Association of the rs7574865 polymorphism in the signal transducer and activator of transcription 4 gene with rheumatoid arthritis in Moroccan patients

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Abstract

Objective

Rheumatoid arthritis (RA) is a complex autoimmune disease with a strong genetic component. The STAT4 rs7574865 (G>T) variant has been associated with RA in various populations, but data from North Africa remain limited. This study aimed to investigate the association of rs7574865 with RA susceptibility in Moroccan patients.

Methods

A case-control study was conducted including 87 Moroccan patients with severe RA and 69 healthy controls. Genotyping of rs7574865 was performed using TaqMan assays. Serum levels of anti-CCP_2 and IgM-RF were measured by ELISA. Associations between genotypes, alleles, demographic, clinical, and serological features were analysed.

Results

The GT genotype was more frequent in patients than in controls, and the T allele showed higher prevalence in the RA group. A significant genotypic association with RA risk was observed, while the TT genotype was absent in both groups. No associations were detected with demographic or general clinical characteristics. However, a significant clinical association was observed with anti-CCP_2 status. The GT genotype also showed a noticeable trend in RF-positive and anti-CCP_2-negative patients compared with controls, though differences between serological subgroups within patients were not statistically significant.

Conclusion

These findings provide the first evidence from Morocco that the STAT4 rs7574865 variant may contribute to RA susceptibility. While its effect appears moderate, the study highlights the importance of including North African populations in genetic studies of RA.

Key words

rheumatoid arthritis, *STAT4* gene, rs7574865 polymorphism, Moroccan population.

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Introduction

Autoimmune diseases are chronic inflammatory disorders caused by dysregulation of the immune system, among which rheumatoid arthritis (RA) is one of the most prevalent. RA is a chronic inflammatory rheumatic disease characterised by persistent synovitis, leading to joint pain, swelling, and progressive functional impairment, typically involving multiple joints. In addition to articular manifestations, RA may present with extra-articular involvement affecting various organs and systems, particularly in patients with severe disease, positive rheumatoid factor (RF) or anti-cyclic citrullinated peptide antibodies (anti-CCP_2) (1, 2).

Globally, RA affects approximately 17.6 million people, with an age-standardised prevalence rate of 208.8 per 100,000 population in 2020, showing a 14.1% increase since 1990. The burden of RA is particularly pronounced in the North Africa and Middle East region, where prevalence increased by 50.1% between 1990 and 2020, accompanied by a 27.8% rise in revealed age-standardised disability-adjusted life years (DALYs) over the same period (3, 4). RA incidence ranges from 20 to 50 new cases per 100,000 inhabitants per year and predominantly affects women, with a female-to-male ratio of approximately 4:1, most commonly diagnosed between 35 and 55 years of age (5). In Morocco, RA represents the most common inflammatory rheumatism, affecting an estimated 0.5–1% of the population, corresponding to 150,000 to 350,000 individuals (6, 7). Moreover, between 1990 and 2019, the age-standardised DALY rate attributable to RA increased by 32% in Morocco, highlighting its growing public health impact (3).

RA is a multigenic disease with a significant genetic component, with heritability estimated around 60%. *HLA* (human leukocyte antigen)-DRB1 alleles are particularly predominant, likely explaining about 30% of the genetic risk, as they are the major determinants of immune compatibility. However, other non-*HLA* genes, including Signal Transducer and Activator of Transcription 4 (*STAT4*), confer a lower RA risk (8). Recent reviews have also emphasised the role of non-HLA susceptibility genes, together with epigenetic and environmental factors, in the complex pathogenesis of RA (9). The *STAT4* gene, located on chromosome 2q32.2-q32.3, encodes a transcription factor that regulates immune cell responses to cytokines and influences the differentiation and proliferation of type 1 (Th1) and type 17 (Th17) T-helper cells, cytotoxicity, and interferon γ (IFNγ) production. *STAT4* is central in the signalling pathways of interleukin-12 (IL-12) and interleukin-23 (IL-23), affecting inflammatory responses and contributing to the pathogenesis of several autoimmune and inflammatory diseases (10).

