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**ORIGINAL ARTICLE**

## Polymorphism of the Endoplasmic Reticulum Aminopeptidase 1 Gene in Egyptian Patients with Rheumatoid Arthritis

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### ABSTRACT

**Background:** Rheumatoid arthritis (RA) is a systemic autoimmune disease caused by the interplay of hereditary and environmental factors. Extreme polymorphism is assumed for the Endoplasmic Reticulum Aminopeptidase 1 Gene Polymorphism (ERAP1) gene. ERAP1 single-nucleotide polymorphisms (SNPs) profoundly modify its immunological, clinical, and biochemical features. The study aims to ascertain how ERAP1 Gene single-nucleotide polymorphisms (SNPs) affect RA susceptibility, disease activity, and severity.

**Methods:** The study enrolled 98 subjects, 49 RA patients, and 49 healthy volunteers. Real-time PCR was used to genotype all subjects for the ERAP1 SNPs (rs27037, rs27044, and rs30187).

**Results:** With reference to rs27037, the T allele, the TT, and GT genotypes were linked to an increased risk of RA ( $P < 0.001$ ,  $P 0.006$ , and  $P 0.002$ , respectively). In relation to rs30187, RA risk was higher in those with TT, CT, and T alleles than in the healthy controls. The risk genotypes TT and GT of rs27037 ( $P 0.02$  and  $P 0.03$ , respectively) were linked to anticitrullinated antibody seropositivity. On the other hand, the risk genotypes TT and CT of rs30187 were associated with rheumatoid factor seropositivity ( $P < 0.001$  for both).

**Conclusions:** Our study showed that rs27037 and rs30187, two ERAP1 SNPs, increase the incidence of seropositive RA.

**Keywords:** Rheumatoid arthritis; ERAP1; Autoimmunity; Genetic predisposition.

### INTRODUCTION

Chronic systemic immune-derived disease, rheumatoid arthritis (RA), affects 0.5 to 1% of people worldwide. Female affection is two to three times more common than male affection, peaking in the third and fifth decades of life [1].

The present understanding of the etiology of RA is based on the idea that it is a complex condition where immunological tolerance is broken by the combination of several hereditary and environmental risk factors. Following the development of autoantibodies in the form of rheumatoid factors (RF) and/or anti-cyclic citrullinated peptide (Anti-CCP), the autoimmune process culminates in the appearance of arthritis [2]. The variables with the highest documented

correlations are female gender, a positive family history of RA, genetic risk factors, and tobacco use [3].

Over 150 loci have been linked to RA due to a recent genetic study, with HLA DRB1 having the greatest association [4]. However, only 30% of the genetic susceptibility to RA is explained by HLA linkage. Hence, a lot of research has been done on non-HLA genes [5].

One enzyme that is a member of the zinc metallopeptidases is called endoplasmic reticulum aminopeptidase-1 (ERAP1). The endoplasmic reticulum (ER) peptides are fragmented by ERAP1 to be loaded into the antigen-binding groove of HLA class I [6]. Tumor necrosis factor receptor 1,

IL1R2, and other proinflammatory cytokine receptors are cleaved by ERAP1, which lowers the transmission of inflammatory signals [7]. Furthermore, through inflammasome activation mediated by Nod-like receptor protein 3, ERAP1 variants contribute to the innate immune system's generation of inflammatory chemokines and cytokines [8]. Furthermore, via Toll-like receptor 4 signal transduction and natural killer cell generation, ERAP1 is involved in the upregulation of macrophage phagocytosis [9].

The ERAP1 gene is thought to be extremely polymorphic. Its immunological, clinical, and biochemical characteristics are significantly altered by ERAP1 polymorphisms. According to genome-wide association studies (GWAS), autoimmune and autoinflammatory disorders are associated with ERAP1 polymorphisms [10].

On the ERAP1 gene, five SNPs (rs27044, rs17482078, q22rs10050860, rs30187, rs2287987) were found to be associated with an increased risk of developing Ankylosing spondylitis (AS). It's interesting to note that the rs30187 SNP has been connected to a number of autoimmune disorders, including multiple sclerosis, psoriasis, diabetes, and Crohn's disease. Consequently, it has been discovered that rs10050860 and rs17482078 are connected to Behcet's illness [11].

Pharmaceutical research has focused on ERAP1's basic function in immune response modulation, hoping to modify its activity for the purpose of managing autoimmunity and cancer immunotherapy [12]. Even with the advancements in modern medicine, RA is still regarded as an unmet medical necessity. In order to create better treatment regimens and comprehend the pathophysiology of the disease, a thorough analysis of the genetic connections associated with RA would be advantageous [13].

## METHODS

The present investigation was carried out from November 2022 to August 2023 at Zagazig University Hospital. A total of 98 participants were included, divided between 49 RA patients who met the American College of Rheumatology's recommendations for RA diagnosis [14] and 49 healthy volunteers who were matched for age and sex. The study procedure was approved by the Zagazig University Faculty of Medicine's Ethics Committee (IRB Approval No. (9819). The study excluded patients with a history of cancer or other

autoimmune illnesses. Written informed consent has been granted by each participant.

