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Original article

Association between the rs7700944 polymorphism in the TIM-4 gene and rheumatoid arthritis in Zahedan, southeast Iran

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ABSTRACT

Introduction: Recently, an association between rheumatoid arthritis (RA) and the rs7700944 G>A variant in the T-cell immunoglobulin and mucin domains 4 (TIM-4) has been reported. **Objective:** The present study aimed at investigating the impact of that polymorphism on susceptibility to RA in a sample of the Iranian population.

Patients and methods: This case-control study was conducted on 120 patients with RA and 120 healthy subjects. The rs7700944 polymorphism in the TIM-4 gene was determined using tetra amplification refractory mutation system-polymerase chain reaction (T-ARMS-PCR) assay.

Results: No significant difference was observed regarding the rs7700944 polymorphism of the TIM-4 gene between patients with RA and normal individuals. In females, no significant association was found between the groups concerning the rs7700944 polymorphism of the TIM-4 gene. In males, the GA+AA genotype increased the risk of RA in comparison with the GG genotype (OR = 5.15, 95% CI = 1.30-20.48, P = 0.020). Furthermore the results showed that the rs7700944 A allele increased the risk of RA (OR = 4.39, 95% CI = 1.43-13.54, P = 0.009).

Conclusion: Our results do not support an association between the rs7700944 polymorphism of the TIM-4 gene and RA. An interaction between this polymorphism and sex suggests a sex-specific association between this single nucleotide polymorphism and RA, which remains to be fully elucidated.

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Associação entre o polimorfismo rs7700944 no gene TIM-4 e artrite reumatoide em Zahedan, sudeste do Irã

R E S U M O

Palavras-chave:
Artrite reumatoide
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Polimorfismo

Introdução: Recentemente, relatou-se uma associação entre artrite reumatoide (AR) e a variante rs7700944 G>A nos domínios imunoglobulina e mucina de células T (TIM-4).

Objetivo: Investigar o impacto desse polimorfismo na suscetibilidade a AR em uma amostra da população iraniana.

Pacientes e métodos: Este estudo caso-controle foi conduzido em 120 pacientes com AR e 120 indivíduos saudáveis. O polimorfismo rs7700944 do gene TIM-4 foi determinado usando-se o ensaio tetra *amplification refractory mutation system-polymerase chain reaction* (T-ARMS-PCR).

Resultados: Não se observou diferença significativa quanto ao polimorfismo rs7700944 do gene TIM-4 entre os pacientes com AR e os indivíduos saudáveis. Nas mulheres, não houve associação significativa quanto ao polimorfismo rs7700944 do gene TIM-4 nos dois grupos. Nos homens, o genótipo GA+AA, em comparação ao GG, aumentou o risco para AR (OR = 5,15; IC 95% = 1,30-20,48; P = 0,020). Além disso, os resultados mostraram que o alelo rs7700944 A aumentou o risco para AR (OR = 4,39; IC 95% = 1,43-13,54; P = 0,009).

Conclusão: Nossos resultados não confirmam a existência de associação entre AR e o polimorfismo rs7700944 do gene TIM-4. Uma interação entre esse polimorfismo e sexo sugere uma associação sexo-específica entre AR e esse polimorfismo de nucleotídeo único, que ainda requer elucidação.

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Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease that results in severe cartilage damage and bone destruction in synovial joints. Despite the etiology of the disease is still unknown, RA is considered to be influenced by the combination of genetic and environmental factors.^{1,2} It has been estimated that RA affect about 1% of adult population worldwide, and genetic factors have been estimated to account for 60% of the disease risk.³

The T-cell immunoglobulin domain and mucin domain (TIM) gene family consists of three genes located on chromosome 5q23. Three of the family members (TIM-1, TIM-3, and TIM-4) are conserved between mouse and man, encode for cell surface glycoproteins with common structural motifs. TIM family of genes encodes proteins that are expressed by T cells and contain an IgV-like and a mucin-like domain and have been shown to critically regulate adaptive immunity.^{4,5}

TIM-4 was identified as a natural ligand for TIM-1, and the interaction of TIM-1 and TIM-4 stimulate T cell proliferation and activation.⁶ TIM-4 is a phosphatidylserine (PS) receptor expressed in mature antigen presenting cells that enhances phagocytosing activity of apoptotic cells by macrophages to maintain the homeostasis.⁷ TIM-4 plays an essential role in controlling of adaptive immunity by regulating the clearance of antigen-specific cells.⁸ Mice lack of TIM-4 causes failure to clean apoptotic bodies in vivo, leading to dysregulated lymphocyte activation and signs of systemic autoimmunity,⁹ implying that TIM-4 may be associated with the susceptibility to allergic and autoimmune diseases. TIM-1 and TIM-3 gene polymorphisms have been shown to be associated with susceptibility to RA.¹⁰⁻¹⁴

There is few data regarding the association of TIM-4 gene polymorphisms and risk of RA. Recently, Xu et al.¹⁵ have found an association between TIM-4 rs7700944 polymorphism with RA susceptibility in Chinese Han and Hui populations. Therefore, the present study was aimed to evaluate the impact of TIM-4 rs7700944 polymorphism on susceptibility to RA in a sample of Iranian population.

