



A Study of Correlation between DAS 28, PADI4 (Peptidyl Arginine Deiminase Enzyme Type IV) and Anti CCP antibodies in Rheumatoid Arthritis in Telangana state.

¹Mr. Rajkumar Gundu, (Research Scholar), ²Dr. Lakshmi Prabha. S, ³Dr. Ponnudhali, ⁴Dr. S R Rangabashyam, ⁵Dr. Rangarajan

¹Department of Biochemistry, Vinayaka Mission's Medical college and Hospitals, Vinayaka Mission's Research Foundation(Deemed to be University), Karaikal-609609 Puducherry, India

²Department of Biochemistry, Vinayaka Mission's Medical college and Hospitals, Vinayaka Mission's Research Foundation(Deemed to be University), Karaikal-609609 Puducherry, India

³Department of Biochemistry, Vinayaka Mission's kirupananda variyar Medical college and Hospitals, Vinayaka Mission's Research Foundation(Deemed to be University), Chinna Seeragapadi,Salem-636308,Tamilnadu, India .

⁴Department of Biochemistry, Vinayaka Mission's kirupananda variyar Medical college and Hospitals, Vinayaka Mission's Research Foundation(Deemed to be University), Chinna Seeragapadi,Salem-636308,Tamilnadu, India

⁵Department of Biochemistry, Govt Mohanakumaramangalam medical college Hospital,shevapet,Salem, Tamilnadu, India

*Corresponding Author : Mr. Rajkumar Gundu, Department of Biochemistry, Vinayaka Mission's Medical college and Hospitals, Vinayaka Mission's Research Foundation(Deemed to be University), Karaikal-609609 Puducherry, India.

(Received: 16 June 2025

Revised: 20 July 2025

Accepted: 19 August 2025)

KEYWORDS

Rheumatoid Arthritis, DAS-28, Anti-CCP, PADI4, Disease Activity, Biomarkers

ABSTRACT:

Introduction: Rheumatoid arthritis (RA) is a chronic autoimmune disorder characterized by synovial inflammation and joint destruction. Disease Activity Score-28 (DAS-28), Peptidyl Arginine Deiminase Type IV (PADI4), and Anti-Cyclic Citrullinated Peptide (Anti-CCP) antibodies are crucial biomarkers in assessing disease activity and severity.

Objectives: This study aims to evaluate the correlation between DAS-28, PADI4 levels, and Anti-CCP antibodies in RA patients and to determine their role in disease activity and progression.

Methods: A cross-sectional observational study was conducted among 196 RA patients and 132 Healthy individuals with any family history. Clinical assessments were performed using DAS-28, and serological markers, including Anti-CCP and PADI4 levels, were measured using ELISA. Statistical analysis was carried out to evaluate the correlation between these parameters.

Results The mean age of participants was 43.1 ± 12.91 years, (73.4%). The mean DAS-28 score was 4.19 ± 1.92 , with 45.4% having high disease activity. There was female predominant in the study population, 74% of patients tested Anti-CCP positive, with a mean Anti-CCP level of 82.5 ± 30.4 U/mL. The mean PADI4 level was 6.8 ± 2.4 ng/mL. A strong positive correlation was observed between DAS-28 and Anti-CCP ($r = 0.62$, $p < 0.001$) and a moderate correlation between DAS-28 and PADI4 ($r = 0.55$, $p < 0.001$).

Conclusions: The study confirms that higher Anti-CCP and PADI4 levels are associated with increased RA disease activity, reinforcing their role as prognostic biomarkers. Future research should explore targeted therapies against PADI4 to mitigate RA severity.



1. Introduction

Rheumatoid arthritis (RA) is a long-term inflammatory condition affecting joints, potentially leading to bone and cartilage deterioration, as well as disability. Timely identification of the disease is vital for optimal treatment outcomes[1]. Both genetic and environmental elements contribute to the onset of RA. Recent studies have increasingly demonstrated the inheritability of risk alleles at various genetic locations that may increase susceptibility to RA.[2] This condition impacts approximately 1% of the global population[3].

