Original Research Article

Association Assessment of Peptidylarginine Deiminase Type 4 (PADI4) rs1748033 polymorphism and susceptibility to rheumatoid arthritis in Gorgan, Northeast of Iran

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ABSTRACT

Introduction: Rheumatoid arthritis (RA) is a chronic autoimmune disease in which both genetic and environmental factors could be involved. Peptidyl arginine deiminase type IV (PADI4) is an enzyme responsible for the posttranslational conversion of arginine residues into citrulline. The association between PADI4 single nucleotide polymorphisms (SNPs) and RA susceptibility have been reported. Here, we aimed to assess the association of PADI4-104 (rs1748033) variant with the susceptibility to RA in an Iranian population in northeast of Iran. **Materials and methods:** A total of 130 RA patients and 128 age- and sex-matched healthy donors were recruited. The amplification-refractory mutation system with allele specific primers was used to detect PADI4-104 SNP. Disease activity was calculated using Disease Activity Scale 28a. SPSS 22.0 and SNPstat online software were used to analyze data using relevant statistical tests. **Results:** The CC genotype was more frequent in healthy subjects compared to RA patients. Setting the CC genotype as the reference, the TT genotype was significantly associated with increased risk of RA [OR = 2.11, 95% CI (1.45–3.07), P-value = 0.0001]. Moreover, no significant association was observed between genotypes and the disease activity score (P=0.154). **Conclusions:** The present study suggests that the PADI4-104 genetic variants are associated with RA susceptibility but not with the disease activity. While this is the first time to report such association in an Iranian population in northeast of Iran, further studies are required to confirm these findings.

KEYWORDS: Amplification-refractory mutation system; Peptidylarginine Deiminase Type 4; Rheumatoid arthritis; Single nucleotide polymorphism

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INTRODUCTION

Rheumatoid arthritis (RA [MIM no. 180300]) is a multi-factorial, systemic and chronic inflammatory disease in which the immune response is dysregulated [1]. RA predominantly affects the synovial joints and may lead to progressive destruction of affected organs [2]. As a growing health burden that leads to limitation of daily activities, RA affects approximately up to 1% of the adult population worldwide [3]. Although the etiology of RA is not thoroughly elucidated, both genetic and environmental factors are thought to be involved in susceptibility to the disease [4, 5]. The genetic component may account for approximately 60% of RA pathogenesis [4]. Recent findings have represented growing evidence on heritability of risk alleles at a number of genetic loci, which may predispose individuals to RA [6]. Human leukocyte antigen (HLA) class II molecules and related genetic loci have been considered as the most powerful genetic factors associated with RA [7]. However, several genes outside the HLA region including cytotoxic T-lymphocyte-associated antigen 4 (CTLA4) [8], PTN22 [9], STAT4 [10, 11] and peptidyl arginine deiminase type IV (PADI4) [12,13] have been proposed according to linkage-based approaches for assessment of genetic susceptibility and associations with RA.

Peptidyl arginine deiminase type IV (PADI4) (NM_012387) is a member of the *PADI* gene family (located on human chromosome 1p36; a linkage region for RA), which encodes

enzymes responsible for the posttranslational conversion of arginine residues into citrulline [12,14]. Autoantibodies directed against cyclic citrullinated peptides (CCPs) may result in breakdown of immunological tolerance due to the subsequent changes in their conformation and antigenicity, which play a crucial role in the RA pathogenesis [15]. PADI4 mRNA and protein is overexpressed in pathological synovial tissues and peripheral blood mononuclear cells (PBMCs) of RA patients [16,17]. Furthermore, several studies have addressed the association of PADI4 single nucleotide polymorphisms (SNPs) and functional variants with RA susceptibility [13, 18]. However, the results of such studies in various populations have been controversial. Considering the lack of sufficient data on this association in Iran, we aimed to assess the association of PADI4-104 (rs1748033) genetic variants with the susceptibility to RA in an Iranian population.

MATERIALS AND METHODS

Patients and controls

A total of 128 patients fulfilling the revised criteria of the American College of Rheumatology for RA were selected from the Rheumatology Research Center of Sayyad Shirazi educational hospital in Gorgan, Iran. The primary diagnosis and clinical manifestations were confirmed and Disease Activity Scale (DAS) 28a [19] was calculated by a rheumatologist. A total of 130 age- and sex-matched healthy subjects were also enrolled. Informed consent was obtained from all individuals. A volume of 5mL whole blood was taken from all participants. The samples were transferred to the laboratory and plasma was isolated immediately and stored at -80 ºC. Anti-CCP was measured by enzyme-linked immunosorbent assay (ELISA) using Human CCP ELISA Kits (Mybioscource, SD, USA). PBMCs were separated using Ficoll-Paque (Baharafshan, Tehran, Iran) gradient centrifugation as described previously [20]. Clinical and laboratory data were also obtained using filed documents and latest laboratory test results.