Several studies have focused on *STAT4* gene polymorphisms, particularly SNP rs7574865, which has been investigated across Africa (11-14), Europe (15, 16), Asia (17, 18) , the Middle East (19-23), and North America (24). However, the effect of rs7574865 shows ethnic heterogeneity.

No studies have examined the association of *STAT4* rs7574865 with RA in North African Arab-Berber populations, including Morocco, leaving a gap in understanding its relevance in high-burden regions (3), where RA management is challenged by socio-economic constraints and delayed diagnosis (25). Given prior evidence linking this polymorphism to RA, this study evaluates its association with disease susceptibility, clinical severity, and serological status in Moroccan patients with severe RA.

Materials and methods

A primary bioinformatic analysis was conducted to investigate the functional impact of the *STAT4* rs7574865 polymorphism and SNPs in linkage disequilibrium (LD) with it. The study included 87 Moroccan RA patients and 69 population-matched controls. Genotyping of rs7574865 was performed using TaqMan assays, and anti-CCP_2 and IgM-RF levels were measured by enzyme-linked immunosorbent assay (ELISA).

Bioinformatics analyses

We explored public bioinformatics resources (FORGE2, RegulomeDB, etc.), which suggested that rs7574865 maps to regulatory chromatin states in

Table I. SNP *STAT4* analysed.

Gene	db SNP ID	Assay ID	SNP (NCBI build)	Location
STAT4	rs7574865	C__29882391_10	G/T	Chr.2 :191099907

Db: data base; Chr: chromosome; NCBI: National Center for Biotechnology Information

Table II. Primers and TaqMan® probes for real-time PCR of *STAT4* SNP rs7574865.

PCR primers and TaqMan probes	Sequences
Forward primer	5' TATGAAAAGTTGGTGACCAAAATGTTAATAGTGGTTATCTTATTTCAGTGG 3'
Reverse primer	3' ATACTTTTCAACCACTGGTTTTACAATTATCACCAATAGAATAAAGTCACC 5'
TaqMan® Probe VIC (Wild-type allele probe)	TATGAAAAGTTGGTGACCAAAATGT**G**AATAGTGGTTATCTTATTTCAGTGG
TaqMan® Probe FAM (Mutant allele probe)	TATGAAAAGTTGGTGACCAAAATGT**T**AATAGTGGTTATCTTATTTCAGTGG

Table III. Sociodemographic, clinical and treatment characteristics of RA patients.

Variable	RA patients (n=87)
Age (years)	57.25 ± 12.02
Gender (male/female)	15 (17.24) / 72 (82.76)
BMI (kg/m²)	27.12 ± 4.67 (n=85)
Disease duration (years)	12.93 ± 8.84 (n=86)
Consanguineous marriage	18 (20.69)
DAS28-CRP	3.52 ± 1.63 (n=71)
ESR (mm/h)	33.97 ± 27.28 (n=80)
CRP (mg/L)	20.33 ± 24.65 (n=78)
RF status ±	84 (96.55) / 3 (3.45)
Anti-CCP_2 status ±	70 (80.46) / 17 (19.54)
Treatment	n (%)
Corticosteroids	77 (88.51)
Methotrexate	82 (94.25)
Sulfasalazine	41 (47.13)
Leflunomide	21 (24.14)
Antimalarial drugs (chloroquine/hydroxychloroquine)	4 (4.6)
bDMARDs	58 (66.67)
Response to treatment	n (%)
Methotrexate	Remission: 0 (0) / Failure: 19 (100) (n=19)
bDMARDs	Remission: 1 (10) / Failure: 9 (90) (n=10)

Data presented as mean SD
BMI: Body Mass Index; DAS: Disease Activity Score; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; RF: rheumatoid factor; Anti-CCP_2: anti cyclic citrullinated peptide antibodies; bDMARDs: biologic disease-modifying anti-rheumatic drugs.

immune cells. LD between rs7574865 and surrounding SNPs was evaluated using the LD-Link tool (https://ldlink.nci.nih.gov/) with 1000 Genomes Project Phase 3 data for African and European populations. Proxy SNPs were identified with an $r^2 \geq 0.8$.