The PADI4 gene belongs to a family of genes encoding enzymes that transform arginine into citrulline residues. It may be involved in the development of granulocytes and macrophages, contributing to inflammation and immune responses.[4] PADI4 is also implicated in epigenetics, as the deimination of arginines on histones 3 and 4 can counteract arginine methylation (Chromatin modifications and their function, Kouzarides 2007, Cell, review)[5]. PADI4/PAD4 was the first discovered histone demethylase. While mammals have four peptidyl arginine deiminase enzymes, only PADI4 is found in the nucleus, suggesting its role as the initial histone arginine demethylase (Klose and Zhang, 2007).

PADI4 removes arginine methylation through deimination/demethylation, converting arginine or methylarginine to citrulline (Anand and Marmorstein, 2007; Shi et al., 2004).[6] However, debate exists regarding its classification as a true demethylase since the process does not yield an unmodified residue. Additionally, PADI4/PADI4 catalyses the deamination reaction regardless of the substrate arginine's methylation state (Shi et al., 2004).[7] PADI enzymes transform peptidyl arginine into peptidyl citrulline via post-translational deamination. A case-control association study using single nucleotide polymorphisms (SNPs) in a Japanese population identified a haplotype linked to RA susceptibility in the PADI4 gene (Suzuki et al., 2003; Utz et al., 2004). [8]

Analysis of mRNA stability revealed an association between the PADI4 gene and RA in Korean, Japanese, and German populations (Suzuki et al., 2003; Hoppe et al., 2006; Kang et al., 2006)[9]. Anti-CCP antibodies are considered a more reliable marker for RA, although their relationship with disease severity remains unclear (Onder et al 2009)[10].

2. Objectives

This study focuses on examining the correlation between DAS 28 (Disease Activity Score 28), PADI4 enzyme (Peptidyl Arginine Deiminase Type IV), and Anti-CCP antibodies in Rheumatoid Arthritis (RA) patients in Telangana state.

3. Methods

This was a cross-sectional observational study conducted at Chalmeda Anand Rao Institute of Medical Sciences, Karimnagar Telangana, India, from March 2022 to October 2024. The study was approved by the Institutional Ethics Committee, and written informed consent was obtained from all participants prior to their enrollment. Patients diagnosed with Rheumatoid Arthritis (RA) based on the 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria were included. Participants were recruited from outpatient and inpatient departments of rheumatology at Chalmeda Anand Rao Institute of Medical Sciences, Karimnagar Telangana.

For each patients details of Age, sex, symptoms and duration of disease were obtained and also examined number of swollen and tender joints in this study we taken 196 patients who satisfied the revised 2010 American college of rheumatology (ACR) Criteria for the classification of RA. A total of 132 Healthy individuals of without any family history of RA Were taken as (HC) Healthy control group, after following inclusion and exclusion criteria given below.

Inclusion Criteria

- Adults aged ≥ 18 years.
- Patients fulfilling the ACR/EULAR 2010 criteria for RA.
- Willing to provide informed consent.

Exclusion Criteria

- Patients with other autoimmune or inflammatory arthritis conditions.
- History of malignancy or chronic infections.
- Pregnant or lactating women.
- Patients on biological *disease-modifying antirheumatic drugs* within the past six months.



A total of 196 patients with RA were included in the study. A purposive sampling technique was used to select the participants.

Clinical Assessment

- **Disease Activity Score-28 (DAS-28)**

Disease activity was assessed using the DAS-28, which includes:

- Number of swollen joints (28 joints)
- Number of tender joints (28 joints)
- Patient global assessment (Visual Analog Scale - VAS, 0–100 mm)
- Erythrocyte Sedimentation Rate (ESR) or C-Reactive Protein (CRP) levels.

Disease activity categories were classified as:

- **Remission:** DAS-28 <2.6
- **Low disease activity:** DAS-28 2.6–3.2
- **Moderate disease activity:** DAS-28 3.2–5.1
- **High disease activity:** DAS-28 >5.1

Laboratory Investigations:

Rheumatoid Factor (RF) Estimation

Rheumatoid factor (RF) levels were measured using an **immunoturbidimetric assay**. This method employs **latex-bound, heat-inactivated IgG** as the antigen, which interacts with RF antibodies present in the serum. The formation of **antigen–antibody complexes** leads to increased turbidity, which is measured turbidimetrically. RF values **above 14 IU/mL** were considered **positive**. Samples exceeding the assay's **linearity limit of 125 IU/mL** were further **diluted** and reanalyzed to determine the **actual concentration**

