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Genetic Insights Into Rheumatoid Arthritis: Investigating PADI4 And CD40 Single Nucleotide Polymorphism (SNP) In The Coimbatore District Population Of Tamil Nadu

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Abstract:
Rheumatoid arthritis (RA) is an autoimmune disease in patients affected nearly less than 1% of the global population. Peptidyl arginine deiminase 4 (PADI4) and Clustered Differentiation 40 (CD40) gene also has a significant role to play in the pathophysiology of RA. this study is conducted to detect the association between the two genetic markers PADIV and CD 40 in the RA positive patients in Coimbatore, Tamil Nadu. This is a cross sectional study performed in a tertiary care hospital in Coimbatore district of Tamil Nadu. The period of study was about four years from March 2020 – April 2024. About 3ml of blood samples from 80 seropositive and 80 seronegative were collected and stored in -20°C. Single Nucleotide Polymorphism (SNP) primers for allelic specific TaqMan probe is designed for the detection of PADI4 (rs2240340) in chromosome 1 and CD40 (rs4810485) in chromosome 20 at position 17336144 and 46119308 respectively. All 160 (80+80) patient's samples were employed for the detection of PADI4 and CD40 gene. SNPs of PADIV are more significant contributors of the disease development in RA conditions in the Asian population. In the present study, CD40 has expressed low in RF positive and negative patients compared to PADI4 gene. The combination of both markers CD40 and PADI4 do not show a statistically significant relationship with RF status in the present study. The study concludes that PADI4 shows a significant association with RF positivity and could be explored further as a genetic marker for disease risk.
Keywords: *PADI4, CD40, Single Nucelotide Polymorphisms (SNPs), Rheumatoid Arthritis, Anti-CCP antibodies*

INTRODUCTION:

Rheumatoid arthritis (RA) is an autoimmune disease in patients with inflammatory and painful episodes which are also chronic, affecting diarthrodial joints. The disease has been affected nearly less than 1% of the global population. Female is the predominant gender when compared to male, which could be characterized by the production of autoantibody, synovial hyperplasia, systemic inflammation, infiltration of joints by inflammatory cells and cardiovascular complications (1). There are many hypotheses, but the exact trigger mechanism for RA is still unknown. Different research studies have targeted on various genes which are more sensitive to this arthritic condition in patients, including KIF5A, PRKCQ, TRAF1/C5, IL2RB and 6q (2). Single nucleotide polymorphism (SNP) is an alteration at a single position in a DNA sequence among individuals. Peptidyl arginine deiminase 4 (PADI4) and Clustered Differentiation 40 (CD40) gene also has a significant role to play in the pathophysiology of RA among the non-HLA genes and there are various studies to support this hypothesis (2-3). PADI4 is a significant enzyme which helps in the conversion of the amino acid arginine to a non-essential amino acid citrulline, as a result of post-translational modification (4-5). There has been a report of many populations with RA risk pertaining to the PADI4 genes genetically associated SNPs. Complementing this, antibodies have also been discovered in the RA patients which are called as anti-cyclic-citullinated protein antibodies (anti-CCP). CD40 plays a major role in the development of the symptoms of RA in the susceptible patients. This gene is recognized to be member of tumor necrosis factor (TNF) and plays a crucial role in the signaling pathway that causes

chronic inflammatory autoimmune disease, as revealed by a recent discovery (3). This transmembrane protein is expressed on the immune cells including antigen-presenting cells (APCs), T cells, monocyte and non-immune cells such as fibroblasts, smooth muscle, epithelial and endothelial cells as an inducible or constitutive manner. Hence this study is conducted to detect the association between the two genetic markers PADIV and CD 40 in the RA positive patients in Coimbatore, Tamil Nadu.

MATERIALS AND METHODS:

This is a cross sectional study performed in a tertiary care hospital in Coimbatore district of Tamil Nadu. The period of study was about four years from March 2020 – April 2024. After getting approval from the Institutional Human Ethics Committee (EC/2020/0411/CR/85) and consent from the patients, 3ml of blood samples from 80 seropositive and 80 seronegative were collected and stored in -20^{o}C. These samples were collected based on our national prevalence of RA is around 16%. In the study, RF positive and negative patients shows the characteristic symptoms of symmetric joint pain, joint stiffness, fever etc., were included. Antenatal women, pediatric age group, patients above 80 years, pain only in large joints etc., were excluded from the study. The basic information of the patients was collected viz., gender, age, ethnicity etc., along with the clinical conditions of the patients. The consent and medical history was collected by face to face interview of the patients and they were made aware of the study details and its outcomes. The genomic DNA was extracted from the commercially available extraction kit (QIAmp Blood Mini Kit, Qiagen) and performed as per the manufacturer's instructions. The extracted DNA was checked with purity of range from 1.7 to 1.8 in NanoDrop, a spectrophotometer, Thermoscientific. Single Nucleotide Polymorphism (SNP) primers for allelic specific TaqMan probe is designed for the detection of PADI4 (rs2240340) in chromosome 1 and CD40 (rs4810485) in chromosome 20 at position 17336144 and 46119308 respectively. The allele position for PADI4 (rs2240340) is C/T and CD40 (rs4810485) G/T (6). The primers and probes of these two genes were designed from Primer 3 software. PAD14 (rs2240340) Primers are Forward: 5'-CTGAGCTTCTGATGCTGACAGC-3', Reverse: 5'-CTGACGCTAGCAGCAGTCTGAC-3' and Probe: FAM-AGACCGCAGCTTAGCTACG. CD40 (rs4810485) primers are Forward: 5'-AGTCTGATCGACTGACTCGA-3', Reverse: 5'-ACTGACTGACTCGTAGCATG-3' and Probe: FAM-TACGTAGCTTAGCTCTAGCATG. SNP TaqMan Real Time PCR (Applied Biosystem, Thermofisher Scientific) was performed based on the amplification conditions which constitutes 20 µl includes 10µl TaqMan Universal master mix followed by 1µl of forward, reverse primer, and probe each, 5 µl of Template DNA and 2 µl of Nuclease free water was added. About 1µl of GAPDH, an internal control was added to the patient sample during the genomic DNA extraction to check the purity. The cyclic conditions are Initial Denaturation at 95°C for 10 minutes followed by 45 cycles of denaturation at 95°C for 10 seconds, Annealing & Extension at 65°C for 5 seconds. The amplified products were shown as an amplification curve. Data was collected from the patients and entered in the excel sheet. Mean age and standard deviation of the population was calculated. Chi-square and Fisher exact test were analyzed for small number of samples in the GraphPad Prism Online statistical software. $p \leq 0.05$ is statistically significant.

RESULTS:

In the present study 160 patients were collected and categorized into 80 RA positive and equal number of RA negative. Mean ± SD age of the patients was 41.5 ± 15.6 with 95% Confidence Interval (41.56 ±3.44). The age of the RA positive patients ranges from 28 to 70 years and for negative patients about 20 to 70 years. The statistical significance of joint pain and swelling in RA positive patients (P=0.0019) and anemia in RA negative patients (P=0.0207) can be seen in Table 1. There were no statistically significant differences ($P > 0.05$) between other symptoms, such as joint pain, fever, stiffness, fever, malaise, and morning stiffness. All 160 (80+80) patient's samples were employed for the detection of PADI4 and CD40 gene. Expression of PADI4 and CD40 genes in RF positive patients were shown in Figure – 1 & 2. Comparison between Molecular SNP Markers viz., CD40 and PADI4 of RF positive and negative patients were shown in Table – 1. There is a statistically significant association between the PADI4 gene variant and RF positivity (p=0.0428). Other combinations like only CD40 and both gene positivity had no statistical difference was observed between RF positive and negative patients.