Material and methods

Patients

We investigated the possible association between rs7700944 polymorphism of TIM-4 and RA susceptibility in 120 patients (104 female and 16 male) with an average age of 44.8 ± 12.8 years fulfilling the American College of Rheumatology (ACR) criteria for RA.¹⁶ All the subjects were patients of the Rheumatology Clinic at Zahedan University of Medical Sciences.^{2,17,18} The control group consisted of 120 healthy individual (85 female and 35 male) with a mean age of 44.9 ± 12.4 years and unrelated to RA patients. The ethics committee of Zahedan University of Medical Sciences approved the project and informed consent was taken from all patients and healthy individuals. Blood samples from patients and healthy control were collected in Na-EDTA tubes. Genomic DNA was extracted from peripheral blood samples that had been collected into tubes containing EDTA as described previously.¹⁸

The TIM-4 genomic sequences (NT_023133.13) were obtained from the National Center for Biotechnology Information (NCBI, <http://www.ncbi.nlm.nih.gov>). We searched the polymorphisms and designed the primers for T-ARMS-PCR assay, which is a simple and rapid method for detection of

Table 1 – The primers sequences used for detection of the rs7700944 polymorphism in the TIM-4 gene by using T-ARMS-PCR assay.

Primers	Sequence (5' to 3')	Amplicons size
FO	TCTGGTGTCTTCTGTCTAGCTCCTTAG	364 bp
RO	TTGTGAGCAATTGTCTGAATCTATGAGG	
FI (G allele)	CTAATGAGGCAAGACAATAAAGTGTGTCAG	168 bp
RI (A allele)	TCTCTTTGGTGCTTGAACCTGAGATT	252 bp

FO, forward outer; RO, reverse outer; FI, forward inner; RI, reverse inner.

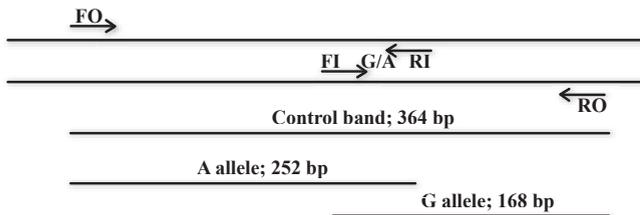


Fig. 1 – Schematic representation of the tetra amplification refractory mutation system-polymerase chain reaction (Tetra-ARMS-PCR) assay for detection of the rs7700944 polymorphism in the TIM-4 gene. Two forward and two reverse primers are used to generate three potential PCR products. Primers FO and RO give a 364 bp product (control band). Primers FO and RI amplify the A allele, generating a 252 bp product, and primers FI and RO generate a 168-bp product for the G allele.

single nucleotide polymorphism (SNP)¹⁹ (Table 1). The schematic representation was shown in Fig. 1.

PCR was performed by using commercially available PCR premix (AccuPower PCR PreMix; BIONEER, Daejeon, Korea) according to the manufacturer's instructions. Briefly, 1 µL template DNA (~100 ng/µL), 1 µL of each primer (10 pmol/µL), and 15 µL DNase-free water were added to AccuPower PCR PreMix.

Amplification was done with an initial denaturation step at 95 °C for 5 minutes, followed by 30 cycles of 30 seconds at 95 °C, 22 seconds at 62 °C, and 25 seconds at 72 °C, with a final step at 72 °C for 10 minutes. PCR products were verified on a 2.0% agarose gel contained 0.5 µg/mL ethidium bromide and photographs was taken (Fig. 2). To confirm genotyping quality, all polymorphisms in random samples were re-genotyped.

Statistical analysis

Statistical analysis was performed using statistical software package SPSS 18 software. We estimated the Hardy-Weinberg equilibrium (HWE) separately for cases and controls. The associations between genotypes of TIM-4 gene and RA were assessed by computing the *odds ratio* (OR) and 95% confidence intervals (95% CI) from logistic regression analyses adjusted for sex and age.