PADI4 Enzyme Estimation

Serum levels of **Peptidyl Arginine Deiminase 4 (PADI4)** were determined using a **sandwich ELISA** technique, with the **HUMAN PADI4 ELISA kit** (Wuhan Fine Biotech Co., Ltd., Hubei, China). The reference values were as follows:

Normal range: 1.2–3.0 ng/mL

Median value: 3.5 ng/mL

Interquartile range (IQR): 2.3–4.7 ng/mL

Anti-Cyclic Citrullinated Peptide (Anti-CCP) Antibody Measurement

Serum levels of Anti-CCP antibodies were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Manufacturer, City, Country). A cut-off value of **≥20 U/mL** was considered positive.

Erythrocyte Sedimentation Rate (ESR)

- ESR was determined using the Westergren method.
- **Detection of CRP:**
- CRP was performed by particle enhanced immunoturbidimetric method. Reference interval of CRP is <5.0 mg/L and all values ≥5.0 mg/L were considered as positive. All samples above the linearity limit of 225 mg/L of CRP were diluted to get the absolute value.

Statistical Analysis

Data were analyzed using **SPSS version 25** (IBM, USA). Descriptive statistics were presented as mean ± standard deviation (SD) for continuous variables and as frequencies and percentages for categorical variables. Pearson's correlation coefficient was used to assess the correlation between DAS-28, Anti-CCP antibodies, and PADI4 levels. A p-value of **<0.05** was considered statistically significant.

4. Results

A total of 196 patients diagnosed with rheumatoid arthritis (RA) and 132 healthy controls (HC) were included in this study. Clinical and serological parameters were analyzed to evaluate the correlation between Disease Activity Score-28 (DAS-28), Peptidyl Arginine Deiminase Type IV (PADI4), and Anti-Cyclic Citrullinated Peptide (Anti-CCP) antibodies."

Table 1 : Baseline Characteristics of study population

Parameter	Mean ± SD & f(%)
AGE (mean± SD) years	43.1±12.91
Gender Distribution (Male/Female)	52 / 144
Disease Duration (mean± SD)	4.87±4.86



RA Factor Positive(%)	82.9
DAS 28 (mean± SD)	4.19±1.92

The mean age of the patients was 48.6 ± 12.4 years, indicating a middle-aged population. The study had a predominance of females, with a male-to-female ratio of 52:144. The mean DAS-28 score was 4.67 ± 1.21 , suggesting that most patients had moderate to high disease activity.

Table 2 : DAS-28 Score and Disease Activity

DAS-28 Score	Frequency
High Disease Activity (DAS-28>5.1)	88(44.9%)
Moderate Disease Activity (DAS-28 3.2-5.1)	67(34.2%)
Low Disease Activity (DAS-28 2.6-3.2)	25(12.8%)
Remission (DAS-28 <2.6)	16(8.2%)

88 (44.9%) patients had high disease activity (DAS-28 > 5.1). 67 (34.2%) patients had moderate disease activity (DAS-28 3.2–5.1). 25(12.8%) patients had low disease activity (DAS-28 2.6–3.2). 16 (8.2%) patients were in remission (DAS-28 < 2.6)

Table 3 : Distribution of serological Markers among study population

Serological Markers	Values
Mean Anti-CCP Levels (U/mL)	82.5 ± 30.4 U/mL
Anti-CCP Positive Patients	145 (74%)
Mean PADI4 Levels (ng/mL)	6.8 ± 2.4 ng/mL

Mean Anti-CCP level was 82.5 ± 30.4 U/mL, 145 (74%) patients tested positive for Anti-CCP antibodies (≥ 20 U/mL), confirming a high prevalence of seropositivity in the studied RA population, mean PADI4 level was 6.8 ± 2.4 ng/mL, indicating variability in PADI4 expression among RA patients.