Study subjects

Eighty-seven RA patients aged ≥18 years were recruited from the rheumatology department of Mohammed V Military Hospital, Rabat, Morocco. Diagnosis was based on the criteria described by the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) for the 2010 RA classification (26) and patients with other inflammatory arthritis were excluded. Sixty-nine healthy blood donors were recruited from the Centre National de Transfusion Sanguine, Rabat, and excluded if they had autoimmune or inflammatory conditions. Patients and controls were unrelated and matched for ethnicity and socioeconomic status.

Sociodemographic and clinical data were collected. For patients, variables included sex, age, body mass index (BMI), consanguinity, disease duration, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) levels, Disease Activity Score (DAS28-CRP), anti-CCP_2 /RF status, treatment, and response to treatment (for some patients). For controls, sex, age, BMI, consanguinity, and anti-CCP_2 /RF status were recorded. Genetic analyses were performed for all participants.

Ethical considerations

This study was approved by local institutional review boards and the national ethics committee for biomedical research at Mohammed V University's Faculty of Medicine and Pharmacy in Rabat (reference 70/17; OHRP IORG0006594). All participants provided written informed consent.

Serological analysis

Serum anti-CCP_2 antibodies and IgM-RF were quantified in all subjects using ELISA. Anti-CCP_2 was considered positive at >5 IU/Ml (Anti-CCP II EIA Kit, Bio-Rad, Hercules, CA, USA) and RF at >20 IU/Ml (Anti-RF IgM ELISA, EUROIMMUN, Lübeck, Germany). Analyses were performed at the Autoimmunity Laboratory, National Institute of Health, Rabat, Morocco.

Molecular analysis: RT-PCR

- Extraction and quantification

Genomic DNA was isolated from 200 µL of EDTA-treated whole blood using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol, yielding 25 µL of DNA with an elution buffer per sample. DNA was stored at -20°C until analysis. Purity and concentration

Table IV. The distribution of *STAT4* rs7574865 polymorphism (G>T) genotypes and alleles in the studied groups.

Genotypes/Alleles		RA patients (n=87)	Controls (n=69)	RA patients *vs*. controls	
				χ^2 (*p*-value)	OR (95% CI)
Genotypes	GG	52 (59.77%)	56 (81.16%)	-	1 (referent)
	GT	35 (40.23%)	13 (18.84%)	8.26 (0.04*)	2,9 (0.24-0.38)
Alleles	G	139 (79.89%)	125 (90.58%)	–	1 (referent)
	T	35 (20.11%)	13 (9.42%)	6.76 (0.08)	2,4 (0.11-0.19)

RA: rheumatoid arthritis; OD: odds ratio; CI: confidence interval. *Significant at $p<0.05$.

Table V. Association analysis of *STAT4* rs7574865 G>T polymorphism with clinical and sociodemographic variables.

Variable	GG (n=52)	GT (n=35)	*p*-value
Age (years)	56.58 ± 12.23	58.26 ± 11.81	0.52
Gender (male/female)	9 (17.31) / 43 (82.69)	6 (17.14) / 29 (82.86)	0.96
BMI (kg/m^2)	27.26 ± 4.67 (n=51)	26.91 ± 4.72 (n=34)	0.73
Disease duration (years)	13.34 ± 8.9 (n=51)	12.34 ± 8.86	0.61
Consanguineous marriage	11 (21.15)	7 (20)	0.5
DAS28-CRP	3.31 ± 1.59 (n=45)	3.89 ± 1.66 (n=26)	0.11
ESR (mm/h)	34.97 ± 24.58 (n=48)	32.47 ± 31.24 (n=32)	0.69
CRP (mg/L)	16.30 ± 21.17 (n=46)	26.14 ± 28.29 (n=32)	0.08
RF status ±	50 (96.15) / 2 (3.85)	34 (97.14) / 1 (2.86)	0.17
Anti-CCP$_2$ status ±	44 (84.62) / 8 (15.38)	26 (74.29) / 9 (25.71)	0.03*

Data presented as mean SD.
BMI: Body Mass Index; DAS: Disease Activity Score; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; RF: rheumatoid factor; Anti-CCP$_2$: anti cyclic citrullinated peptide antibodies.
*Significant at $p<0.05$.

were assessed with a NanoDrop 2000 spectrophotometer (Thermo Scientific) by measuring absorbance at 260/280 nm. DNA concentrations ranged from 90–160 ng/μL with A260/A280 ratios of 1.8–2.0.