Results

There was no significant difference between the groups regarding age ($P = 0.815$), but the sex was significantly different between the groups ($P < 0.05$).

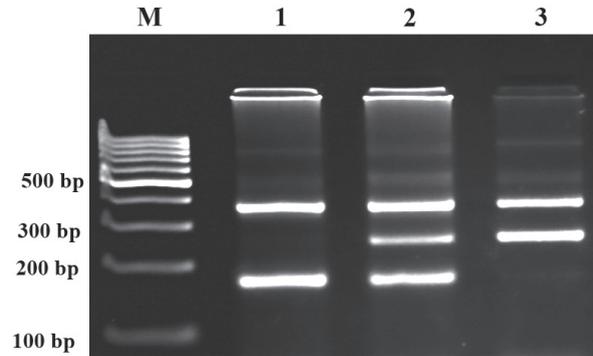


Fig. 2 – Photograph of the PCR product of the the rs7700944 polymorphism in the TIM-4 gene. Product sizes were 168 bp for G allele, 252 bp for A allele, and 364 bp for two outer primers (control band). M, DNA marker; Lane 1, rs7700944 GG; Lane 2, GA; Lane 3, AA.

Table 2 shows the genotype and allele frequencies of rs7700944 polymorphism in RA patients and in controls. No significant differences were found between the groups regarding TIM-4 rs7700944 polymorphism ($\chi^2 = 2.253$, $P = 0.324$). This polymorphism was not associated with RA susceptibility/protection in codominant (OR = 1.17, 95% CI = 0.68-2.01, $P = 0.557$, GA vs. GG; and OR = 3.07, 95% CI = 0.55-17.02, $P = 0.200$, AA vs. GG), dominant (OR = 1.25, 95% CI = 0.74-2.13, $P = 0.410$,

Table 2 – Genotype and allele frequencies of the rs7700944 polymorphism in the TIM-4 gene in rheumatoid arthritis and normal subjects.

rs7700944 G>A	RA n (%)	Control n (%)	^a OR (95% CI)	P
Codominant				
GG	68 (56.6)	77 (64.2)	1.00	—
GA	47 (39.2)	41(34.2)	1.17 (0.68-2.01)	0.557
AA	5 (4.2)	2 (1.6)	3.07 (0.55-17.02)	0.200
Dominant				
GG	68 (56.6)	77 (64.2)	1.00	—
GA+AA	52 (43.4)	43 (35.8)	1.25 (0.74-2.13)	0.410
Recessive				
GG+GA	115 (95.8)	118 (98.4)	1.00	—
AA	5 (4.2)	2 (1.6)	2.90 (0.53-15.98)	0.221
Allele				
G	183 (76.3)	195 (81.3)	1.00	—
A	57 (23.7)	45 (18.7)	1.35(0.87-2.09)	0.181

RA, rheumatoid arthritis; OR, odds ratio; 95% CI, 95% confidence intervals.

^aAdjusted for sex and age.

Table 3 – Genotype and allele frequencies of the rs7700944 polymorphism in the TIM-4 gene in rheumatoid arthritis and normal subjects within sex.

rs7700944	Female				Male			P
	Case (n)	Control (n)	^a OR (95%CI)	P	Case (n)	Control (n)	^a OR (95%CI)	
Codominant								
GG	60	48	1.00	—	8	29	1.00	—
GA	41	35	0.94 (0.52-1.69)	0.85	6	6	3.77 (0.89-15.97)	0.071
AA	3	2	1.19 (0.19-7.77)	0.89	2	0	—	—
Dominant								
GG	60	48	1.00	—	8	29	1.00	—
GA+AA	44	37	0.95 (0.53-1.70)	0.87	8	6	5.15 (1.30-20.48)	0.020
Recessive								
GG+GA	101	83	1.00	—	14	35	1.00	—
AA	3	2	1.17 (0.20-7.20)	0.87	2	0	—	—
Allele								
G	161	131	1.00	—	22	58	1.00	—
A	47	39	0.98 (0.60-1.59)	0.96	10	6	4.39 (1.43-13.54)	0.009

OR, odds ratio; 95%CI, 95% confidence intervals.
^aAdjusted for age.

GA+AA vs. GG) and recessive (OR = 2.90, 95% CI = 0.53-15.98, P = 0.221, AA vs. GG+GA) tested inheritance models (Table 2).

The allele frequency was no significantly different between the groups ($\chi^2 = 1.79$, P = 0.180). The genotype in TIM-4 rs7700944 polymorphism in case and control group was in HWE ($\chi^2 = 0.794$, P = 0.372, and $\chi^2 = 1.762$, P = 0.183, respectively).

