings. There are a variety of potential mechanisms that may be responsible for differences that are specific to a population, including the following: i) when compared to East Asians, Iraqi and Middle Eastern populations may have lower or different minor allele frequencies. This may reduce the capacity to detect small effects or obscure the genetic background that determines risk. Additionally, allele frequencies may vary across different groups; ii) distinct linkage disequilibrium (LD) patterns: because the investigated SNPs are intronic, they have the potential to tag functional causal variants in certain populations but not in others. This occurs because LD blocks vary significantly among different ancestries. However, the LD structure in Arab populations may be different, potentially decoupling these variants from actually causal factors within *CASP1* or neighboring genes. For example, rs580253 and rs501192 exhibit moderate-to-high LD in Mexican cohorts, which have been analyzed in previous research on the inflammasome (17); iii) RA pathways exclusive to a particular ethnic group: the susceptibility and course of RA among Middle Eastern populations may entail distinct inflammatory or autoimmune pathways.

Genome-wide association studies in Arab populations have verified that European and Asian groups share contributions from HLA and documented non-HLA loci, while also suggesting the existence of population-specific modifiers or rare variations not captured here (32).

Activation of *CASP1* may play a less prominent role in the pathogenesis of RA in Iraqis compared with East Asians, or environmental and epigenetic factors may regulate the influence of this process. These factors highlight the importance of conducting genetic research on under-represented populations, such as Iraqis, to improve our understanding of RA variability. To determine whether these

CASP1 variations contribute modestly or whether other inflammasome-related genes increase risk in this demographic, larger replication studies involving many ethnic groups and including functional assays are required.

Beyond its function in apoptosis, *CASP1* serves as the principal inflammatory caspase mediating the generation of pro-inflammatory cytokines IL-1β and IL-18 through the activation of the inflammasome (20,33,34). Yang et al. (21) found that hyperactivated *CASP1* in neutrophils drives IL-18 and enhances RA activity, irrespective of NLRP3 inflammasome signaling, a pathway with direct relevance to RA etiology.

Preclinical evidence demonstrates that the *CASP1* inhibitor VX765 reduces the severity of collagen-induced arthritis in mouse models (35). Collectively, these findings position *CASP1* as a biologically plausible candidate for influencing RA susceptibility and warrant further investigation as a potential therapeutic target. This finding lends credence to the therapeutic possibility of targeting this axis.

Rheumatology practice is significantly impacted by the high prevalence of TMJ dysfunction among patients with RA: i) patients diagnosed with RA should undergo routine TMJ screening as part of the standard rheumatologic examination, utilizing established tools such as the Helkimo index. Early detection of TMJ involvement may allow for intervention before irreversible damage occurs; ii) treatment responses must be analyzed independently for TMJ symptoms, since improvements in peripheral joint counts or inflammatory markers may not correlate with TMJ outcomes; and iii) multidisciplinary management strategies are vital to provide comprehensive care.

These strategies should include rheumatologists, dentists, and maxillofacial experts. In addition to systemic immunomodulation, local therapies, such as intra-articular injections, physiotherapy, and occlusal appliances, must be considered; iv) it may be possible to facilitate earlier reporting and intervention by providing patients with information regarding TMJ symptoms, which include pain during mastication, joint clicking, and limited mouth opening; v) TMJ outcomes should be included as secondary endpoints in future therapeutic trials for RA to better understand how various medications influence this joint.

The present study has certain limitations, which should be mentioned. Its cross-sectional design allows for the identification of associations at a specific moment in time; however, it does not permit the demonstration of causality or monitoring of longitudinal changes in TMJ dysfunction among individual patients.

Due to the low minor allele frequencies of the investigated *CASP1* variants, the sample size was insufficient to perform valid sub-analyses correlating specific genotypes with TMJ severity phenotypes. We recommend this genotype-phenotype correlation as a specific objective for future multi-center studies with larger cohorts. Another limitation of our present study is the absence of baseline laboratory parameters, such as acute-phase reactants (CRP, ESR) and serological markers (RF, anti-CCP). While disease activity was rigorously captured using the acute-phase reactant-free CDAI, and all patients met standard classification criteria, the lack of baseline laboratory data limits the depth of immunological characterization of the cohort. Future studies should integrate these biomarkers to provide a more comprehensive clinical-serological profile.

Two primary findings emerged from this study. First, in our patient cohort, the investigated *CASP1* polymorphisms (rs580253, rs501192, and rs56244680) did not demonstrate a statistically significant association with rheumatoid arthritis in this Iraqi population. Consequently, while these specific variants did not appear to be major risk factors in this population, their potential role as minor modifiers requires further validation in larger cohorts. Second, the involvement of TMJ is relatively common in RA, affecting the vast majority of patients. Objective TMJ dysfunction did not differ significantly between treated and newly diagnosed patients, while subjective symptoms were more severe in the treated group, potentially reflecting a prolonged disease course and cumulative damage. This clinical dissociation underscores the necessity of routinely assessing the TMJ using both patient-reported outcomes and clinical examination, as well as the significance of adjuvant local therapies particularly targeting the TMJ. Taken together, these findings have two key implications for the management of RA: i) routine clinical assessment of TMJ function should be integrated into rheumatology practice; and ii) further large-scale multi-center studies are warranted to continue the search for genetic markers.

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Author Contributions

Conceptualization, W.L.A. and A.H.A.H; Methodology, B.H.A.G. and A.H.A.H.; Investigation, W.L.A. and A.H.A.H.; Formal Analysis, B.H.A.G. and A.H.A.H.; Writing – original draft, B.H.A.G. and A.H.A.H.; Writing – review & editing, W.L.A and A.H.A.H. All authors have read and approved the published version of the manuscript.

Statement of Ethics

The study protocol was approved by Baghdad Teaching Hospital Scientific and Ethical Unit (Approval No. 859, issued on February 19, 2023). All participants provided written informed consent prior to enrollment in accordance with the Declaration of Helsinki.

Statement of Competing Interest

The authors declare no relevant conflicts of interest.

Statement of Data Availability

All data analyzed during this study are included in the published article. Additional data are available from the corresponding author upon reasonable request.

Statement of Generative AI Use

No generative AI was used.

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