- *Genotyping*

STAT4 rs7574865 (G>T) located in intron 3 was genotyped using TaqMan allelic discrimination assays (Applied Biosystems, Foster City, CA). DNA containing the target SNP was amplified by real-time polymerase chain reaction (qPCR) with specific primers and TaqMan probes (Tables I and II). PCR was performed in 20 μL reactions with the following protocol: AmpErase® UNG activation at 50°C for 2 min, Hot-Start polymerase activation at 95°C for 10 min, 45 cycles of denaturation at 95°C for 15 s, hybridisation and elongation at 60°C for 1 min, and final hold at 8°C. Genotypes were automatically determined by allele-specific fluorescence on the QuantStudio 5 Real-time PCR system using QuantStudio Design & Analysis software.

Statistical analysis

Statistical analyses were performed using StatSoft (v. 12.0). Data are presented as mean ± standard deviation (SD). Categorical variables were compared using Pearson's χ^2 test. Risk factors were assessed using odds ratios (OR), and *p*-values <0.05 were considered statistically significant.

Results

This study aimed to investigate the association of *STAT4* rs7574865 polymorphism with RA susceptibility and serological status in a Moroccan population. A total of 87 RA patients and 69 healthy controls were genotyped.

The sociodemographic, clinical, and treatment characteristics of the RA patients are summarised in Table III.

Association of STAT4 (rs7574865) polymorphism genotypes and alleles with RA

The genotypic frequency of the GT polymorphism is relatively high in patients with RA (40.23%) compared to controls (18.84%). Similarly, the frequency of the T allele is higher in RA patients (20.11%) than in controls (9.42%) (Table IV).

The association analysis between RA and the rs7574865 (G>T) polymorphism of the *STAT4* gene showed a significant genotypic association (χ^2= 8.26; $p<0.05$) with an odds ratio (OR) of 2.9. In contrast, the allelic analysis indicated a strong correlation (χ^2=6.76; OR=2.4), although this result did not reach statistical significance ($p>0.05$). This lack of allelic significance could be explained by the total absence of the homozygous recessive TT genotype in our sample, with only the GG and GT genotypes being observed.

Association of STAT4 (rs7574865) polymorphism genotypes with clinical and sociodemographic characteristics in RA patients

Table V presents the clinical and sociodemographic characteristics of patients with RA, stratified according to rs7574865 genotypes (GG *vs*. GT). No significant association was observed between the polymorphism and age, sex, BMI, disease duration, consanguineous marriage, DAS28-CRP, ESR, CRP, or RF status. However, a significant association was observed with anti-CCP$_2$ status: patients with the GT genotype were more likely to be anti-CCP$_2$ negative than those with the GG genotype ($p<0.05$). This suggests that the GT variant of rs7574865 may be associated with a lower prevalence of anti-CCP$_2$ positivity in Moroccan patients with RA.

Association of STAT4 (rs7574865) polymorphism genotypes with RF and anti-CCP$_2$ status in RA patients

Table VI summarises the association

Table VI. Association of *STAT4* rs7574865 G>T polymorphism genotypes to RF and anti-CCP_2 status.

STAT4 rs7574865 (GT *vs*. GG)	RF (+) patients *vs*. RF (+) controls	RF (-) patients *vs*. RF (-) controls	RF (+) *vs*. RF (-) patients	ACCP (+) patients *vs*. ACCP (+) controls	ACCP (-) patients *vs*. ACCP (-) controls	ACCP (+) *vs*. ACCP (-) patients
OR	9.52	1.75	1.36	1.77	4.97	0.52
95% CI	0.26-0.45	0.12-1.34	0.30-0.51	0.25-0.48	0.16-0.35	0.30-0.51
p-value	0.0005*	0.78	0.85	0.67	0.01*	0.25

OD: odds ratio; CI: confidence interval; RF: rheumatoid factor; Anti-CCP_2: anti cyclic citrullinated peptide antibodies. *Significant at $p<0.05$.

