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Association between Peptidylarginine Deiminase Type 4 rs1748033 Polymorphism and Susceptibility to Rheumatoid Arthritis in Zahedan, Southeast Iran

Mohammad Hashemi^{1,2}, Zahra Zakeri^{3,4}, Hamed Taheri³, Gholamreza Bahari², and Mohsen Taheri⁵

¹ Cellular and Molecular Research Center, Zahedan University of Medical Sciences, Zahedan, Iran

² Department of Clinical Biochemistry, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran

³ Department of Internal Medicine, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran

⁴ Department of Internal Medicine, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

⁵ Genetics of Non Communicable Disease Research Center, Zahedan University of Medical Sciences, Zahedan, Iran

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ABSTRACT

There are controversial reports regarding the role of peptidylarginine deiminase type 4 (PADI4) gene polymorphisms and risk of Rheumatoid arthritis (RA). The aim of the present study was to investigate the impact of *PADI4* rs1748033 polymorphism and susceptibility to RA in a sample of the Iranian population.

This case-control study was done on 150 patients with RA and 150 healthy subjects. *PADI4* rs1748033 genotyping was done using amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) assay.

The *PADI4* rs1748033 variant increased the risk of RA in codominant (OR=1.67, 95%CI=1.03-2.71, $p=0.048$, CT vs CC; OR=2.73, 95%CI=1.25-5.97, $p=0.013$, TT vs CC) and dominant (OR=1.84, 95%CI=1.15-2.92, $p=0.014$, CT+TT vs CC) tested inheritance models. In addition, the *PADI4* rs1748033 T allele increased the risk of RA (OR=1.63, 95%CI=1.16-2.29, $p=0.006$) in comparison with C allele.

In conclusion, our finding indicated that *PADI4* rs1748033 gene polymorphism increased the risk of RA in a sample of the Iranian population.

Keywords: PADI4; Polymorphism; Rheumatoid arthritis

Corresponding Author: Zahra Zakeri, MD;

Associate Professor of Rheumatology, Department of Internal Medicine, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Tel: (+98 21) 2360 2188, Fax: (+98 21) 2258 0333, E-mail: zah_zakeri@yahoo.com

INTRODUCTION

Rheumatoid arthritis (RA) is a complex autoimmune disease of unknown etiology which affects approximately 0.5-1% of the human population

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worldwide.^{1, 2} Both genetic and environmental factors are involved in the expression and complications of the disease.^{3, 4} Genetic factors believed to be responsible for ~ 60% of the risk of developing RA.²

The *PADI4* gene is located on the short (p) arm of chromosome 1 at position 36.13 (1p36.13). The gene encodes the enzyme catalyses arginine within peptide into citrulline by posttranslational deamination.⁵ The serum titer of antibodies against citrullinated peptides, ACPA, is a prognostic serologic marker for joint destruction in RA patients.^{6, 7}

PADI enzymes convert the arginine within peptide into citrulline by posttranslational deamination. The *PADI4* gene is located on the short (p) arm of chromosome 1 at position 36.13 (1p36.13). Autoantibodies directed against citrullinated proteins (anti-cyclic citrullinated peptide) are highly specific for RA and suggesting the involvement of PADIs in the pathogenesis of RA.⁸

PADI4 is considered as one of the strong candidate RA-susceptibility genes. Association studies between *PADI4* polymorphisms and RA in various populations have produced conflicting results.^{5, 9-13} Therefore, the present study aimed to evaluate the impact of *PADI4* rs1748033 variant on the susceptibility to RA in a sample of Iranian population.