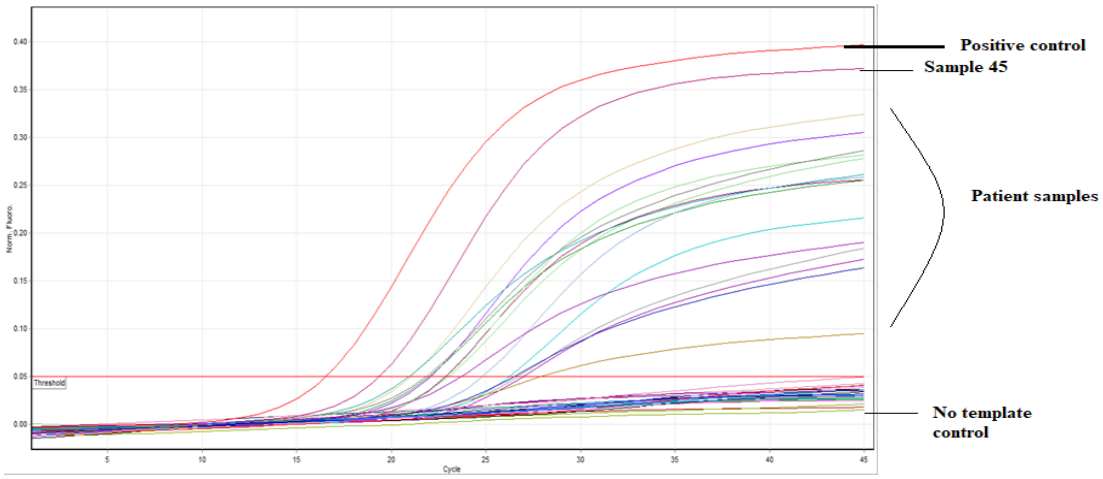

Figure – 1: Expression of PADI4 gene in RF positive patients

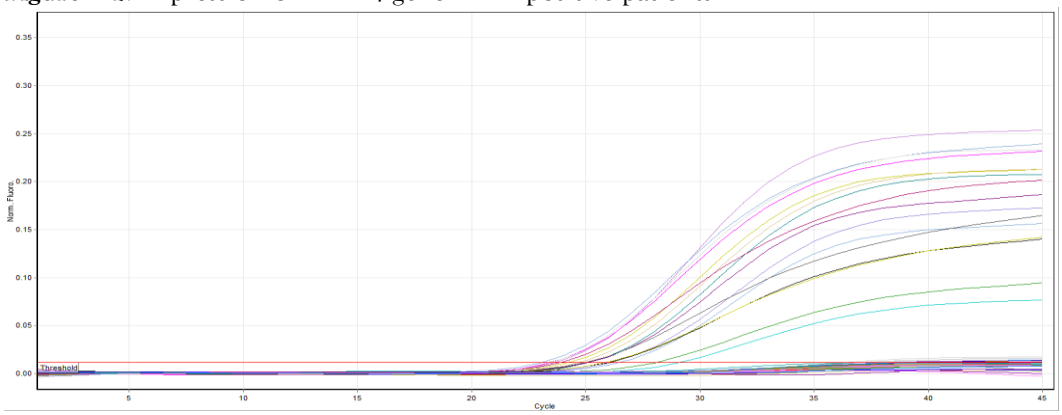

Figure – 2: Expression of CD40 gene in RF positive patients

Table – 1: Comparison between Molecular SNP Markers of RF positive and negative patients

Molecular markers	RF positive (n=80)	RF Negative (n=80)	P value (p≤0.05)
PADI4 (rs2240340)	32	20	0.0428
CD40 (rs4810485)	16	08	0.1199
Both PADI4 & CD40	04	06	0.7459

DISCUSSION:

SNPs of PADIV are more significant contributors of the disease development in RA conditions in the Asian population (7). CD40 has always been very closely associated with the inflammatory diseases and several autoimmune diseases including rheumatoid arthritis (3). In this regards to RA, CD40 has been reported to play a major role in the development of the disease due to its role in the B cell proliferation and activation, inducing the production of pro inflammatory cytokines and major involvement in the Ig class switching (8). Due to these reasons the CD40-CD40L pathway has been identified to be one of the promising therapeutic target for Transplant rejection and several other autoimmune diseases (9-10). In the present study, CD40 has expressed low in RF positive and negative patients compared to PADI4 gene. The literature studies have notably denoted an elevated level of CD40L ligand expression in the female gender from the peripheral blood and also in the synovial fluid CD4 + T cells, in comparison to the healthy controls (11-13). There are many literature studies which have pointed out that the CD40 signalling pathway is responsible for the IgM (ACPA) anti-cyclic citrullinated peptide antibodies by the B cells, not only in RA patients but also in healthy individuals (14). A meta- analysis study including European and Asian population performed to analyze the association between PADIV and RA reveals strong association of PADI4-94 (rs2240340), PADI4-104 (rs1748033) and PADI4-90 (rs11203367) with RA in Asian population and only PADI4-94 (rs2240340) showing stronger association in European population (15). The literature says Suzuki and his colleagues, in the year 2003, uncovered the very first clue about the association among the Japanese population with RA and the SNPs of PADI4gene (16). Another study revealed the increase in the stability of the mRNA in PADI4, in the mononuclear cells

when the RA patients peripheral blood sample was tested (17). The combination of both markers CD40 and PADI4 do not show a statistically significant relationship with RF status in the present study. The study is limited to small number of samples for analysis of two SNPs. The sample size should be increased for further evaluation in future

CONCLUSION:

The study concludes that PADI4 shows a significant association with RF positivity and could be explored further as a genetic marker for disease risk.