Each patient had a comprehensive history taken, a general clinical examination, and a local assessment of the musculoskeletal system. The modified 28-joint Disease Activity Score (DAS 28) was used to assess the level of RA activity [15]. The Rheumatoid Arthritis Severity Scale (RASS) was used to determine the severity of RA [16]. The laboratory investigation involved measuring erythrocyte sedimentation rate (ESR) using a vision ESR Analyzer (YHLO, China) on sodium citrate anticoagulated whole blood sample. Serum anticitrullinated antibody (Anti-CCP) level by electrochemiluminescence immunoassay on a Cobas e411 (Roche Diagnostics, Germany), and both serum rheumatoid factor (RF) and serum C-reactive protein (CRP) levels by immunoturbidimetric assay using the Cobas 6000-c501 auto analyzer (Roche Diagnostics, Germany). The cutoff used for Anti-CCP positivity was  $\geq 17$  IU/ml, while for RF positivity, the cutoff was  $> 14$  IU/ml.

## Total DNA isolation and RT-PCR analysis for ERAP SNPS

For all patients and the control group, two milliliters of peripheral venous EDTA-anticoagulated blood were taken. The manufacturer's protocol was followed while extracting DNA using the QIAamp® DNA Mini Kit (QIAGEN GmbH, Hilden, Germany). The quality of DNA was assessed using agarose gel electrophoresis. ERAP1 single-nucleotide polymorphisms (rs30187, rs27044, and rs27037) were then identified using Real-Time - PCR (QuantoStudio 5, ThermoFisher, Singapore) in a 20  $\mu$ l final volume that contained 10  $\mu$ l of TaqMan Universal PCR Master Mix, 0.5  $\mu$ l of 20 $\times$  working stock of SNP Genotyping Assay, and 9.5  $\mu$ l of DNA. **Table S1** contains a list of the TaqMan probe sequences for ERAP1 SNPs.

The thermal amplification cycles were initiated by PCR activation step at 95°C for 15 min, then 40 denaturation cycles at 95 °C for 30 sec, annealing at 60°C for 30 sec, followed by extension at 72°C for 30 sec, and final extension at 72 °C for 5 min, then 95 °C for 15 sec for melting curve analysis.

## STATISTICAL ANALYSIS

SPSS version 28 (IBM Co., Armonk, NY, USA) was used for the statistical analysis. The unpaired student t-test was used to compare the two groups under study in quantitative parametric data that

were reported as mean and standard deviation (SD). The interquartile range (IQR) and median were used to display quantitative non-parametric data, which were then evaluated using the Mann-Whitney test for two-group comparisons and the Kruskal-Wallis test for more than two groups. A Chi-square test was employed for analysis after categorical data were presented using percentages and frequencies. To determine the degree of correlation between the exposure and the result, the odds ratio was computed. For comparisons, a 0.05 p-value cutoff was established for significant differences.

## RESULT

### *Demographics and laboratory results*

Forty-nine RA patients total, 46 (93.9%) female and 3 (6.1%) male were included in the current study. Furthermore, 49 gender- and age-matched healthy volunteers—42 females (85.7%) and seven men (14.3%)—were included. Age and sex differences were not found to be statistically significant among the groups under study. Table 1 provides an overview of all participants' demographic and clinical features.

### *Frequency of genotypes in studied groups*

Based on genotyping analysis, there was a statistically significant difference in the distribution of genotypes for rs27037 and rs30187 between RA cases and controls. When it came to rs27037, RA cases had greater frequencies of the risk genotypes TT (16.3%, OR 22.4, CI 2.48-202.31, P 0.006) and GT (63.3%, OR 4.34, CI 1.74-10.84, P 0.002) than the healthy controls (2% and 40.8%, respectively). An elevated disease risk of three times was linked to the risk T allele (OR 3.18, CI 1.72-5.91, P<0.001). Concerning rs30187, the findings showed that RA cases had significantly higher frequencies of risk TT (28.6%) and CT (69.4%) genotypes than the healthy controls (20.4% and 49%, respectively). This resulted in an increased disease risk association (OR 21, CI 2.37-185.94, P 0.006 and OR 21.25, CI 2.63-171.91, P 0.004, for TT and CT, respectively). Additionally, there was a two-fold increase in the

risk of disease attributed to the risk T allele (OR 2.11, CI 1.19-3.74, P 0.01). There was no discernible variation in the distribution of genotypes and alleles for rs27044 among the groups under investigation (**Table 2**) (**Figure 1**).

### *Analysis of genotypes in relation to clinical and laboratory data*

Among RA patients, the ERAP1 gene polymorphism (rs27037) was substantially correlated (P 0.005, P <0.001, respectively) with higher levels of CRP and RASS. Patients with the TT genotype had significantly greater CRP than those with the GG and GT genotypes (P 0.001, P 0.008, respectively). However, patients with TT genotypes had considerably higher RASS than those with GT genotypes (P<0.001) (**Table 3**). The ERAP1 gene polymorphism rs27044, however, did not significantly correlate with the severity or activity of RA. (**Table 4**). With reference to the rs30187 ERAP1 SNP, the risk genotypes TT showed a significant association with higher CRP in comparison to the CC and CT genotypes (P 0.006). (**Table 5**)

With regard to the relationship between ERAP1 SNPs and the serological profile of the patients, ERAP1 SNP (rs27037) and anti-CCP positivity were shown to be statistically significantly associated (P 0.026). Only 50% of individuals with the GG genotype tested positive for Anti-CCP, compared to a higher percentage among TT genotype patients (100%) and GT genotype patients (83.9%). Moreover, a significant association was found between the T allele of ERAP1 SNP (rs27037) and anti-CCP positivity (P 0.02). However, there was a significant correlation found between RF positivity (P<0.001) and the ERAP1 SNP (rs30187). The risk genotypes TT and CT were linked to greater incidences of positive cases of RF (100% & 94.1%). No correlation of significance was seen between the genotypes of ERAP1 rs27044 and positivity for serological markers (**Table 6**).

