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PADI4 Gene Polymorphism and Citrullination in Rheumatoid Arthritis: A Review study

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Abstract

Rheumatoid Arthritis (RA) is a chronic autoimmune disorder affecting 0.5–1% of the global population, characterized by synovial inflammation and autoantibody (RF, ACPA) production. Genetic factors, especially PADI4 variants, and environmental triggers like smoking increase susceptibility. A literature review was conducted on genetic and environmental factors influencing RA, focusing on PADI4 variants, their role in citrullination and ACPA formation, and the effect of smoking on synovial citrullination. PADI4 variants are overexpressed in RA, promoting citrullination, ACPA production, and resistance to apoptosis in synovial fibroblasts by inhibiting p53 and p21, leading to synovial hyperplasia. Smoking further enhances citrullination, aggravating disease severity. PADI4 variants and smoking synergistically elevate RA risk and progression by promoting citrullination and fibroblast survival. Their interaction highlights potential biomarkers and therapeutic targets for improved RA management.

Key words : PADI4, Citrullination, ACPA, Rheumatoid factor, Single nucleotide polymorphism.

Rheumatoid Arthritis (RA) is a chronic autoimmune inflammatory joint disease that is characterized by synovial inflammation and hyperplasia. It is associated with autoantibody production (rheumatoid factor and anti-citrullinated protein antibody) against various modifications such as citrullination (ACPA), carbamylation (aCarP), acetylation (AAPA) as well as the migration of T and B lymphocytes in the synovium, cartilage and bone destruction[23]. The occurrence of autoantibodies is due to abnormalities in the cellular and humoral immune response[32]. Rheumatoid arthritis is known to be the most common inflammatory joint disease affecting 0.5-1 percent of the population in the world[14]. Rheumatoid arthritis synovitis causes joint pain, morning stiffness, and swelling due to synovial inflammation and infiltration of articular bone and cartilage. RA not only affects the joints, but eventually progresses to the skin, heart, lungs, kidneys, and eyes, thus resulting in permanent disabilities of internal organs. Additional signs and symptoms include weight loss, swollen rheumatoid nodules under the skin, tenderness in the joints, fatigue and low

fever[6]. Multiple genetic and epigenetic factors have been associated with rheumatoid arthritis. It has become evident that environmental factors such as cigarette smoking and dust exposure also play an important role in the onset of the disease[9]. Findings suggest that genetic factors including class II major histocompatibility antigens/ Human leukocyte antigens (HLA-DR), as well as non-HLA genes, are responsible for the pathogenesis of RA. Several studies have confirmed 31 non-HLA loci that contribute to RA susceptibility[18].

Genetic studies report that single-nucleotide polymorphisms (SNPs) in the PADI4 gene are strongly associated with the development of RA. According to the GWAS studies, other important genes with single-nucleotide polymorphisms linked to rheumatoid arthritis include TRAF1, CTLA4, IRF5, STAT4, FCGR3A, IL6ST, IL2RA, IL2RB, CCL21, CCR6 and CD40. It has also been estimated that the heritability of RA is about 60 percent[18]. A study conducted on a twin cohort concluded that environmental factors such as tobacco smoking may be considered more important in determining the development of ACPAs in RA affected individuals[15]. Smoking is the major risk factor for RA that interacts with HLA-DR shared epitope genes and can trigger immune reactions such as autoantibodies to citrullinated peptides[34]. There is a significant interaction between HLA-DRB1 SE and smoking resulting in the development of anti-CCP positive RA.

The genetic variants in PADI4 are responsible for the association of the PADI locus with RA. Strong evidence support that overexpression of PADI4 in synovial organisation and peripheral blood mononuclear cells is the key factor responsible for the development of RA. PADI4 is responsible for increased citrullination of histone proteins (H3 and H4) at arginine residues and modulates the transcription of key genes during the development of RA. PADI4 is known to negatively regulate the apoptosis in RA-FLSs via inhibiting the p53 expression and its target p21 protein[11]. Studies further concluded that single nucleotide polymorphisms (SNPs) of PADI4 gene causes citrullination which is responsible for the production of ACPAs (29). According to the presence or absence of ACPAs, RA is divided into two major subtypes such as ACPA-positive and ACPA-negative. The ACPA-positive subset of RA has a more aggressive clinical phenotype compared to ACPA-negative subset of RA[19].