Analysis of TIM-4 rs7700944 polymorphism with covariate sex (adjusted for age) was done (Table 3). In females, no significant association was found between the groups regarding TIM-4 rs7700944 polymorphism. In males, the GA+AA increased risk of RA in comparison with GG (OR = 5.15, 95% CI = 1.30-20.48, P = 0.020). Furthermore the results showed the rs7700944 A allele increased the risk of RA (OR = 4.39, 95% CI = 1.43-13.54, P = 0.009). We also found an interaction between rs7700944 polymorphism and sex, which suggests a sex-specific association between this SNP and RA.

Discussion

In the present study, we evaluated the association of the rs7700944 polymorphism in the TIM-4 gene and susceptibility to RA in a sample of Iranian population. No significant association was found between rs7700944 polymorphism and RA. Our finding showed the GA+AA increased risk of RA in comparison with GG in males, which propose a sex-specific association between this SNP and RA. There is little and controversial studies regarding TIM-4 polymorphisms and RA. Xu et al.¹⁵ investigated the rs7700944 polymorphism of the intron region of TIM-4 gene in Chinese Han and Hui populations. They found that the rs7700944 GA genotype increased the risk of RA in Chinese Han population, while it might be a protective genotype in Chinese Hui ethnicity. Likewise, GG was the risk genotype for RA in Hui ethnicity, but it might be a protective genotype in Chinese Han population.¹⁵ Although the G allele have been found to be more frequent in both populations, but the risk allele of this polymorphism for RA in these two populations were different, the risk allele in Hui was G (OR = 1.823; 95% CI = 1.330-2.498; P < 0.01), while indi-

viduals with A allele in this SNP site apparently were more susceptible to RA in Chinese Han (OR = 1.930; 95% CI = 1.412-2.636; P < 0.01).¹⁵

Our finding showed that the genotype of AA was not common. Our results are in agreement with the findings of Xu et al.¹⁵ which have found that AA genotype was not common in Chinese Han and Hui populations. We also found an interaction between rs7700944 polymorphism and sex, which suggests a sex-specific association between this SNP and RA.

Polymorphisms of TIM-4 have been associated with asthma²⁰ and atopic dermatitis (AD).²¹ TIM-3 polymorphisms have been shown to be associated with RA,¹⁰⁻¹² pancreatic cancer,²² renal cell carcinoma,²³ gastric cancer²⁴ as well as disease susceptibility and hepatocellular carcinoma (HCC) traits associated with hepatitis B virus infection.²⁵ TIM-1 polymorphism have been shown to be associated with RA,^{13,14,26} allergic rhinitis²⁷ and AD.²¹

One of the limitations of our study is relatively small sample size, particularly in the genotype analysis in sex group. Other limitation of our study is the statistical significant differences between the sex of patients and controls. Though, this difference probably does not have a significant impact on the results, since we used sex as a covariate in regression analysis.

In conclusion, we found no significant association between TIM-4 rs7700944 polymorphism and RA in a sample of Iranian population. While, an interaction between rs7700944 polymorphism and sex was observed, which suggests a sex-specific association between this SNP and RA. The findings regarding sex-specific association between rs7700944 and RA should be interpreted cautiously because of the small sample size. Further association studies with large sample size and different ethnicities are needed to confirm our findings.

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Conflicts of interest

The authors declare no conflicts of interest.