**Table 1:** Demographic and clinical characteristics of the studied groups

Variable	Cases (n=49)	Controls (49)	P
Age (years)	39.41 ± 10.77	38.86 ± 10.5	0.798 <sup>a</sup>
Sex			0.182 <sup>b</sup>
Male	3(6.1%)	7(14.3%)	
Female	46 (93.9%)	42 (85.7%)	
Duration (years)	6.24 ± 3.46		
MS (min)	38.78 ± 33.78		
NTJ	13 (0-28)		
NSJ	0 (0-12)		
Hb (g/dL)	12.22 ± 1.04		
PLT (x10 <sup>3</sup> cells/μl)	295.98 ± 76.81		
WBCs (x10 <sup>3</sup> cells/μl)	7.82 ± 2.82		
RF (IU/mL) (+ve in 46 cases)	114.29 ± 124.48		
Anti-CCP (U/mL) (+ve in 39 cases)	164.78 ± 134.25		
CRP (mg/L)	10.59 ± 9.28		
ESR (mm/hr)	35.06 ± 24.19		
DAS ESR	4.89 ± 1.39		
RASS	5.42 ± 1.59		
DAS Grade			
Remission	3(6.1%)		
Low disease activity	4(8.2%)		
Moderate disease activity	15(30.6%)		
High disease activity	27(55.1%)		
Family history	13(26.5%)		
Medication			
Biological	3(6.1%)		
DMARDs	6(12.2%)		
NSAIDs	1(2%)		
DMARDs + NSAIDs	33(67.3%)		
DMARDs + GCs	3 (6.1%)		
DMARDs + NSAIDs +GCs	3 (6.1%)		

Data were presented as mean ± SD, median, and (range) or No. (%). At-Test. b Chi-square test.

**Table 2:** Distribution of ERAP1 gene SNPs genotypes and alleles among the studied groups

Variable	Cases (n=49)	Control (n=49)	X <sup>2</sup>	P	OR (95%CI)	
rs27037	GG <sup>®</sup>	10(20.4%)	28 (57.1%)		---	Ref
	TT	8 (16.3%)	1(2%)	4.58	0.006*	22.4 (2.48 to 202.31)
	GT	31 (63.3%)	20 (40.8%)	5.22	0.002*	4.34 (1.74 to 10.84)
	Allele					
	G <sup>®</sup>	51 (52%)	76 (77.6%)	5.67	<0.001*	3.18 (1.72 to 5.91)
T	47 (48%)	22 (22.4%)				
rs27044	CC <sup>®</sup>	25 (51%)	24(49%)		---	Ref

	GG	7 (14.3%)	1(2%)	1.73	0.085	6.72 (0.77 to 58.79)
	CG	17 (34.7%)	24(49%)	1.48	0.366	0.68 (0.3 to 1.57)
	<b>Allele</b>					
	C®	67(68.4%)	72 (73.5%)	0.45	0.432	1.28 (0.69 to 2.38)
	G	31(31.6%)	26 (26.5%)			
<b>rs30187</b>	CC®	1(2%)	15(30.6%)		---	
	TT	14 (28.6%)	10 (20.4%)	4.85	0.006*	21 (2.37 to 185.94)
	CT	34 (69.4%)	24 (49%)	5.05	0.004*	21.25 (2.63 to 171.91)
	<b>Allele</b>					
	C®	36(36.7%)	54 (55.1%)	4.44	0.01*	2.11 (1.19 to 3.74)
	T	62(63.3%)	44 (44.9%)			

X<sup>2</sup> chi-square test, OR odds ratio, CI confidence interval, ® reference group, P value for comparing between the studied groups.\*Statistically significant at p <0.05.

**Table (3): Relation between ERAP1 rs27037 and RA activity and severity**

Variable	rs27037						
	GG (n=10)	TT (n=8)	GT (n=31)	P	G (n=51)	T (n=47)	P
RF (IU/mL)	60 26.1-406.3	94.8 54.9-169.5	55.7 26.3-116.7	0.477	57.8 28.8-146	63 37.8-119.9	0.948
Anti-CCP (U/mL)	130 13.6-203.3	192 106.8-265.3	102 40.5-311.5	0.355	108 35-213	124 81.5-301.5	0.437
CRP (mg/L)	3 1.67-9.4	23 20-26	5.47 2.4-12.49	0.005*	5.47 2.1-12.3	10 2.4-23	0.134
	P1 0.001* P2 0.008* P3 0.186						
DAS ESR	5.2 4.5-5.7	5.2 4.8-5.9	4.6 3.2-6.2	0.539	5.2 4.8-5.9	5.1 3.6-6.1	0.954
RASS	6.3 6-7.9	6.8 6.1-7.8	5 3.6-6	<0.001*	5 4-6.3	5 4-6.3	0.977
	P1=0.523 P2<0.001* P3=0.003*						

KW Kruskal Wallis test, U Mann–Whitney test, p p-value for comparing between different categories, p1 p-value for comparing between GG and TT, p2 p-value for comparing between TT and GT, p3 p-value for comparing between GG and GT.