MATERIALS AND METHODS

Patients

We investigated the possible association between *PADI4* rs1748033 polymorphism and RA susceptibility in 150 patients fulfilling the American College of Rheumatology (ACR) criteria for RA.¹⁴ All the subjects were patients of the Rheumatology Clinic at Zahedan University of Medical Sciences.^{15, 16} The control group consisted of 150 age and sex matched healthy individuals unrelated to RA patients and had no known autoimmune diseases and from the same geographical origin as the patients with RA (Zahedan, Iran). The ethics committee of Zahedan University of Medical Sciences approved the project and informed consent was obtained from all participants. Blood samples from patients and healthy control were collected in Na-EDTA tubes. Genomic DNA was extracted from peripheral blood samples by salting out method.¹⁷

The *PADI4* genomic sequences (NT_004610.2) were obtained from the National Center for

Biotechnology Information (NCBI) (<http://www.ncbi.nlm.nih.gov>). We searched the polymorphism and the primers for amplification refractory mutation system polymerase chain reaction (ARMS-PCR) was designed.

The primer sequences were as follow: generic primers, 5'-GAGGGATGTCTTGAACCTGTGT-3'; T allele, 5'-GGGTGATGTCTGCGCACTA-3'; C allele, 5'-GGGTGATGTCTGCGCACTG-3'. The toll-like receptor 2 (TLR2F: 5'-GATGCATTTGTTTCTTACAGTGAGCG-3', TLR2R: 5'-TCTCATCAAAAAGACGGAAATGGG-3') was used as internal control. In each 0.20ml reaction, 1µl of genomic DNA (~100ng/ml), 1µl of each primer (10µM), 10µl of 2X Prime Taq Premix (Genet Bio, Korea) and 5 µl ddH₂O were added.

Amplification was done with an initial denaturation step at 95°C for 5min, followed by 30 cycles of 30 s at 95°C, 25s at 58°C and 30s at 72°C with a final step at 72°C for 10 min. PCR products were verified on a 2.5% agarose gel contained 0.5 µg/ml ethidium bromide and photographs was taken in Figure 1. The product sizes were 190-bp for either the alleles, and 259-bp for the internal control. To verify genotyping quality, all polymorphisms in random samples were regenotyped.

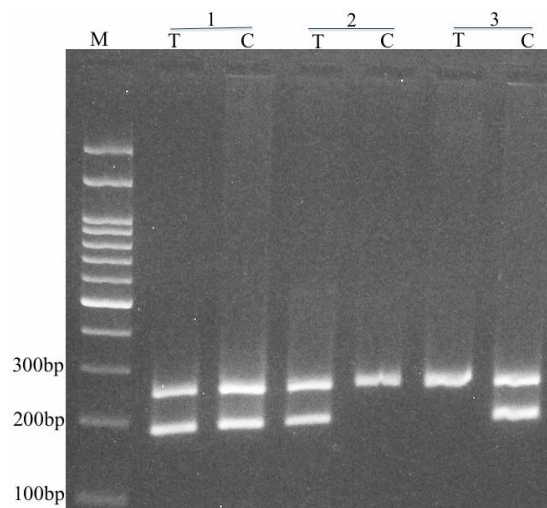


Figure 1. Photograph of the PCR products of *PADI4* rs1748033 C/T polymorphism by amplification refractory mutation system polymerase chain reaction (ARMS-PCR) method. M: DNA Marker; Lane 1: CT; Lane 2: TT; Lane 3: CC.