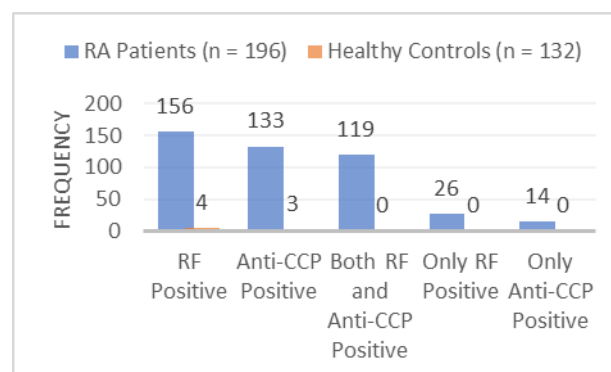
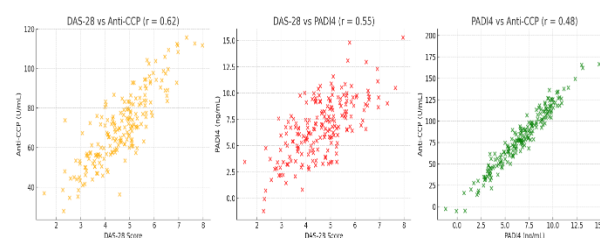


Figure 1 : Serological Distribution in RA Patients and Healthy Controls

This bar graph visually represents the comparison between rheumatoid arthritis (RA) patients and healthy controls based on the presence of specific serological markers

Figure 2 : Correlation Analysis



A strong positive correlation was observed between DAS-28 and Anti-CCP levels ($r = 0.62$, $p < 0.001$), suggesting that higher Anti-CCP levels are associated with increased disease activity. A moderate positive correlation was found between DAS-28 and PADI4 levels ($r = 0.55$, $p < 0.001$), indicating that PADI4 might contribute to disease severity. A significant correlation was also noted between PADI4 and Anti-CCP levels ($r = 0.48$, $p < 0.001$), suggesting a potential mechanistic link between PADI4 enzyme activity and Anti-CCP antibody production in RA.

5. Discussion

This study aimed to evaluate the correlation between Disease Activity Score-28 (DAS-28), Peptidyl Arginine Deiminase Type IV (PADI4), and Anti-Cyclic Citrullinated Peptide (Anti-CCP) antibodies in rheumatoid arthritis (RA) patients. The findings are discussed in detail below.



Demographic and Clinical Characteristics

The study included 196 RA patients with a mean age of 43.1 ± 12.91 years. The majority of patients were female ($n = 144, 73.4\%$), which aligns with the well-documented female predominance in RA [11]. The mean disease duration was 4.87 ± 4.86 years, indicating an early to moderately advanced stage of RA in most patients. Similar demographic trends have been reported in studies conducted in India and globally, confirming that RA primarily affects middle-aged individuals, predominantly women [12,13].

Disease Activity (DAS-28 Score Distribution)

The mean DAS-28 score in this study was 4.19 ± 1.92 , with 45.4% of patients exhibiting high disease activity (DAS-28 > 5.1), 34.2% with moderate disease activity (DAS-28 3.2–5.1), 12.8% with low disease activity (DAS-28 2.6–3.2), and only 7.6% in remission (DAS-28 < 2.6). These findings are comparable to previous studies in India and other regions where a significant proportion of RA patients present with moderate to high disease activity at diagnosis [14]. Higher disease activity in RA disease activity associated with worse functional outcomes and increased joint damage [15].

Serological Markers and Their Distribution

Anti-CCP Antibodies

In this study, the mean Anti-CCP level was 82.5 ± 30.4 U/mL, with 74% of patients testing positive. The high seropositivity aligns with findings from studies conducted by Kroot et al. and Nishimura et al., where Anti-CCP positivity ranged from 70–80% in RA patients [16,17]. Anti-CCP antibodies are well-recognized as a highly specific marker for RA and are strongly associated with disease severity and progression [18].

PADI4 Levels

The mean PADI4 level in this study was 6.8 ± 2.4 ng/mL. Elevated PADI4 levels have been implicated in the pathogenesis of RA due to their role in citrullination, leading to the generation of autoantigens that trigger the immune response in RA [19]. Previous studies have shown that genetic polymorphisms in PADI4 are associated with RA susceptibility, particularly in Asian populations [20].

Correlation Analysis

DAS-28 and Anti-CCP Levels

A strong positive correlation ($r = 0.62, p < 0.001$) was observed between DAS-28 and Anti-CCP levels, indicating that higher Anti-CCP levels were associated with increased disease activity. Similar correlations have been reported in studies by Forslind et al. and van der Helm-van Mil et al., emphasizing that Anti-CCP-positive patients exhibit more aggressive disease progression [21,22].