\*Statistically significant at p < 0.05.

**Table 4:** Relation between ERAP1 rs27044 and RA activity and severity

Variable	rs27044						
	CC (n=25)	GG (n=7)	CG (n=17)	P	C (n=67)	G (n=31)	P
RF (IU/mL)	97.25 38.25-230	90.1 43-103.1	54 24.75-89.25	0.188	60 30-188	60 30-98.38	0.406
Anti-CCP (U/mL)	165 87-248.2	124 101-289.2	35 13.8-350	0.581	130 35-260	102 16-300	0.912
CRP (mg/L)	3.2 1.95-15.8	20 2.2-26	8 3.8-12.12	0.239	5.89 2.33-13.59	8.5 3.7-23	0.302
DAS ESR	5.2 3.9-5.6	5.2 4.3-6.1	4.7 3.7-6.6	0.988	5.2 3.9-5.8	4.9 4.4-6.1	0.942
RASS	6 3.8-6.6	6.1 6-7.3	5 4-6.3	0.28	5 4-6.3	5 4-6.3	0.544

KW Kruskal Wallis test, U Mann–Whitney test p p value for comparing between different categories.

\*Statistically significant at p <0.05.

**Table 5:** Relation between ERAP1 rs30187 and RA activity and severity

Variable	rs30187						
	CC (n=1)	TT (n=14)	CT (n=34)	P	C (n=36)	T (n=62)	P
RF (IU/mL)	7 ---	63 39.7-103.1	60 24.25-214.2	0.82	61.5 30-146	60 24.2-214.2	0.927
Anti-CCP (U/mL)	5 ---	116 37.25-260	130 46-300	0.615	124 46-260	130 46-300	0.615
CRP (mg/L)	1.5 ----	20 5.47-26	3.8 2.02-12.77	0.006*	8 2.4-20	3.6 1.79-12.49	0.192
DAS ESR	3.2 ----	4.9 4.53-5.2	5.2 3.9-6.4	0.363	5.2 4-5.98	5.2 3.9-6.4	0.839
RASS	5 ----	6.1 4-7.3	5.5 4-7.3	0.344	6 4.08-6.53	5 4.3-6.3	0.665

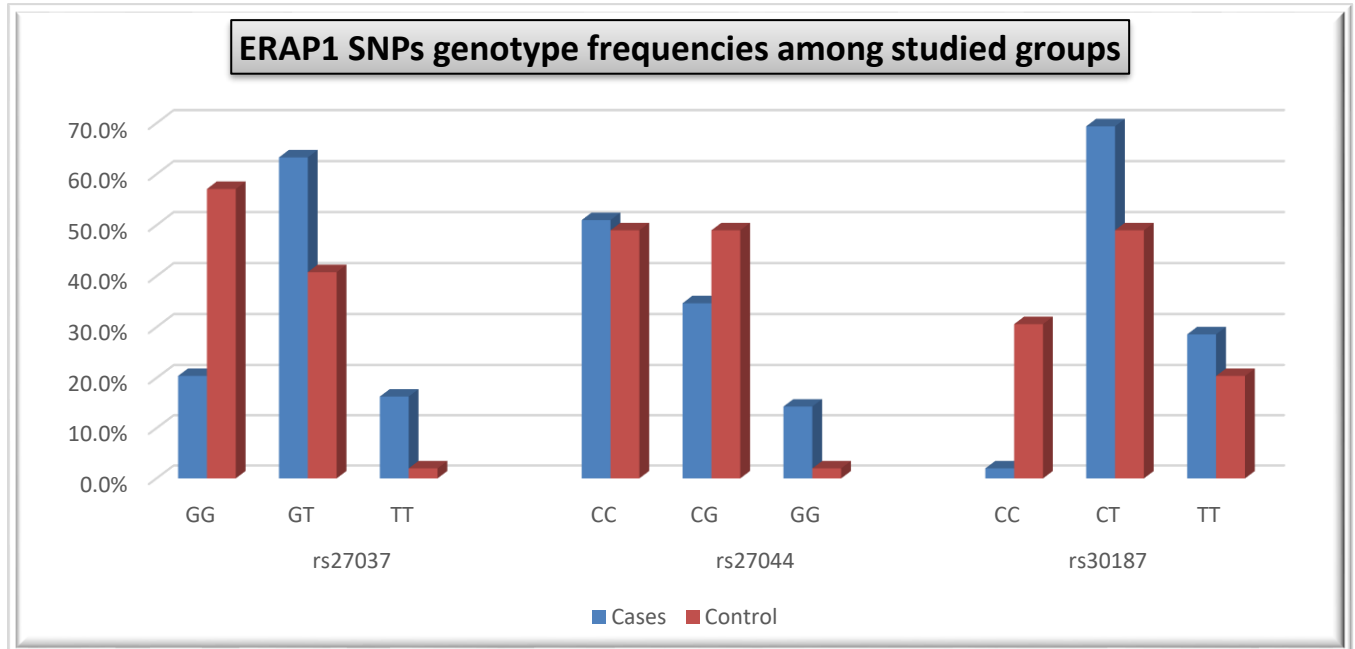
KW Kruskal Wallis test, U Mann–Whitney test, \*Statistically significant at p <0.05