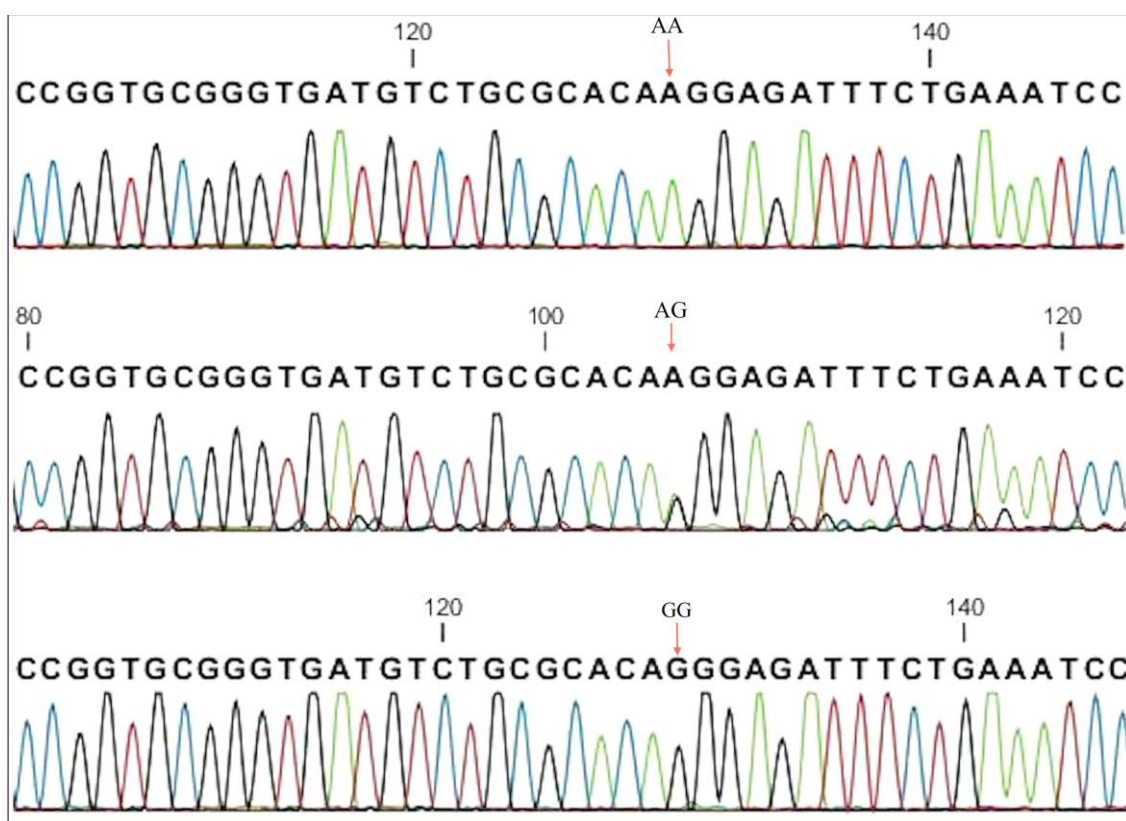


Figure 2. Sequencing results of *PADI4* rs1748033 C/T (G/A) genomic DNA. homozygous rs1748033 AA (TT), heterozygous rs1748033 AG (TC) and homozygous rs1748033 GG (CC) variants are shown.

To confirm the genotyping results, 3 samples for each genotype were sequenced. The forward and reverse primers for sequencing were 5'-GCAGAGGCTTCACATCGAAC-3' and 5'-AACACGGAATACGTGGGACA-3', respectively.

Amplification was done with an initial denaturation step at 95°C for 5 min, followed by 30 cycles at 95°C for 30 s, 63°C for 30 s, and 72°C for 30 s with a final extension at 72°C for 5 min. The results determined by ARMS-PCR were concordant with those determined by sequencing (Figure 2).

Statistical Analysis

Statistical analysis was done using statistical software package SPSS 20 software. The associations between genotype of *PADI4* gene and RA were assessed by computing the odds ratio (OR) and 95% confidence intervals (95% CI) from logistic regression analyses. We estimated the Hardy Weinberg equilibrium (HWE) separately for cases and controls.

RESULTS

In this study, we recruited 150 RA patients (139 female and 11 male; mean age 44.9 ± 13.5 years) and 150 control individuals (134 female and 16 male; mean age: 46.1 ± 12.6 years). There was no significant difference between the groups concerning sex and age ($p=0.815$, $p=0.465$, respectively). The genotype and allele frequencies of *IPAD4* rs1748033 polymorphism in RA patients and in controls are shown in table 1. Significant differences were found in genotype frequencies between the groups regarding *PADI4* rs1748033 polymorphism ($\chi^2=8.17$, $p=0.017$).

