**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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**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. 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.

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.

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. 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. TIM-4 plays an essential role in controlling of adaptive immunity by regulating the clearance of antigen-specific cells. Mice lack of TIM-4 causes failure to clean apoptotic bodies in vivo, leading to dysregulated lymphocyte activation and signs of systemic autoimmunity, 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.

There is few data regarding the association of TIM-4 gene polymorphisms and risk of RA. Recently, Xu et al. 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. All the subjects were patients of the Rheumatology Clinic at Zahedan University of Medical Sciences. 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 controls 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.

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
single nucleotide polymorphism (SNP)\(^9\) (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 regenotyped.

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).

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

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequence (5’ to 3’)</th>
<th>Amplicons size</th>
</tr>
</thead>
<tbody>
<tr>
<td>FO</td>
<td>TCTGGTGTCTTCTGCTAGCTCCTTAG</td>
<td>364 bp</td>
</tr>
<tr>
<td>RO</td>
<td>TTGTGACAAATTGTGCAGATCTATGAGG</td>
<td></td>
</tr>
<tr>
<td>FI (G allele)</td>
<td>CTAATGAGGCAAGACATAAAAGTGTGTCAG</td>
<td>168 bp</td>
</tr>
<tr>
<td>RI (A allele)</td>
<td>TCTCCTTGGGTGCTGGACCTGAGATT</td>
<td>252 bp</td>
</tr>
</tbody>
</table>

Statistical analysis

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 (χ² = 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).
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 not 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 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.

### Acknowledgements

This work was supported by a dissertation grant from Zahedan University of Medical Sciences. We thank all subjects who willingly participated in the study.

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

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

OR, odds ratio; 95%CI, 95% confidence intervals.

$^*$Adjusted for age.
Conflicts of interest

The authors declare no conflicts of interest.

REFERENCES