**Table 6:** Association between ERAP1 SNPs and RF & Anti-CCP positivity

	RF		X <sup>2</sup>	P	Anti-CCP		X <sup>2</sup>	P	
	Negative	Positive			Negative	Positive			
<b>rs27037</b>			0.395	0.621	0(0%)	8 (100%)	14.4	0.026*	
TT (n=8)	0 (0%)	8 (100%)							
GG (n=10)	0 (0%)	10 (100%)							
GT (n=31)	3 (9.7%)	28 (90.2%)			5 (16.1%)	26 (83.9%)			
					P1= 0.02* P2=0.22, P3=0.031*				
<b>Allele</b>			0.03	>0.99	5(10.6%)	42 (89.4%)	5.31	0.02*	
T (n=47)	3 (6.4%)	44 (93.6%)							
G (n=51)	3 (5.9%)	48 (94.1%)			15 (29.4%)	36 (70.6%)			
<b>rs27044</b>			4.06	0.604			2.16	0.875	
CC (n=25)	1 (4%)	24 (96%)				5 (20%)			20 (80.4%)
GG (n=7)	1 (14.3%)	6 (85.7%)				1 (14.3%)			6 (85.7%)
CG (n=17)	1 (5.9%)	16 (94.1%)			4 (23.5%)	13 (76.5%)			
<b>Allele</b>			4.62	0.377			0.58	0.86	
C (n=67)	3 (4.5%)	64 (95.5%)			14 (20.9%)	53 (79.1%)			
G (n=31)	3 (9.7%)	28 (90.3%)			6 (19.4%)	25 (80.6%)			
<b>rs30187</b>			25.71	<0.001*			7.01	0.121	
TT (n=14)	0 (0%)	14 (100%)				2 (14.3%)			12 (85.7%)
CC (n=1)	1 (100%)	0 (0%)				1 (100%)			0 (0%)
CT (n=34)	2 (5.9%)	32 (94.1%)			7 (20.6%)	27 (79.4%)			
	P1<0.001*, P2=0.354, P3<0.001*								
<b>Allele</b>			5.06	0.188			2.05	0.39	
T (n=62)	2 (3.2%)	60 (96.8%)			11 (17.7%)	51 (82.3%)			
C (n=36)	4 (11.1%)	32 (88.9%)			9 (25%)	27 (75%)			

X<sup>2</sup> chi-square test, p p-value for comparing between the studied groups, p1 p-value for comparing between genotype 1 and genotype 2, p2 p-value for comparing between genotype 1 and genotype 3, p3 p-value for comparing between genotype 2 and genotype 3.

\*Statistically significant at p <0.05.



**Figure 1:** ERAP1 SNPs genotype frequencies among studied groups

**DISCUSSION**

One well-known systemic inflammatory rheumatic illness is rheumatoid arthritis (RA). Although a clear etiology is still unknown, the current consensus is that a number of factors contribute to the pathophysiology of the disease. The total risk of RA susceptibility, clinical presentation, and severity are all influenced by the interaction between host and environmental variables [3]. Genetics is the main risk factor for RA [4].

ERAP1 single nucleotide polymorphisms have been linked to viral, autoimmune, and cancerous conditions. Changes in enzyme activity are triggered by ERAP 1 SNPs, which affects how the antigen is presented [17]. As far as we can tell, this is the first study to look at the connection between the Egyptian population's RA susceptibility and severity and the polymorphism of the ERAP1 gene.

According to our findings, there was a higher risk of developing RA in those with the ERAP1 SNP (rs27037) risk genotypes TT and GT (OR 22.4, CI 2.48-202.31; OR 4.34, CI 1.74-10.84, respectively). Additionally, the T allele was regarded as a risk allele for RA susceptibility. To the best of our knowledge, no other research has assessed the relationship between RA risk and the ERAP1 SNP rs27037.

However, a number of studies have discovered a strong correlation between the ERAP1 SNP rs27037

and vulnerability to Ankylosing spondylitis (AS), shedding light on its role in the development of autoimmunity [18,19]. Research on populations in China and Turkey was unable to establish a link between the ERAP1 rs27037 polymorphism and the risk of AS [20, 21].

The current study did not find a significant correlation between RA susceptibility and the missense SNP rs27044 in ERAP1. The data collected by Akbulut and his colleagues, in contrast to our findings, showed that in the Turkish population, the rs27044 polymorphism was linked to an elevated risk of RA (p 0.037, OR 1.583, 95% CI 1.028–2.440). We propose that the ethnic difference between the two populations may be the cause of the disparity between our results and the findings of Akbulut et al. [22].