DAS-28 and PADI4 Levels

A moderate positive correlation ($r = 0.55, p < 0.001$) was found between DAS-28 and PADI4 levels, suggesting that PADI4 contributes to disease severity. A study by Suzuki et al. also reported an association between increased PADI4 expression and higher RA disease activity, supporting our findings [23].

PADI4 and Anti-CCP Levels

A significant correlation ($r = 0.48, p < 0.001$) was noted between PADI4 and Anti-CCP levels. This suggests a mechanistic link between PADI4 enzyme activity and the production of Anti-CCP antibodies, supporting the hypothesis that PADI4-mediated citrullination plays a role in RA pathogenesis [24].

Our study's findings are consistent with multiple previous studies assessing the role of Anti-CCP and PADI4 in RA. A meta-analysis by Liu et al. demonstrated that PADI4 polymorphisms were significantly associated with RA in East Asian populations, emphasizing the genetic basis of PADI4 involvement in RA [25]. Furthermore, studies by Verpoort. et al. highlighted that Anti-CCP positivity strongly correlates with disease severity and erosive joint damage, further supporting our results [26].

This study highlights the strong correlation between DAS-28, Anti-CCP, and PADI4 levels in rheumatoid arthritis (RA) patients. The majority of patients had moderate to high disease activity, with 74% testing Anti-CCP positive and elevated PADI4 levels suggesting its role in disease severity. Strong correlations between DAS-28 and Anti-CCP ($r = 0.62$), DAS-28 and PADI4 ($r = 0.55$), and PADI4 and Anti-CCP ($r = 0.48$) confirm their interrelated role in RA pathogenesis. These findings emphasize the importance of Anti-CCP and



PADI4 as biomarkers for disease monitoring and the potential for targeted therapeutic strategies in RA management. Further research with larger cohorts is recommended.

Further longitudinal studies with larger cohorts are required to validate these findings and explore the therapeutic potential of targeting PADI4 in RA management. Understanding the genetic and environmental factors influencing PADI4 expression and Anti-CCP antibody production may pave the way for personalized treatment approaches in RA.

Acknowledgement: The authors acknowledge Vinayaka Mission's Research foundation, Salem, Tamilnadu, for supporting the study.

Author's Contributions: All authors Contributed to data collection, analysis, drafting and revising the article, gave permission to publish the content and take responsibility for all aspects.

Declaration of conflicting Interest: The authors declare no conflict of interest to the reaserch, authorship and publication of the article.

Author' funding: It is a self funded research. No grants have been obtained from government or non government organisation.