REFERENCES

- 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.
- Turesson C, Matteson EL. Genetics of rheumatoid arthritis. *Mayo Clin Proc*. 2006;81(1):94-101.
- Kuchroo VK, Umetsu DT, DeKruyff RH, Freeman GJ. The TIM gene family: emerging roles in immunity and disease. *Nat Rev Immunol*. 2003;3(6):454-62.
- Meyers JH, Sabatos CA, Chakravarti S, Kuchroo VK. The TIM gene family regulates autoimmune and allergic diseases. *Trends Mol Med*. 2005;11(8):362-9.
- Meyers JH, Chakravarti S, Schlesinger D, Illes Z, Waldner H, Umetsu SE, et al. TIM-4 is the ligand for TIM-1, and the TIM-1-TIM-4 interaction regulates T cell proliferation. *Nat Immunol*. 2005;6(5):455-64.
- Nurtanio N, Yang PC. Role of TIM-4 in innate or adaptive immune response. *N Am J Med Sci*. 2011;3(5):217-21.
- Albacker LA, Karisola P, Chang YJ, Umetsu SE, Zhou M, Akbari O, et al. TIM-4, a receptor for phosphatidylserine, controls adaptive immunity by regulating the removal of antigen-specific T cells. *J Immunol*. 2010;185(11):6839-49.
- Rodriguez-Manzanet R, Sanjuan MA, Wu HY, Quintana FJ, Xiao S, Anderson AC, et al. T and B cell hyperactivity and autoimmunity associated with niche-specific defects in apoptotic body clearance in TIM-4-deficient mice. *Proc Natl Acad Sci USA*. 2010;107(19):8706-11.
- Chae SC, Park YR, Shim SC, Yoon KS, Chung HT. The polymorphisms of Th1 cell surface gene Tim-3 are associated in a Korean population with rheumatoid arthritis. *Immunol Lett*. 2004;95(1):91-5.
- Xu J, Yang Y, Liu X, Wang Y. The -1541 C>T and +4259 G>T of TIM-3 polymorphisms are associated with rheumatoid arthritis susceptibility in a Chinese Hui population. *Int J Immunogenet*. 2011;38(6):513-8.
- Song YW, Im CH, Park JH, Lee YJ, Lee EY, Lee EB, et al. T-cell immunoglobulin and mucin domain 3 genetic polymorphisms are associated with rheumatoid arthritis independent of a shared epitope status. *Hum Immunol*. 2011;72(8):652-5.
- Xu JR, Yang Y, Liu XM, Sun JY, Wang YJ. Polymorphisms of the TIM-1 gene are associated with rheumatoid arthritis in the Chinese Hui minority ethnic population. *Genet Mol Res*. 2012;11(1):61-9.
- Chae SC, Song JH, Shim SC, Yoon KS, Chung HT. The exon 4 variations of Tim-1 gene are associated with rheumatoid arthritis in a Korean population. *Biochem Biophys Res Commun*. 2004;315(4):971-5.
- Xu J, Yang Y, Liu X, Wang Y. Genetic variation and significant association of polymorphism rs7700944 G>A of TIM-4 gene with rheumatoid arthritis susceptibility in Chinese Han and Hui populations. *Int J Immunogenet*. 2012;39(5):409-13.
- 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.
- Sandoughi M, Fazaeli A, Bardestani G, Hashemi M. Frequency of HLA-DRB1 alleles in rheumatoid arthritis patients in Zahedan, southeast Iran. *Ann Saudi Med*. 2011;31(2):171-3.
- 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.
- Hashemi M, Moazeni-Roodi A, Bahari A, Taheri M. A Tetra-Primer Amplification Refractory Mutation System-Polymerase Chain Reaction for the Detection of rs8099917 IL28B Genotype. *Nucleosides Nucleotides Nucleic Acids*. 2012;31(1):55-60.
- Zhao B, Abdelmoudjib G, Li J, Li H, Wei C, Gong Y, et al. Two polymorphisms in the TIM-4 gene are associated with asthma in a Chinese Han population. *Int J Immunogenet*. 2011;38(1):31-5.
- Page NS, Jones G, Stewart GJ. Genetic association studies between the T cell immunoglobulin mucin (TIM) gene locus and childhood atopic dermatitis. *Int Arch Allergy Immunol*. 2006;141(4):331-6.
- Tong D, Zhou Y, Chen W, Deng Y, Li L, Jia Z, et al. T cell immunoglobulin- and mucin-domain-containing molecule 3 gene polymorphisms and susceptibility to pancreatic cancer. *Mol Biol Rep* 2012.
- Cai C, Wang L, Wu Z, Li M, Chen W, Sun Y. T-cell immunoglobulin- and mucin-domain-containing molecule 3 gene polymorphisms and renal cell carcinoma. *DNA Cell Biol*. 2012;31(7):1285-9.
- Cao B, Zhu L, Zhu S, Li D, Zhang C, Xu C, et al. Genetic variations and haplotypes in TIM-3 gene and the risk of gastric cancer. *Cancer Immunol Immunother*. 2010; 59(12):1851-7.
- Li Z, Liu Z, Zhang G, Han Q, Li N, Zhu Q, et al. TIM3 gene polymorphisms in patients with chronic hepatitis B virus infection: impact on disease susceptibility and hepatocellular carcinoma traits. *Tissue Antigens*. 2012;80(2):151-7.
- Chae SC, Park YR, Song JH, Shim SC, Yoon KS, Chung HT. The polymorphisms of Tim-1 promoter region are associated with rheumatoid arthritis in a Korean population. *Immunogenetics*. 2005;56(10):696-701.
- Mou Z, Shi J, Tan Y, Xu R, Zhao Z, Xu G, et al. Association between TIM-1 gene polymorphisms and allergic rhinitis in a Han Chinese population. *J Investig Allergol Clin Immunol*. 2010;20(1):3-8.