Additionally, a number of investigations were carried out to evaluate the relationship between psoriasis, Bechet's syndrome, and AS and ERAP1 SNP rs27044. But the findings were wildly inconsistent. [20, 23-29].

In the current investigation, we discovered that RA cases had considerably greater frequencies of the TT (28.6%) and CT (69.4%) genotypes in relation to the SNP rs30187 compared to the healthy controls (20.4% and 49%, respectively). With a frequency of 2% in RA patients compared to 30.6% in healthy controls, the CC genotype was thought to



be a protective genotype. Additionally, the T allele was linked to a 2.11-fold increase in risk of RA (CI 1.19-3.74). Our results aligned with the findings of Akbulut et al., who found that the rs30187 polymorphism was associated with an increased risk of developing RA illness (P 0.006, OR 1.849, 95% CI 1.191–2.870) [22].

The closure of domain 4, which consists of alpha helices on the active site, enhances ERAP1 catalytic activity [30]. Increased protonation capacity results from the change from an open to a closed shape following the mutation in the Lys528 site [31]. Thus, it clarifies the notable modification in peptide processing characteristics associated with the breakdown of interdomain connections linked to the ERAP1 Lys528Arg (rs30187) polymorphism [22].

Regarding the relationship between rs30187 and various autoimmune diseases, a number of studies have demonstrated a connection between rs30187 and psoriasis, ankylosing spondylitis, type 1 diabetes, multiple sclerosis, and inflammatory bowel disease [17, 18, 22, 29, 32-34].

According to our findings, the TT and CT risk genotypes of the ERAP1 SNP rs30187 were substantially correlated with RF positivity. However, there was no correlation discovered between RF positivity and the ERAP1 SNPs rs27037 and rs27044.

Normally generated during secondary immune responses, rheumatoid factors are naturally occurring autoantibodies. Natural IgM antibodies with low affinity and polyspecificity are secreted by B1 cells, a subset of B lymphocytes, on their own. Though RA patients have higher B1 cell counts than healthy individuals, there is evidence that B1 cells contribute to the generation of RF since a positive association has been documented between the frequency of peripheral blood B1 cells and RF serum level in RA. [35].

In mice deficient in ERAP1, O'Connell and associates observed a marked decrease in the frequency of B1 cell subsets. Furthermore, they mentioned that apparent protein antigen processing could be caused by ERAP1 variations that aren't functioning properly. This could help to clarify how these B cell dysregulated pathways and autoimmunity are related [36].

In this investigation, the ERAP1 SNP rs27037 risk TT and GT genotypes were linked to anti-CCP positivity. Furthermore, a noteworthy correlation was discovered between anti-CCP positivity and the

risk T allele of ERAP1 SNP rs27037. Anti-CCP positivity and both the rs27044 and rs30187 ERAP1 SNPs, however, did not appear to be related.

Normal citrullination takes place in dying cells. Nevertheless, the immune system can override the immunological tolerance and cause an autoimmune illness if it perceives the citrullinated peptide as foreign. [37].

Following their passage to the endoplasmic reticulum, protein fragments are trimmed and loaded onto MHC class I molecules by the catalytic activity of ERAP1 and ERAP2, after which they are transported to the cell surface for antigen presentation [38]. SNPs in the ERAP1 gene have been shown to impact its function, which results in aberrant peptide trimming [39].

Paldino and Fierabracci addressed the theory that autoantigens are inappropriately trimmed into altered peptides and presented to the CD8 + T cells during the early stages of the autoimmune disease. Cell lysis is the outcome of activating cytotoxic T cells. The first generation of autoantibodies occurs when antigen-presenting cells are drawn to the site of injury, where they grab the altered autoantigenic components and transport them to the draining lymph nodes, where T and B lymphocytes will encounter them [40]. These findings may clarify how the ERAP1 gene SNPs contribute to the development of RA and how they affect the patient's serological profile.

## CONCLUSION

A higher vulnerability to RA was linked to risk genotypes TT and GT of ERAP1 SNP rs27037 and risk genotypes TT and CT of ERAP1 SNP rs230187. Moreover, the risk genotypes of ERAP1 SNPs rs27037 and rs30187 were linked to an increased likelihood of the seropositive RA phenotype. However, rs27044 showed no discernible impact on the vulnerability to RA.

**Limitations of the study:** One potential weakness of our study could be its relatively small sample size. Consequently, in order to elucidate the relationship between ERAP1 SNPs and RA susceptibility and disease progression, we advise conducting additional research on the examined SNPs.