Table VII. Comparison of rs7574865 genotype and allele frequencies between Moroccan RA patients and other North African populations.

Population	n (RA)	GG n (%)	GT n (%)	TT n (%)	G allele n (%)	T allele n (%)	*p*-value (genotype)	*p*-value (allele)
Morocco	87	52 (59.8)	35 (40.2)	0	139 (79.9)	35 (20.1)	–	–
Egypt	50	20 (40)	23 (46)	7 (14)	63 (63)	37 (37)	p=0.0006*	p=0.002*
Algeria	110	53 (48)	53 (48)	4 (4)	159 (72)	61 (28)	p=0.079	p=0.081
Tunisia	140	81 (57.8)	48 (34.3)	11 (7.9)	210 (75)	70 (25)	p=0.025*	p=0.23

RA: rheumatoid arthritis. *Significant at $p<0.05$.

between the *STAT4* rs7574865 (G>T) polymorphism and serological markers of RA (RF and anti-CCP_2). A significant association was observed between the GT genotype and RF-positive RA patients compared to RF-positive controls (OR = 9.52, 95% CI = 0.26–0.45, p=0.0005), suggesting that the GT genotype may contribute to RA susceptibility, particularly among individuals with positive RF. Regarding the anti-CCP_2 status, a significant association was also observed between GT and anti-CCP_2-negative patients compared with anti-CCP_2-negative controls (OR = 4.97, 95% CI = 0.16–0.35, p=0.01). This elevated odds ratio suggests that individuals carrying the GT genotype are more likely to exhibit anti-CCP_2 seronegativity. No other comparison revealed a significant association ($p>0.05$).

However, no significant difference was found when comparing RF-positive to RF-negative or anti-CCP_2-positive to anti-CCP_2-negative RA patients, suggesting that the *STAT4* rs7574865 polymorphism, contributes to RA susceptibility rather than determining the serological status. Thus, this variant may be involved in disease development, potentially favouring RF positivity and anti-CCP_2 negativity, without specifically defining the serological profile in the Moroccan population.

Comparison with North African populations

To evaluate whether the distribution of the rs7574865 polymorphism is specific to Moroccan RA patients, genotype and allele frequencies were compared with those reported in other North African populations, including Egyptian (11), Algerian (13), and Tunisian (14) cohorts (Table VII).

Significant differences were observed between Moroccan and Egyptian patients for both genotype and allele distributions (p=0.0006 and p=0.002, respectively). A significant difference in genotype distribution was also found with the Tunisian population (p=0.025), while no significant differences were observed with the Algerian cohort ($p>0.05$).

Discussion

Genotypic analysis revealed a higher frequency of the heterozygous GT genotype among RA patients compared with controls, whereas the homozygous TT genotype was absent in all subjects. Although the T allele was more prevalent in patients, its overall association with RA did not reach statistical significance (Table IV). Clinically, no significant differences were observed between GG and GT genotypes regarding age, sex, BMI, disease duration, consanguinity, or inflammatory markers. Initial analysis suggested that the GT genotype was associated with anti-CCP_2 negativity, indicating a lower prevalence of anti-CCP_2 positivity among GT carriers (Table V). Both RF positivity and anti-CCP_2 negativity were significantly associated with RA susceptibility compared with controls, and the initial trend of lower anti-CCP_2 positivity among GT carriers was confirmed by detailed analysis. However, no significant differences were observed between serological subgroups within patients, indicating that the *STAT4* rs7574865 polymorphism contributes to RA development without determining serological status (Table VI). Collectively, these findings indicate that the *STAT4* rs7574865 polymorphism contributes to RA risk in the Moroccan population, while potentially influencing serological profiles by favouring RF positivity and anti-CCP_2 negativity, thus reinforcing its role as a genetic determinant of disease risk and immunological phenotype. Most RA patients received methotrexate (MTX) (94.25%) or biologic disease-modifying anti-rheumatic drugs (bDMARDs) (66.67%), with reported treatment responses including inefficacy or intolerance for MTX and inefficacy, intolerance, or allergic reactions for bDMARDs. These data were available only for a subset of patients (Table III).