DNA extraction and genotyping

DNA was extracted from PBMCs using a genomic DNA isolation kit (Dena Zist, Iran) and kept at -20 ºC until use. The amplification-refractory mutation system with allele specific primers (Table 1) was used to amplify the polymorphic region and to detect rs1748033 SNP (C/T) of the *PADI4* gene. Polymerase chain reaction (PCR) was performed in a 25 mL reaction solution containing 50 ng of template DNA, 10X PCR buffer (GeNet Bio, Korea), 2 mM $MgCl_2$, 0.5 mM of each primer and 1.5 U Taq polymerase (GeNet Bio, Korea). Amplification conditions were as follows: initial denaturation at 95 ºC for 5 minutes, 35 cycles of denaturation at 95 ºC for 25 seconds, annealing at 58 ºC for 30 seconds, extension at 72 ºC for 30 seconds and final extension at 72 ºC for 10 minutes. PCR products were electrophoresed on 1.5% agarose gel and presence of a 190 bp band represented both alleles (C and T). A 422-bp fragment was used as internal control for the PCR experiment.

Table1. List of primers used in the amplification-refractory mutation system PCR for detection of rs1748033 SNP (C/T) in the *PADI4* gene

Name	Sequence (5'>3')	Value
PADI4-104F	GAGGGATGTCTTGAACCTGTGT	Generic forward
PADI4-104T	GGGTGATGTCTGCGCACTA	T allele
PADI4-104C	GGGTGATGTCTGCGCACTG	C allele
hGH422-F	TTCCCAACCATTCCCTTA	Internal control F
hGH422-R	GGATTTCTGTTGTGTTTC	Internal control R

Statistical analysis

The Hardy-Weinberg equilibrium was checked by Pearson's goodness of fit test. SPSS 22.0 (SPSS, Chicago, USA) and SNPstat online software (https://www.snpstats.net/start.htm) were used for statistical analysis of data. Odds ratio (OR) and 95% confidence interval (CI) were determined to evaluate case-control study associations. The Fisher's exact test was used to compare genotype frequencies between groups. One-way analysis of variance (ANOVA) with Tukey's post hoc test or nonparametric Kruskal-Wallis with Dunn-Bonferroni post hoc test was used to compare means of multiple samples. P-values less than 0.05 were considered as statistically significant.

RESULTS

PADI4-104 alleles and genotypes are associated with the increased risk of RA

The distribution of genotypes and alleles under different inheritance models (co-dominant, dominant, recessive and over-dominant) in RA patients and normal subjects were in the Hardy–Weinberg equilibrium. Comparison of the PADI4-104 (C/T) genotypes showed that the CC genotype was more frequent in healthy subjects. Setting the CC genotype as the reference, the TT genotype was significantly associated with increased risk of RA under co-dominant model [OR = 2.11, 95% CI (1.45–3.07), P-value = 0.0001] after adjusting for sex and age. These findings were confirmed under dominant [OR = 2.20, 95% CI (1.34–2.63), P-value = 0.0017] and recessive [OR = 4.48, 95% CI (1.87–10.74), P-value = 0.0001] models. Moreover, comparison of allele frequencies in patients and healthy donors suggested a significant association between T allele and RA susceptibility [OR = 2.11, 95% CI (1.45–3.07), P-value = 0.0001] (Table 2).

Table2. The genotype and allele frequencies of PADI4-104 (C/T) rs1748033 in RA patients and healthy subjects under different inheritance models

Genotypes and alleles		Healthy subjects *(n=130)*	RA patients *(n=128)*	OR (95% CI)	P-value
		Number (%)	*Number (%)*		
rs1748033	C	347 (67%)	150 (59%)	Reference	
	T	169 (33%)	106 (41%)	**2.11 (1.45-3.07)**	**0.0001**
	Co-dominant model				
	CC	74 (56.9%)	48 (37.5%)	Reference	
	CT	49 (37.7%)	54 (42.2%)	1.70 (1.00-2.89)	
	TT	7 (5.4%)	26 (20.3%)	**5.73 (2.30-14.23)**	**0.0001**
	Dominant model				
	CC	74 (56.9%)	48 (37.5%)	Reference	
	CT+TT	56 (43.1%)	80 (62.5%)	**2.20 (1.34-3.63)**	**0.0017**
	Recessive model				
	CC+CT	123 (94.6%)	102 (79.7%)	Reference	
	TT	7 (5.4%)	26 (20.3%)	**4.48 (1.87-10.74)**	**0.0001**
	Over-dominant model				
	CC+TT	81 (62.3%)	74 (57.8%)	Reference	
	CT	49 (37.7%)	54 (42.2%)	1.21 (0.73-1.99)	0.46
X^2 HWE* (*P*-value)		2.18 *(0.13)*	0.91 *(0.76)*		