**Conflict of interest:** None

**Financial Disclosure:** None

## REFERENCES

1. Black, R.J., Cross M, Haile LM, Culbreth GT, Steinmetz JD, Hagins H, et al., Global, regional,

- and national burden of rheumatoid arthritis, 1990–2020, and projections to 2050: a systematic analysis of the global burden of disease study 2021. *Lancet Rheumatol*, 2023. 5(10): p. e594-e610.
2. Petrovská, N., Prajzlerová K, Vencovský J, Šenolt L, Filková M. The pre-clinical phase of rheumatoid arthritis: from risk factors to prevention of arthritis. *Autoimmun Rev*, 2021. 20(5): p. 102797.
  3. Romão, V.C. and J.E. Fonseca, Etiology and risk factors for rheumatoid arthritis: a state-of-the-art review. *Front Med*, 2021. 8: p. 689698.
  4. Padyukov, L. Editor. Genetics of rheumatoid arthritis. *Semin Immunopathol*. 2022. (44). P. 47-62.
  5. Dedmon, L.E., The genetics of rheumatoid arthritis. *Rheumatology (Oxford)*, 2020. 59(10): p. 2661-2670.
  6. Giastas, P., M. Neu, P. Rowland, and E. Stratikos, High-resolution crystal structure of endoplasmic reticulum aminopeptidase 1 with bound phosphinic transition-state analogue inhibitor. *ACS Med Chem Lett*, 2019. 10(5): p. 708-713.
  7. Sharip, A. and J. Kunz, Understanding the Pathogenesis of Spondyloarthritis. *Biomolecules*. 2020; 10 (10).
  8. Blake, M.K., O'Connell P, Pepelyayeva Y, Godbehere S, Aldhamen YA, Amalfitano A, ERAP1 is a critical regulator of inflammasome-mediated proinflammatory and ER stress responses. *BMC Immunol*, 2022. 23(1): p. 1-14.
  9. Reeves, E., Y. Islam, and E. James, ERAP1: A potential therapeutic target for a myriad of diseases. *Expert Opin Ther Targets*, 2020. 24(6): p. 535-544.
  10. Liu, S., Lu J, Wu J, Feng D, Wang Y, Su X, Cao H, Structural and biochemical insights into the association between ERAP1 polymorphism and autoimmune diseases. *Biochem Biophys Res Commun*, 2022. 632: p. 189-194.
  11. Pepelyayeva, Y. and A. Amalfitano, The role of ERAP1 in autoinflammation and autoimmunity. *Hum Immunol*, 2019. 80(5): p. 302-309.
  12. Giastas, P., Mpakali A, Papakyriakou A, Lelis A, Kokkala P, Neu M, et al., Mechanism for antigenic peptide selection by endoplasmic reticulum aminopeptidase 1. *Proc Natl Acad Sci U S A*, 2019. 116(52): p. 26709-26716.
  13. Ainsworth, R.I., Hammaker D, Nygaard G, Ansalone C, Machado C, Zhang K, et al., Systems-biology analysis of rheumatoid arthritis fibroblast-like synoviocytes implicates cell line-specific transcription factor function. *Nat Commun*, 2022. 13(1): p. 6221.
  14. Aletaha, D., Neogi T, Silman AJ, Funovits J, Felson DT, Bingham III CO, et al., 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/ European League Against Rheumatism collaborative initiative. *Arthritis Rheum*, 2010. 62(9): p. 2569-2581.
  15. Prevoo, M., Van'T Hof MA, Kuper H, Van Leeuwen M, Van De Putte L, Van Riel P, Modified disease activity scores that include twenty-eight-joint counts development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. *Arthritis Rheum*, 1995. 38(1): p. 44-48.
  16. Bardwell, W., Nicassio P, Weisman M, Gevitz R, Bazzo D, Rheumatoid Arthritis Severity Scale: a brief, physician-completed scale not confounded by patient self-report of psychological functioning. *Rheumatology (Oxford)*, 2002. 41(1): p. 38-45.
  17. Babaie, F., Hosseinzadeh R, Ebrazeh M, Seyfizadeh N, Aslani S, Salimi S, et al., The roles of ERAP1 and ERAP2 in autoimmunity and cancer immunity: New insights and perspective. *Mol Immunol*, 2020. 121: p. 7-19.
  18. Jiang, Y., Y. Ren, D. Zhou, and Y. Xu, Associations between ERAP1 polymorphisms and susceptibility to ankylosing spondylitis: a meta-analysis of East Asian Population. *Medicine (Baltimore)*, 2018. 97(47).
  19. Wang, X., Ma J, Ma J, Wen Y, Meng L, Yang H, et al., Bioinformatics analysis of genetic variants of endoplasmic reticulum aminopeptidase 1 in ankylosing spondylitis. *Mol Med Rep*, 2017. 16(5): p. 6532-6543.
  20. Tang, Y., Yang P, Wang F, Xu H, Zong S-Y, Association of polymorphisms in ERAP1 and risk of ankylosing spondylitis in a Chinese population. *Gene*, 2018. 646: p. 8-11.
  21. Akbulut, E., Erdemir A., Özgen M, Baskin Y, Güleç F, Turgut Balik D. Genotyping and analysis of rs27037 polymorphism for ankylosing spondylitis. *Sigma J Eng Nat Sci*, 2015. 6(1): p. 117-25.
  22. Akbulut, E., T. Yıldırım, and O. Öztürk, Endoplasmic reticulum aminopeptidase-1 polymorphism increases the risk of rheumatoid arthritis. *Turkish J. Biochem.*, 2022. 47(4): p. 465-473.
  23. Wang, J.-X., X. Zhao, and S.-Q. Xu, Screening Key lncRNAs of Ankylosing Spondylitis Using