Conflict of Interest: None to declare by all authors
Acknowledgement: None

REFERENCES:

1. McInnes IB and Schett G (2011). The pathogenesis of rheumatoid arthritis. N. Engl. J. Med. 365: 2205-2219. http://dx.doi. org/10.1056/NEJMra100496523.
2. Abbasifard M, Imani D, Bagheri-Hosseinabadi Z. PTPN22 gene polymorphism and susceptibility to rheumatoid arthritis (RA): updated systematic review and meta-analysis. J Gene Med. 2020;22(9):e3204.14.
3. Peters, A.L., Stunz, L.L. and Bishop, G.A., 2009. Semin. Immunol., 21: 293–300. https://doi.org/10.1016/j.smim.2009.05.012
4. Cornelis, F., Faure, S., Martinez, M., Prud'homme, J. F., Fritz, P., Dib, C., Alves, H., Barrera, P., de Vries, N., Balsa, A., et al. (1998) New susceptibility locus for rheumatoid arthritis suggested by a genome-wide linkage study. Proc. Natl. Acad. Sci. USA 95, 10746–10750.
5. Anzilotti C, Pratesi F, Tommasi C, Migliorini P. Peptidylarginine deiminase 4 and citrullination in health and disease. Autoimmun rev. 2010;9(3):158–6014. Peters, A.L., Stunz, L.L. and Bishop, G.A., 2009. Semin. Immunol., 21: 293–300.
6. Budlewski T, Sarnik J, Galita G, Dragan G, Brzezińska O, Popławska M, Popławski T, Makowska J. SNP in PTPN22, PADI4, and STAT4 but Not TRAF1 and CD40 Increase the Risk of Rheumatoid Arthritis in Polish Population. Int J Mol Sci. 2023 Apr 20;24(8):7586.
7. Burr ML, Naseem H, Hinks A, Eyre S, Gibbons LJ, Bowes J, et al. PADI4 genotype is not associated with rheumatoid arthritis in a large UK caucasian population. Ann Rheum Dis. 2010;69(4):666–70.
8. Orozco G, Eyre S, Hinks A, Ke X, et al.; Wellcome Trust Case Control consortium YEAR Consortium (2010). Association of CD40 with rheumatoid arthritis confirmed in a large UK case-control study. Ann. Rheum. Dis. 69: 813-816. http:// dx.doi.org/10.1136/ard.2009.109579
9. Elgueta, R., M. J. Benson, V. C. de Vries, A. Wasiuk, Y. Guo, and R. J. Noelle. 2009. Molecular mechanism and function of CD40/CD40L engagement in the immune system. Immunol. Rev. 229: 152–172.
10. Kirk, A. D., L. C. Burkly, D. S. Batty, R. E. Baumgartner, J. D. Berning, K. Buchanan, J. H. Fechner, Jr., R. L. Germond, R. L. Kampen, N. B. Patterson,et al. 1999. Treatment with humanized monoclonal antibody against CD154 prevents acute renal allograft rejection in nonhuman primates. Nat. Med. 5: 686–693.
11. MacDonald, K. P., Y. Nishioka, P. E. Lipsky, and R. Thomas. 1997. Functional CD40 ligand is expressed by T cells in rheumatoid arthritis. J. Clin. Invest. 100: 2404–2414.
12. Raychaudhuri, S., E. F. Remmers, A. T. Lee, R. Hackett, C. Guiducci, N. P. Burtt, L. Gianniny, B. D. Korman, L. Padyukov, F. A. S. Kurreeman, et al. 2008. Common variants at CD40 and other loci confer risk of rheumatoid arthritis. Nat. Genet. 40: 1216–1223.
13. Berner, B., G. Wolf, K. M. Hummel, G. A. M€uller, and M. A. Reuss-Borst. 2000. Increased expression of CD40 ligand (CD154) on CD4+ T cells as a marker of disease activity in rheumatoid arthritis. Ann. Rheum. Dis. 59: 190–195.
14. Reparon-Schuijt CC, van Esch WJ, van Kooten C, Schellekens GA, et al. (2001). Secretion of anti-citrulline-containing peptide antibody by B lymphocytes in rheumatoid arthritis. Arthritis Rheum. 44: 41-47.
15. Lee YH, Rho YH, Choi SJ, Ji JD, Song GG. PADI4 polymorphisms and rheuma¬toid arthritis susceptibility: a meta-analysis. Rheumatol Int. 2007;27(9):827–33.
16. Habib A, Jafri L, Hussain MA, Ishaq S. Diagnostic utility of anti-citrullinated protein antibody and its comparison with rheumatoid factor in rheumatoid arthritis. JCPSP: J Coll Physicians Surg Pakistan. 2012;22(11):711.
17. Suzuki A, Yamada R, Chang X, Tokuhiro S, Sawada T, Suzuki M, et al. Functional haplotypes of PADI4, encoding citrullinating enzyme peptidyl¬arginine deiminase 4, are associated with rheumatoid arthritis. Nat Genet. 2003;34(4):395–402.