The human lung is a potential originating site of autoimmunity in RA. The increased risk of RA is associated with cigarette smoking being the most important extrinsic risk factor for the development and severity of the disease[10]. Tobacco smoking has been associated with autoimmunity and increased ACPA production which results in increased levels of PADI4 protein in RA-ILD patients[26]. Cigarette smoking affects both cellular and humoral aspects of immune system to trigger various morphological, biochemical and enzymatic changes that lead to impaired cellular regulatory activity and inflammatory responses. Smoking results in increase in oxidative stress and is responsible for the activation of endogenous sources of free radicals. It leads to both increased and decreased apoptosis due to increased levels of Fas (CD95) and CD4 T-cells resulting in high levels of cellular debris.

HLA-DR- restricted immune reactions to autoantigens are triggered by smoking[41].

Studies have reported that smoking could lead to extensive epigenetic changes,

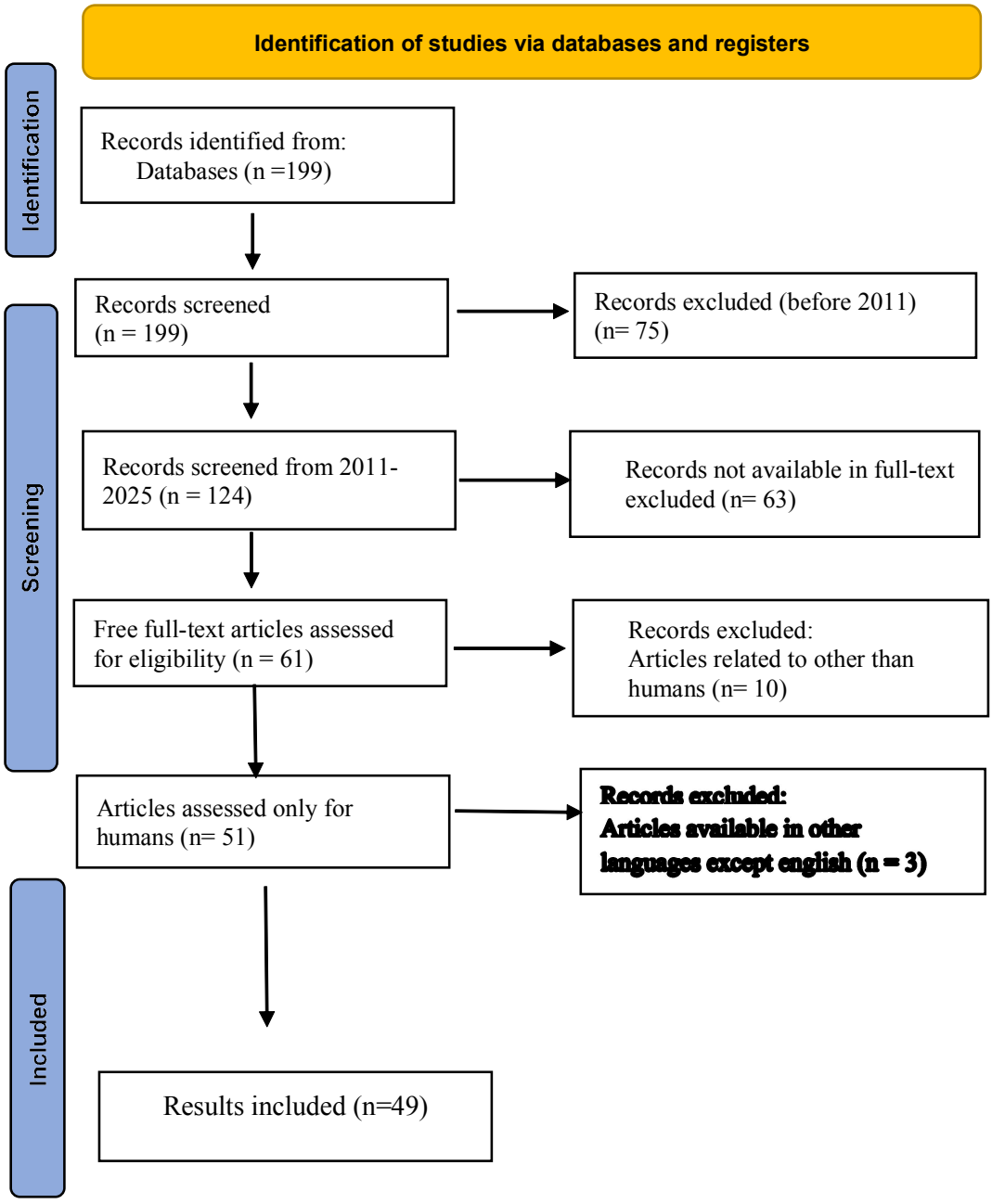

Figure 1. *PRISMA* flowchart of the review's results.

such as DNA methylation, as they play a crucial role in gene regulation and development of RA. It has been observed that cigarette smoke condensate (CSC) induces proinflammatory cytokines, such as IL-1α, IL-1β, IL-6 and IL-8. Among these cytokines, IL-1 and TNF-α are to be strongly associated with the pathogenesis in RA [38].

Objectives :

The current literature review has been framed to analyse the role of genetic polymorphism of *PADI4* gene in causing citrullination of peptidyl arginine, resulting in rheumatoid arthritis. The considered candidate gene might be involved in triggering citrullination of bronchoalveolar lavage cells in smokers which results into increased expression of PADI4 enzymes which may directly or indirectly lead to onset of RA. The RA etiology with a genetic basis may pave a novel path in treating RA and understanding of how the environmental factors, such as smoking may interact with gene in triggering arthritogenic immunity. Therefore, the main objective of this review is to emphasise and discuss the involvement of genetic polymorphism of *PADI4* gene and smoking in RA.