The *PADI4* rs1748033 variant increased the risk of RA in codominant (OR=1.67, 95%CI=1.03-2.71, $p=0.048$, CT vs CC; OR=2.73, 95%CI=1.25-5.97, $p=0.013$, TT vs CC) and dominant (OR=1.84, 95%CI=1.15-2.92, $p=0.014$, CT+TT vs CC) tested inheritance models.

Table 1. Comparison of genotype frequency of *PADI4* rs1748033 polymorphism in RA and controls.

rs1748033 C/T	RA n (%)	Control n (%)	OR (95%CI)	p-value
Codominant				
CC	52 (34.7)	74 (49.3)	1.00	-
CT	75 (50.0)	64 (42.7)	1.67 (1.03-2.71)	0.048
TT	23 (15.3)	12 (8.0)	2.73 (1.25-5.97)	0.013
Dominant				
CC	52 (34.7)	74 (49.3)	1.00	-
CT+TT	98 (65.3)	76 (50.7)	1.84 (1.15-2.92)	0.014
Recessive				
CC+CT	127 (84.7)	138 (92.0)	1.00	-
TT	23 (15.3)	12 (8.0)	2.01 (0.99-4.36)	0.071
Alleles				
C	179 (59.7)	212 (70.6)	1.00	-
T	121 (40.3)	88 (29.4)	1.63 (1.16-2.29)	0.006

The *PADI4* rs1748033 T allele increased the risk of RA (OR=1.63, 95%CI=1.16-2.29, $p=0.006$) in comparison with C allele.

The genotype of *PADI4* rs1748033 polymorphism in controls and cases were in HWE ($\chi^2=0.127$, $P=0.720$ and $\chi^2=0.226$, $P=0.634$, respectively).

DISCUSSION

The findings of this study showed an association between *PADI4* rs1748033 polymorphism and RA in our population. The *PADI4* rs1748033 variant increased the risk of RA in codominant (CT vs CC; TT vs CC) and dominant (CT+TT vs CC) tested inheritance models. Also, the rs1748033 T allele increased the risk of RA in our population.

Suzuki et al⁵ first reported that polymorphisms of *PADI4* including rs1748033 increased the risk of RA in a Japanese population. *PADI4* rs1748033 polymorphism was found to be associated with RA in Korean¹⁰ and Japanese population.^{5, 9, 18} While this variant was not associated with RA in UK Caucasian population¹⁹, German²⁰ and Spanish population²¹. Pantani et al¹¹ have found that the rs1748033 CT+CC decreased the risk of RA in comparison with TT genotype in Indian population. They reported the rs1748033 C allele of *PADI4* increased the risk of RA in Indian population. They did not find any association between rs11203366, rs11203367 and rs874881 variants of *PADI4* and RA. The sample size in their

study was too small (95 RA patients and 56 healthy blood donors).

Chen et al¹² have found no significant association between *PADI4* polymorphisms (rs2240340, rs11203366, rs11203367 and rs874881) and RA in Chinese Han population. While Du et al²² reported that *PADI4* rs2240340 and rs1748033 polymorphisms were significantly associated with RA susceptibility in Chinese Han RA patients. *PADI4* rs1748033 polymorphism has been shown to be associated with greater risk of RA in men than in women and in ever-smokers than in never-smokers.²³

A meta-analysis performed by Burr et al²⁴ showed that *PADI4* rs2240340 polymorphism was not a significant risk factor for RA in people of European ancestry, in contrast to Asian populations. A meta-analysis performed by Okada et al¹³ showed that *PADI4* rs766449 variant is a risk factor for RA even in Caucasian populations although its impact on disease susceptibility is lower than in Asian populations. Recently, it has been shown that the *PADI4* rs2240340 A allele increased the risk of RA in Japanese population.²⁵

Several investigations using Caucasian samples showed no association between RA susceptibility and *PADI4* polymorphisms, though the association was repeatedly established in Asian populations.^{10, 18, 19, 21, 24, 26} The controversial findings in various studies might be due to the heterogeneity of populations and complicating environmental factors.