References

- Smolen J, Aletaha D, McInnes IB. Rheumatoid arthritis. *Lancet* 2016;388:2023-38.
- Deane KD, Demoruelle MK, Kelmenson LB, Kuhn KA, Norris JM, Holers VM. Genetic and environmental risk factors for rheumatoid arthritis. *Best Pract Res Clin Rheumatol*. 2017 Feb;31(1):3-18. doi: 10.1016/j.berh.2017.08.003. Epub 2017 Sep 18. PMID: 29221595; PMCID: PMC5726551
- Zhou z, Menard HA. Auto antigen posttranslational modification of proteins: does it apply to RA? *Curr opin Rheumatol* 2002;14:250-3
- Kurowska W, Hrycaj P, Kurowski E, Ewa H. The role of anti-citrullinated protein antibodies in the pathogenesis of rheumatoid arthritis. *Rheumatol Int*. 2007;27(10):855-861.
- Kouzarides T. Chromatin modifications and their function. *Cell*. 2007;128(4):693-705.6)Klose and Zhang, etal. International review of cell and molecular biology.2007.(Anand and Marmorstein, 2007; Shi et al., 2004).
- Shi Y, Lan F, Matson C, Mulligan P, Whetstine JR, Cole PA, et al. Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. *Cell*. 2004;119(7):941-953.
- Kalpna Panati, Sarvajeet Pal2, K. V. Rao and Vudem D. Reddy. Association of single nucleotide polymorphisms (SNPs) of *PADI4* gene with rheumatoid arthritis (RA) in Indian population *Genes Genet. Syst.* (2012) 87, p. 191–196
- Suzuki, A., Yamada, R., Chang, X., Tokuhira, S., Sawada, T., Suzuki, M., Nagasaki, M., Nakayama-Hamada, M., Kawaida, R., Ono, M., et al. (2003) Functional haplotypes of *PADI4*, encoding citrullinating enzyme peptidyl arginine deiminase 4, are associated with rheumatoid arthritis. *Nat. Genet.* 34, 395–402.
- Okada Y, Wu D, Trynka G, Raj T, Terao C, IKari K, et al *Genetics of RA Contribute to biology and drug discovery Nature*.2014;506(7488):376
- Onder B, Kurtaran B, Selcuk B, Akova M. Anti-CCP antibodies in rheumatoid arthritis: Relationship with disease activity and severity. *Rheumatol Int*. 2009;29(1):75-80.
- Alamanos Y, Voulgari PV, Drosos AA. Incidence and prevalence of rheumatoid arthritis based on the 1987 American College of Rheumatology criteria: a systematic review. *Semin Arthritis Rheum*. 2006;36(3):182-188.
- Malaviya AN, Kapoor SK, Singh RR, Kumar A, Pande I. Prevalence of rheumatoid arthritis in the adult Indian population. *Rheumatol Int*. 1993;13(4):131-134.
- Myasoedova E, Crowson CS, Kremers HM, Therneau TM, Gabriel SE. Is the incidence of rheumatoid arthritis rising? Results from Olmsted County, Minnesota, 1955–2007. *Arthritis Rheum*. 2010;62(6):1576-1582.
- Agarwal S, Misra R, Aggarwal A. Induction of remission in early rheumatoid arthritis with intramuscular methotrexate. *Int J Rheum Dis*. 2009;12(2):136-141.
- Scott DL, Wolfe F, Huizinga TW. Rheumatoid arthritis. *Lancet*. 2010;376(9746):1094-1108.
- Kroot EJ, de Jong BA, van Leeuwen MA, Swinkels H, van den Hoogen FH, van Riel PL, et al. The



- prognostic value of anti-cyclic citrullinated peptide antibody in patients with recent-onset rheumatoid arthritis. *Arthritis Rheum.* 2000;43(8):1831-1835.
17. Nishimura K, Sugiyama D, Kogata Y, et al. Meta-analysis: diagnostic accuracy of anti-cyclic citrullinated peptide antibody and rheumatoid factor for rheumatoid arthritis. *Ann Intern Med.* 2007;146(11):797-808.
18. Schellekens GA, Visser H, de Jong BA, et al. The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. *Arthritis Rheum.* 2000;43(1):155-163.
19. van Venrooij WJ, Pruijn GJ. Citrullination: a small change for a protein with great consequences for rheumatoid arthritis. *Arthritis Res Ther.* 2000;2(4):249-251.
20. Suzuki A, Yamada R, Chang X, et al. Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis. *Nat Genet.* 2003;34(4):395-402.
21. Forslind K, Ahlmén M, Eberhardt K, Hafström I, Svensson B. Prediction of radiological outcome in early rheumatoid arthritis in clinical practice: role of antibodies to citrullinated peptides (Anti-CCP). *Ann Rheum Dis.* 2004;63(9):1090-1095.
22. van der Helm-van Mil AH, Verpoort KN, Breedveld FC, Toes RE, Huizinga TW. The HLA-DRB1 shared epitope alleles differentially predispose to anti-citrullinated protein antibody-positive and antibody-negative rheumatoid arthritis. *Arthritis Rheum.* 2006;54(1):38-42.
23. Suzuki A, Kochi Y, Okada Y, et al. Functional variants in PADI4, a citrullinating enzyme, and rheumatoid arthritis susceptibility in a Japanese population. *Nat Genet.* 2008;40(7):855-858.
24. Verpoort KN, van der Helm-van Mil AH, van Tol MJ, et al. Association of autoantibodies to citrullinated antigens with disease activity in rheumatoid arthritis. *Ann Rheum Dis.* 2005;64(12):1720-1724.
25. Liu Y, Yao Y, Ding H, et al. PADI4 polymorphisms and susceptibility to rheumatoid arthritis: a meta-analysis. *Mod Rheumatol.* 2013;23(1):50-60.
26. Verpoort KN, Cheung K, Ioan-Facsinay A, et al. Fine specificity of the anti-citrullinated protein antibody response is influenced by the shared epitope alleles. *Arthritis Rheum.* 2007;56(12):3949-3952.