*P-values lower than 0.05 are considered as statistically significant. Significant associations are shown in Bold. Sex and age adjustment was performed to standardize the risk assessment

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PADI4-104 genotypes are not associated with disease activity score

We evaluated the association of PADI4-104 genotypes with the clinical and laboratory characteristics of RA patients. Regarding the DAS-28a, no significant difference was observed between the genotypes (P=0.154). Erythrocyte sedimentation rate was significantly higher among RA patients with the TT genotype compared to other genotypes (P=0.029). No other significant association was observed regarding the clinical and laboratory characteristics of RA patients and PADI4-104 genotypes (Table 3).

Table3. Clinical and laboratory characteristics of RA patients regarding the PADI4-104 genotypes

Characteristics and genotypes (N=130)		CC	TT	CT	P-value
Age		49.87±2.36	45.58±3.42	51.69±1.99	0.287
DAS28a		3.45±0.31	2.71±0.39	3.65±0.33	0.154
Erythrocyte sedimentation rate		20.12±2.67*	35.11±5.03*	28.11±4.01	**0.029**
Age of onset		44.26±3.31	38.75±2.98	42.31±2.89	0.580
Anti-CCP	**Positive**	52.9%	23.5%	23.5%	0.416
	Negative	39.5%	18.4%	42.1%	
Family history	**Yes**	31%	27.6%	41.4%	0.129
	No	50%	11.4%	38.65	

Data are presented as mean ± standard error for continuous variables and as percentages of positive patients (%) for categorical variables. Significant associations are shown in bold. * *P*-value <0.05

DISCUSSION

Although the etiology of RA is not thoroughly understood, both genetic and environmental factors could be involved in its initiation and progression [4, 5]. Several risk alleles at a number of genetic loci have been associated with predisposition to RA including genetic variants in HLA II [7], CTLA4 [8], PTN22 [9], STAT4 [10, 11] and PADI4 [13]. The *PADI4* gene encodes an enzyme responsible for the posttranslational conversion of arginine residues into citrulline [12, 14], which plays a crucial role in the RA pathogenesis [15-17]. Several studies have addressed the association between PADI4 genetic variants and RA susceptibility [13, 18]. However, no study has yet evaluated the relationship between PADI4 SNPs and RA susceptibility in Iran. Therefore, we assessed the association of PADI4-104 (rs1748033) genetic variants with the susceptibility to RA in an Iranian population using different inheritance models.

A study by Suzuki et al. in Japan was the first to report the association of the *PADI4* gene polymorphisms including PADI4-104 and increased risk of RA [13]. Other studies evaluated the association of PADI4-104 genetic variants with RA in Korean [21] and Japanese [18, 22] populations. No significant association was observed between PADI4-104 genetic variants and RA in German [23], Spanish [24] and White British [24] populations. In a recent meta-analysis of 16 studies including 7551 cases and 6394 controls, a significant association between PADI4-104 genetic variants and RA was observed for Asians but not for Europeans [25]. Du et al. reported that the PADI4-104 polymorphism was significantly associated with RA susceptibility in a Chinese Han population [26]. Another meta-analysis performed by Lee et al. revealed that the PADI4-104 was a significant risk factor for Asian RA patients [27]. The TT genotype was associated with the increased risk of RA development in the mentioned studies. Similarly, we revealed that the CC genotype was more frequent in healthy subjects compared to RA patients. Setting the CC

genotype as the reference, the TT genotype was found to be significantly associated with increased risk of RA. In India, Panati et al. showed that CT-CC genotype of PADI4-104 SNP was associated with decreased risk of RA and reported C allele as the risk allele [28], which is in contrast to our findings. However, most investigations on Caucasians revealed no association between RA susceptibility and PADI4 polymorphisms [21, 24]. The inconsistency of findings in various studies might be due to the heterogeneity of populations and complicating environmental factors.

There are not enough data on the association of the *PADI4* gene polymorphisms with RA susceptibility among the Iranian population [29, 30]. Thus far, only one study in Zahedan (southeast of Iran) has investigated the PADI4-104 genetic variants among Iranian RA patients [30]. In line with our findings, the mentioned study stated that the PADI4-104 variant increased the risk of RA in codominant and dominant inheritance models. Moreover, the T allele was reported to increase the risk of RA compared to the C allele, which is consistent with our findings.

CONCLUSION

Our findings reveal that the PADI4-104 genetic variants could be associated with the susceptibility to RA but not with the disease activity in Iranians in Gorgan, northeast of Iran. Further clinical and molecular studies are required to better elucidate the role of the *PADI4* gene polymorphisms in the RA pathogenesis.

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