Du et al²² have found that *PADI4* rs2240340 and rs1748033 variants conferred great risk for developing anti-CCP-positive RA. In addition, they reported a trend association between *PADI4* rs2240340 polymorphism and radiographic severity. Suzuki et al²⁵ stated that rs2240340 variant is independent genetic risk for radiographic progression in Japanese rheumatoid arthritis patients. Anti-CCP antibody levels have been reported to be associated with high disease activity scoring at 28 joints (DAS28) values in RA patients.¹¹ The *PADI4* RA risk haplotype have been found to be associated with increased anti-CCP levels in RA patients with disease of short duration.⁶

The limitation of the present study is that we have no data regarding Anti-CCP antibodies, RF antibody, DAS28, HLA-DRB1 shared epitope and smoking history. Thus, we could not determine the association between *PADI4* variant and these factors. Nevertheless, we believe that the results of this study provide an important input into the debate concerning the clinical relevance of studied variant.

Taken together, we found a significant association between *PADI4* rs1748033 variant and susceptibility to RA. Further association studies with large sample size and different ethnicities are needed to confirm our findings.

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CONFLICT OF INTEREST

No competing financial interests exist.

REFERENCES

- Hochberg MC, Spector TD. Epidemiology of rheumatoid arthritis: update. *Epidemiol Rev* 1990; 12:247-52.
- Turesson C, Matteson EL. Genetics of rheumatoid arthritis. *Mayo Clin Proc* 2006; 81(1):94-101.
- Ghelani AM, Samanta A, Jones AC, Mastana SS. Association analysis of TNFR2, VDR, A2M, GSTT1, GSTM1, and ACE genes with rheumatoid arthritis in South Asians and Caucasians of East Midlands in the United Kingdom. *Rheumatol Int* 2011; 31(10):1355-61.
- Hashemi M, Moazeni-Roodi AK, Fazaeli A, Sandoughi M, Taheri M, Bardestani GR, et al. The L55M polymorphism of paraoxonase-1 is a risk factor for rheumatoid arthritis. *Genet Mol Res* 2010; 9(3):1735-41.
- Suzuki A, Yamada R, Chang X, Tokuhiko S, Sawada T, Suzuki M, et al. Functional haplotypes of *PADI4*, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis. *Nat Genet* 2003; 34(4):395-402.
- Cha S, Choi CB, Han TU, Kang CP, Kang C, Bae SC. Association of anti-cyclic citrullinated peptide antibody levels with *PADI4* haplotypes in early rheumatoid arthritis and with shared epitope alleles in very late rheumatoid arthritis. *Arthritis Rheum* 2007; 56(5):1454-63.
- Chang X, Yamada R, Suzuki A, Sawada T, Yoshino S, Tokuhiko S, et al. Localization of peptidylarginine deiminase 4 (*PADI4*) and citrullinated protein in synovial tissue of rheumatoid arthritis. *Rheumatology (Oxford)* 2005; 44(1):40-50.
- Vossenaar ER, Zendman AJ, and Van Venrooij WJ. Citrullination, a possible functional link between susceptibility genes and rheumatoid arthritis. *Arthritis Res Ther* 2004; 6(1):1-5.
- Ikari K, Kuwahara M, Nakamura T, Momohara S, Hara M, Yamanaka H, et al. Association between *PADI4* and rheumatoid arthritis: a replication study. *Arthritis Rheum* 2005; 52(10):3054-7.
- Kang CP, Lee HS, Ju H, Cho H, Kang C, Bae SC. A functional haplotype of the *PADI4* gene associated with increased rheumatoid arthritis susceptibility in Koreans. *Arthritis Rheum* 2006; 54(1):90-6.
- Panati K, Pal S, Rao KV, Reddy VD. Association of single nucleotide polymorphisms (SNPs) of *PADI4* gene with rheumatoid arthritis (RA) in Indian population. *Genes Genet Syst* 2012; 87(3):191-6.
- Chen R, Wei Y, Cai Q, Duan S, Ren D, Shen J, et al. The *PADI4* gene does not contribute to genetic susceptibility to rheumatoid arthritis in Chinese Han population. *Rheumatol Int* 2011; 31(12):1631-4.
- Okada Y, Terao C, Ikari K, Kochi Y, Ohmura K, Suzuki A, et al. Meta-analysis identifies nine new loci associated with rheumatoid arthritis in the Japanese population. *Nat Genet* 2012; 44(5):511-6.
- Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988; 31(3):315-24.
- Hashemi M, Zakeri Z, Eskandari-Nasab E, Atabaki M, Pourhosseini ME, Jahantigh M, et al. CD226 rs763361