The current literature review was conducted according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) statement. The identification of various studies was done by using PubMed database. For the searching strategy, various keywords were included such as PADI4 gene AND rheumatoid arthritis. A total of 199 papers were individually searched on PubMed and papers with adequate information were retrieved according to the inclusion criteria. Records were screened and retrieved using various filters.

PADIs have been implicated in the post-translational conversion of peptidylarginine to peptidylcitrulline which leads to citrullination. Various physiological functions such as skin keratinization, neuron myelination and formation of neutrophil extracellular traps (NETs) involve citrullination but one major pathogenic role of citrullination is the production of anti-citrullinated protein-antibody (ACPA)[16]. There are five existing PAD isoforms in humans that are coded by PADI1-4 and PADI6 genes and each isoform has specific functions, substrates and tissue expression. Out of these five isoforms, PADI2 and PADI4 are expressed by immune cells and are present in the synovium of RA patients. The earlier studies concluded that PADI4 is generally expressed in the granulocytes and monocytes and it generates autoantigens recognized by ACPAs more efficiently as compared to PADI2 gene[4]. It has been concluded that ACPAs can be detected several years before onset of disease and correlate with preclinical inflammation. These autoantibodies are hallmark serological findings in RA. There are several studies that suggest the important role of PADI4 as epigenetic regulator via histone citrullination and as a transcriptional regulator through interactions with transcriptional factors such as p53, ING4 and NF-kB [13].

PADI4 is highly expressed in bone marrow, macrophages, neutrophils and monocytes and produces peptidylcitrulline via modification of protein substrates. The enhanced expression and function of PADI4 could increase the risk of RA. Citrullination is an irreversible process.

The net charge of protein is reduced due to citrullination resulting in loss of one positive charge per modified arginine residue. This leads to an increased protein hydrophobicity, protein unfolding and altered intra- and inter-molecular interactions. These structural changes can lead to either gain or more likely, loss of protein function. The mechanism of citrullination must be tightly regulated to avoid excessive citrullination of physiologic as well as non-physiologic substrates[14].