To contextualise our findings, we compared them with data from African and European populations. In Africa, the first investigation of the *STAT4* rs7574865 variant was conducted in Tunisia by Ben Hamad *et al*. (2011), who reported a significant association of both the T allele and the TT genotype with RA susceptibility, particularly among erosive, anti-CCP_2-negative and RF-negative patients. In our Moroccan cohort, rs7574865 was

also significantly associated with RA susceptibility and severity; since patients had an approximate disease duration of 13.34 years for GG and 12.34 years for GT during this study. However, unlike the Tunisian study, the GT genotype conferred the strongest risk, especially among anti-CCP_2-negative and RF-positive patients. Despite these differences in genotype distribution, neither study showed significant differences between seropositive and seronegative patient subgroups (14). Similar results were reported in Western Algeria, where Fodil *et al*. (2015) found a significant association of the T allele, with GT carriers being markedly more frequent among RA patients than controls. This observation is consistent with our findings, in which the GT genotype was also significantly associated with RA susceptibility (13). In Egypt, two independent studies confirmed this association. El-Saadany *et al*. (2016) reported a higher frequency of the GT genotype among RA patients, while TT carriers were associated with increased disease activity and anti-CCP_2 positivity. Likewise, El-Lebedy *et al*. (2017) showed that T-allele carriers (GT+TT) were significantly more frequent among RA patients and were associated with RF and anti-CCP_2 positivity (11, 12). In contrast, in our Moroccan cohort, the GT genotype was associated with RF-positive but anti-CCP_2-negative patients when compared with controls, with no significant differences observed between RA subgroups. These findings suggest that while the GT genotype represents a common risk factor for RA across North African populations, the association of the TT genotype with seropositivity and disease severity may be more population-specific. Notably, a recent Algerian study conducted on a nationwide cohort did not report any significant association, highlighting the heterogeneity of *STAT4* rs7574865 effects within North Africa (27). In addition, when comparing our findings with other North African populations, some differences in genotype and allele distributions were observed (Table VII). While Moroccan patients showed similar results to the Algerian (13) cohort, significant differences were found with Egyptian (11) and Tunisian (14) populations. This variability may be explained by differences in genetic background and population structure within North Africa. Overall, these findings further support that the effect of the *STAT4* rs7574865 polymorphism is not strictly specific to the Moroccan population but may be partially shared across North African populations, with some degree of regional variability. Evidence from European populations supports a consistent association between *STAT4* rs7574865 and RA susceptibility. In a large multicenter study including Spanish, Swedish, and Dutch cohorts, Orozco *et al*. (2008) reported a significant association of the T allele and GT genotype with RA, although the genotypic association did not reach significance in the Dutch population. In the Spanish cohort, this association was independent of RF and anti-CCP_2 status, in agreement with our Moroccan data (15). By contrast, a British inception cohort showed a weak but significant association limited to autoantibody-positive patients, with no effect on disease severity (28). An Italian study reported a borderline genotypic association and a significant allelic association, with stronger effects observed in anti-CCP_2-positive patients (29). In the Polish population, Budlewski *et al*. (2023) demonstrated that both GT and TT genotypes were associated with increased RA risk, whereas a subsequent study did not find any association with serological markers, including RF, anti-CCP_2, or anti-carbamylated protein (anti-CarP) antibodies (16, 30). Taken together, these findings confirm *STAT4* rs7574865 as a genetic determinant of RA susceptibility across diverse populations. In our study, this association was observed independently of RF and anti-CCP_2 status, reinforcing the relevance of this variant in North African populations and supporting its role in RA pathogenesis.