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- (Gly307Ser) Polymorphism Is Associated with Susceptibility to Rheumatoid Arthritis in Zahedan, Southeast Iran. *Iran Biomed J* 2013; 17(4):194-9.
16. Hashemi M, Eskandari-Nasab E, Zakeri Z, Atabaki M, Bahari G, Jahantigh M, et al. Association of pre-miRNA-146a rs2910164 and pre-miRNA-499 rs3746444 polymorphisms and susceptibility to rheumatoid arthritis. *Mol Med Rep* 2013; 7(1):287-91.
 17. Hashemi M, Moazeni-Roodi AK, Fazaeli A, Sandoughi M, Bardestani GR, Kordi-Tamandani DM, et al. Lack of association between paraoxonase-1 Q192R polymorphism and rheumatoid arthritis in southeast Iran. *Genet Mol Res* 2010; 9(1):333-9.
 18. Iwamoto T, Ikari K, Nakamura T, Kuwahara M, Toyama Y, Tomatsu T, et al. Association between *PADI4* and rheumatoid arthritis: a meta-analysis. *Rheumatology (Oxford)* 2006; 45(7):804-7.
 19. Barton A, Bowes J, Eyre S, Spreckley K, Hinks A, John S, et al. A functional haplotype of the *PADI4* gene associated with rheumatoid arthritis in a Japanese population is not associated in a United Kingdom population. *Arthritis Rheum* 2004; 50(4):1117-21.
 20. Hoppe B, Haupl T, Gruber R, Kiesewetter H, Burmester GR, Salama A, et al. Detailed analysis of the variability of peptidylarginine deiminase type 4 in German patients with rheumatoid arthritis: a case-control study. *Arthritis Res Ther* 2006; 8(2):R34.
 21. Martinez A, Valdivia A, Pascual-Salcedo D, Lamas JR, Fernandez-Arquero M, Balsa A, et al. *PADI4* polymorphisms are not associated with rheumatoid arthritis in the Spanish population. *Rheumatology (Oxford)* 2005; 44(10):1263-6.
 22. Du Y, Liu X, Guo JP, Liu X, Li R, Zhao Y, et al. Association between *PADI4* gene polymorphisms and anti-cyclic citrullinated peptide antibody positive rheumatoid arthritis in a large Chinese Han cohort. *Clin Exp Rheumatol* 2014; 32(3):377-82.
 23. Kochi Y, Thabet MM, Suzuki A, Okada Y, Doha NA, Toes RE, et al. *PADI4* polymorphism predisposes male smokers to rheumatoid arthritis. *Ann Rheum Dis* 2011; 70(3):512-5.
 24. 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.
 25. Suzuki T, Ikari K, Yano K, Inoue E, Toyama Y, Taniguchi A, et al. *PADI4* and *HLA-DRB1* are genetic risks for radiographic progression in RA patients, independent of ACPA status: results from the IORRA cohort study. *PLoS One* 2013; 8(4):e61045.
 26. Too CL, Murad S, Dhaliwal JS, Larsson P, Jiang X, Ding B, et al. Polymorphisms in peptidylarginine deiminase associate with rheumatoid arthritis in diverse Asian populations: evidence from MyEIRA study and meta-analysis. *Arthritis Res Ther* 2012; 14(6):R250.