Pathogenesis of Rheumatoid Arthritis :

Various immune modulators (cytokines and effector cells) and signaling pathways are responsible for the joint damage that begins at the synovial membrane. Environmental factors such as smoking and infection, may also influence the development, rate of progression and severity of RA. Various case-control studies as well as cohort studies have demonstrated that cigarette smoking is a risk factor for RF positive and anti-citrullinated antibody with RA[35]. Studies revealed that smoking can trigger specific and potentially disease inducing immune reactions against citrullinated proteins in RA. The reactivity of autoantibodies to citrullinated antigens in RA is influenced by gene-environment interactions. Studies revealed that ACPAs can be detected long before the onset of joint symptoms which suggest that joints may not be the triggering spot for autoimmunity. It has been stated that lung exposure to various noxious agents such as smoke, silica dust, nanosized silica or carbon-derived nanomaterials can trigger mucosal toll-like receptors (TLRs) which in turn activate calcium-mediated PADs and antigen-presenting cells (ACPs) including classical dendritic cells (DCs) and B-cells[25]. Disruption of endoplasmic reticulum (ER)-Golgi transport due to the coatomer subunit α-gene mutations can cause hereditary autoimmune-mediated lung disease and arthritis, thereby providing a connection between lung and the joint diseases[39].

A number of autoimmune responses are mediated by T-cells and B- cells resulting in initiation of inflammatory cascade. These T-cells, B-cells, macrophages and neutrophils migrate into synovial tissues leading to breakdown of extracellular matrix in cartilage. Migration of immune cells results in synovial hypertrophy and angiogenesis and activates osteoclasts as well as bone erosion[28].

PADI4 gene polymorphism :

The PADI4 gene is member of PADIs gene family that encodes peptidylarginine deiminase enzyme. PADI4 gene is located on chromosome 1p36 and several polymorphisms have been identified in its promoter region. A review study highlighted the role of peptidy-larginine deiminase (PADs) in protein citrullination. Single nucleotide variations in genes encoding PADs often lead to functional alterations in the protein and its enzymatic activity in rheumatoid arthritis[27]. A population-based study in North and South China revealed significant results which concluded that PADI4- 94G/A polymorphism may influence the risk of RA [7]. A novel study determined the strong association of missense variant rs874881 (Gly112Ala) of PADI4 in RA susceptibility along with in-silico analysis of structural and functional impacts of this substitution. The interaction analysis revealed significant interaction of genotype with smoking and gender ($p< 0.05$)[3].

A study was conducted to determine the association of PADI4 SNPs (rs874881, rs11203366, rs11203367, rs2240336, rs2240337, rs2240339, rs1748033 and rs2240340) with RA. All the SNPs except rs11203366 were found to be significantly associated with RA and in addition, two novel mutations have been identified in exon 4 (SCV000804840:C.218T>C and SCV000807675:C.241G>T). Haplotype analysis indicated that GACCACGCC and GACCACGCT were highly significant in disease development and affected the functioning of PADIs by altering their amino acid sequence[20]. Another study revealed a novel mechanism of *Helicobacter pylori* infection in RA patients. Findings suggested *H. pylori* infection induced protein citrullination by upregulation of PADI4 activity via the ROS/HIF-1α pathway. This study resulted in higher DAS28 and ACPA levels of patients with RA[40]. A study on Mexican population revealed higher PAD activity in RA patients carrying the GTG haplotype thereby confirming an association of the GTG haplotype with the risk for RA and higher gene expression in carriers of these haplotypes[22]. The study of correlations of genotype frequencies with clinical characteristics and biochemical parameters of the patients revealed that PADI4 SNPs (rs11203367, rs2240340, rs11203366, rs874881) were significantly associated with the severity of the disease[37]. Nava-Quiroz *et al.* identified that the polymorphism of 3 SNPs (rs11203366, rs11203367 and rs874881) in PADI4 are also associated with developing ILD in patients with rheumatoid arthritis[2]. Hosseinabadi *et al.* confirmed the association of PADI4 gene rs1748033 SNP with increased RA proneness in an Iranian population. Furthermore, anti-CCP and DAS28 was significantly higher in TT carriers for rs1748033 polymorphism[30]. A study conducted by Ciesla *et al* examined 122 European subjects with RA and investigated two SNPs of PADI4 gene using real-time polymerase chain reaction. The study suggested lack of association of these two SNPs of PADI4 gene in Europeans whereas PADI4 gene polymorphism is associated with risk of developing RA in Asian population. PADI4 is known to be involved in the pathogenesis of ACPA-positive RA by catalyzing the formation of citrullinated autoantigens[8]. A study was conducted on the Danish and North American cohorts to investigate the association of PADI4 polymorphism and smoking with Anti-CCP positive RA. This study concluded that Anti-CCP positive RA patients were often smokers than anti-CCP negative patients in both the cohorts and suggested that certain single nucleotide polymorphisms in PADI4 are the risk factors for ACPA-positive RA[20]. Recently, PADI4 rs2240336 polymorphism at chromosome 1p36 has been reported as one of the most important RA susceptibility loci in multiple ethnic groups, including Europeans, Asians and Latin Americans[1]. A case-control study was conducted in Iranian population (including 665 RA patients) to assess the association of two PADI4 SNPs (rs874881 and rs11203367) with susceptibility to RA. The findings revealed lack of association of these PADI4 SNPs which do not significantly determine RA susceptibility in Iranian population. The results were similar with some European populations. Hence, it was reported that RA pathogenesis might be different among various ethnicities[33].