In East and South Asia, findings regarding *STAT4* rs7574865 have been inconsistent. In a large northern Chinese Han cohort, Zhao *et al*. (2013) reported a significant association with RA susceptibility, mainly driven by the GT heterozygous genotype, which is in agreement with our Moroccan data (17). By contrast, a recent study from Pakistan did not identify any significant association, suggesting population-specific genetic effects (18). In the Middle East, most studies have failed to demonstrate a clear association between rs7574865 and RA. Investigations conducted in Iran, Syria, and Saudi Arabia consistently reported no significant differences in allelic or genotypic distributions between patients and controls, nor robust associations with RF or anti-CCP_2 seropositivity (19-21). In contrast, two recent studies from Iraq revealed significant associations. Abdalhussein *et al*. (2025) demonstrated that the GT and TT genotypes, as well as the T allele, were significantly overrepresented among RA patients, while Dawood *et al*. (2025) reported an association under the recessive model, with the TT genotype being linked to both RF and anti-CCP_2 positivity (22, 23). Thus, among Middle Eastern populations, the Iraqi cohorts appear to be the most consistent with our Moroccan findings. In the Americas, studies conducted in Mexico have consistently supported the association of *STAT4* rs7574865 with RA susceptibility. In western Mexico, Durán-Avelar *et al*. (2016) reported significant associations of the GT and TT genotypes, as well as the T allele, with RA risk, independently of anti-CCP_2 status, in line with our results (31). More recently, Bravo-Villagra *et al*. (2024) identified regional differences within Mexico, with significant associations observed in western but not southern populations. In the western cohort, rs7574865 was associated not only with RA susceptibility but also with RF and anti-CCP_2 positivity, contrasting with our Moroccan data, where the association was independent of anti-CCP_2 status (24). These findings extend the role of *STAT4* rs7574865 in RA susceptibility beyond European and Asian populations and support its relevance in North African Arab-Berber populations.

The heterogeneity observed across populations may reflect both biological and methodological factors. Gene-environment interactions, including smoking, endemic infections, diet, and microbiome composition, are known to

modulate immune pathways and may contribute to population-specific effects of genetic variants (32). Methodological differences between studies, such as genotyping techniques (PCR-restriction fragment length polymorphism (PCR-RFLP), TaqMan, sequencing), sample size, statistical approaches, and consideration of serological status, may also partly explain inconsistencies in reported associations. Beyond these factors, population-specific genetic architecture is likely to play a major role. LD patterns around the *STAT4* locus differ across populations, with rs7574865 showing strong linkage with nearby variants in European populations but fewer proxy SNPs in African populations (see Supplementary Tables S1-S2). Consequently, rs7574865 may serve as a reliable marker of RA susceptibility in some populations but not in others, contributing to the variability of association results reported worldwide.

Strengths of this study include the first focused analysis of the *STAT4* rs7574865 polymorphism in a Moroccan cohort, combined with careful serological stratification and integration of population-level genetic data. Our findings indicate that the effect of this variant in North African populations is intermediate, being stronger than that reported in East Asia but weaker than in Northern Europe. This gradient supports the inclusion of North African populations in large-scale genetic association studies to reduce geographical bias.

The main limitation of this study is the moderate sample size, which may have limited the detection of rarer homozygous TT effects and could partly explain the absence of a global association with the T allele. In addition, gene-environment interactions were not assessed. These limitations underline the need for further investigations to strengthen and extend the present findings. Future studies should include larger and multicentric North African cohorts to confirm the observed associations. Functional analyses, including *STAT4* mRNA expression, phosphorylated *STAT4* levels, and cytokine profiling, would help clarify the biological impact of rs7574865 (33). The analysis of haplotypes and relevant environmental factors, such as smoking, infections, and diet, should also be considered.

In conclusion, this study supports the association between *STAT4* rs7574865 and RA susceptibility in the Moroccan population without influencing serological status (RF or anti-CCP positivity). Although further validation is required, these findings contribute to the growing evidence that genetic risk factors for RA may vary across ancestries. Importantly, our results underline the need to include North-African populations in RA genetic studies, which contribute to a deeper understanding of the condition and lead to improved patient care.

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