- Bioinformatics Analysis. *J Inflamm Res*, 2022: p. 6087-6096.
24. Fernández-Torres, J., Zamudio-Cuevas Y, Montañó-Armendariz N, Luján-Juárez IA, Sánchez-Sánchez R, Martínez-Flores K. HLA-B27 may modulate the interaction between ERAP1 polymorphisms and smoking in ankylosing spondylitis patients. *Mol Biol Rep*, 2022. 49(7): p. 6423-6431.
  25. Bai, Y., Zhao N, Sun H, Yin L, Chen J, Hu N, Associations between ERAP1 polymorphisms and ankylosing spondylitis susceptibility in HLA-B27 positive population: a Meta-analysis and bioinformatics analysis. *Nucleosides, Nucleotides & Nucleic Acids*, 2022. 41(4): p. 407-418.
  26. Padula, M.C., Leccese P, Pellizzieri E, Padula AA, Gilio M, Carbone T, et al., distribution of rs17482078 and rs27044 ERAP1 polymorphisms in a group of Italian Behçet's syndrome patients: a preliminary case-control study. *Intern Emerg Med*, 2019. 14: pp. 713-718.
  27. Gao, W., Jin X, Zhou P, Zhu H, Xie K, Jin B, Du L. The correlation of endoplasmic reticulum aminopeptidase 1 polymorphisms with Behçet's disease: a meta-analysis. *Clin Exp Rheumatol*, 2023. 41: p. 2027-2038.
  28. Das, A., Chandra A, Chakraborty J, Chattopadhyay A, Senapati S, Chatterjee G, Chatterjee R. Associations of ERAP1 coding variants and domain specific interaction with HLA-C\* 06 in the early onset psoriasis patients of India. *Hum immunol*, 2017. 78(11-12): p. 724-730.
  29. Wiśniewski, A., Matusiak Ł, Szczerkowska-Dobosz A, Nowak I, Łuszczek W, Kuśnierczyk P The association of ERAP1 and ERAP2 single nucleotide polymorphisms and their haplotypes with psoriasis vulgaris is dependent on the presence or absence of the HLA-C\* 06: 02 allele and age at disease onset. *Hum immunol*, 2018. 79(2): p. 109-116.
  30. Liddle, J., Hutchinson JP, Kitchen S, Rowland P, Neu M, Cecconie T, et al., Targeting the regulatory site of ER aminopeptidase 1 leads to discovering a natural product modulator of antigen presentation. *J Med Chem*, 2020. 63(6): p. 3348-3358.
  31. de Castro, J.A.L., Ivarez-Navarro C, Brito A, Guasp P, Martin-Esteban A, Sanz-Bravo A. Molecular and pathogenic effects of endoplasmic reticulum aminopeptidases ERAP1 and ERAP2 in MHC-I-associated inflammatory disorders: Towards a unifying view. *Mol immunol*, 2016. 77: p. 193-204.
  32. Wu, X. and Z. Zhao, Associations between ERAP1 gene polymorphisms and psoriasis susceptibility: a meta-analysis of case-control studies. *Biomed Res Int.*, 2021: p.1-9.
  33. Giancchetti, E., Crinò A, Palma A, Luciano R, Perri V, Fruci D, et al., Case-control analysis of the ERAP1 polymorphism rs30187 in Italian type 1 diabetes mellitus patients. *Health*, 2013 ; 2013 (5), p. 2150-2155.
  34. Guerini, F.R., Cagliari R, Forni D, Agliardi C, Caputo D, Cassinotti A, et al., A functional variant in ERAP1 predisposes to multiple sclerosis. *PLoS One*, 2012. 7(1): p. e29931.
  35. Song, YW, and E.H. Kang, The pathogenic role of rheumatoid factor in rheumatoid arthritis. *Int. J. Clin. Rheumatol*, 2010. 5(6): p. 651.
  36. O'Connell, P., Blake MK, Godbehere S, Aldhamen YA, Amalfitano A. Absence of ERAP1 in B cells increases susceptibility to central nervous system autoimmunity, alters B cell biology, and mechanistically explains genetic associations between ERAP1 and multiple sclerosis. *J Immunol*, 2021. 207(12): p. 2952-2965.
  37. Alghamdi, M., Alasmari D, Assiri A, Mattar E, Aljaddawi AA, Alattas SG, Redwan EM. An overview of the intrinsic role of citrullination in autoimmune disorders. *J immunol Res*, 2019; 2019: p.1-39.
  38. Admon, A., ERAP1 shapes just part of the immunopeptidome. *Hum immunol*, 2019. 80(5): p. 296-301.
  39. Reeves, E. and E. James, The role of polymorphic ERAP1 in autoinflammatory disease. *Biosci Rep*, 2018. 38(4): BSR20171503.
  40. Paldino, G. and A. Fierabracci, Shedding new light on the role of ERAP1 in Type 1 diabetes: A perspective on disease management. *Autoimmun Rev*, 2023: p. 103291.

**Table S1:** Sequence of TaqMan probes for the studied ERAP1 SNPs

SNP	Context Sequence [VIC/FAM]
rs27044	TGCACACAGGCGAGGAGTAGTAGTT[C/G]ACTCCGCAGCATTTCGCTCTGAGACT
rs30187	TGTGATGGTTATTAGGGGAAAACCC[C/T]TCTGCAGTGTCCAAGTGTTTCATCAT
rs27037	ATTATTATTATTACAATTGTTAGGG[G/T]TGTTT

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