Mergaert *et al* determined single nucleotide polymorphism rs2240335 in PADI4 whose G allele homozygotes were found to

be associated with reduced PAD4 in neutrophils, correlates with NETs, anti-histone antibodies and RA susceptibility in North Americans. It was concluded that the G allele conferred increased risk for rheumatoid arthritis diagnosis, suggesting a complex role for PADI4 in human rheumatoid arthritis[24]. The correlation between the 104 C/T polymorphism in the peptidylarginine deiminase 4 (PADI4) gene and rheumatoid arthritis risk has been analysed in several studies. A meta-analysis indicated that PADI4-104 C/T polymorphism is significantly associated with RA risk in North Chinese population[12]. Positive correlations between anti-PADI4 and disease duration, anti-CCP and erythrocyte sedimentation rate has also been observed with help of comparative analysis. Furthermore, it has been concluded that anti-CCP is genetically associated with the citrullinating enzyme PADI4 and is strongly related to Th1 and Th2 cytokines suggesting a feed-forward loop between cytokines and ACPA production[30]. Case-control association studies and mRNA stability assays reported the association of PADI4 gene with RA in Korean and Japanese populations but no such association was found in Spanish population. A case-control study conducted in a multiethnic population residing in South-east Asia provided evidence of polymorphism in PADI4 gene with RA [36]. However, significant association of PADI4 polymorphism (rs2240340) has been confirmed in the Polish population whereas Ghanaian population excluded it as a risk factor for RA due to lack of SNP association[5,31]. Another significant association of PADI4 rs1748033 polymorphism showed greater risk of RA in men than in women and in ever-smokers than in never-smokers in Japanese and Dutch populations. Polymorphism in exon-4 (padi4_104) rs1748033 of PADI4 showed significant association of 'C' allele with RA in Indian population[17].

This review underscores the central role of citrullination in the generation of anti-citrullinated protein antibodies (ACPA) and highlights PADI4 as a critical factor in RA pathogenesis. PADI4 functions as an epigenetic regulator through histone citrullination and also modulates transcriptional factors such as p53, ING4, and NF-κB. Increased PADI4 expression and activity may therefore elevate RA risk.

Epidemiological studies confirm that cigarette smoking is a significant risk factor for RF- and ACPA-positive RA. Genetic studies further reveal strong but population-specific associations between PADI4 single nucleotide polymorphisms (SNPs) and RA. Variants such as rs874881 and rs11203367 are linked with RA in Russian, Pakistani, and European populations but not in Iranians. rs2240336, rs2240337, rs2240339, rs1748033, and rs2240340 show associations in Pakistanis, while rs1748033 confers higher risk in Japanese men and Indians. rs2240335 is associated with RA in North Americans, and rs2240340 in Russian and Polish cohorts, with weaker links in Ghanaians. Overall, PADI4 variants contribute variably to RA susceptibility across ethnicities, likely reflecting HLA and non-HLA genetic backgrounds. Certain SNPs act as risk factors for ACPA-positive RA, but many remain unexplored. Larger, multi-ethnic studies are needed to clarify their roles.

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