Association Study of Tumor Necrosis Factor Receptor Type II Polymorphism (196R) with Rheumatoid Arthritis in Iranian People

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1. Background

Rheumatoid arthritis (RA) is one of the most common autoimmune diseases. So far, no factor has been found to cause this disease. The prevalence of RA is about 1% in the human population worldwide; moreover, it is twice as prevalent in females than in males (1). A 10-year follow-up study from 2004 to 2014 in the United States showed that the prevalence of RA is increasing (2).

Rheumatoid arthritis is known as a complex disorder characterized by the inflammation of the synovium (the thin lining of a joint). Rheumatoid arthritis is a chronic disease in which genetic and environmental factors contribute to decreasing the tolerance to self-antigens (3). TNF-α is an inflammatory cytokine that plays an important role in the development of RA (4, 5). TNF-α initiates and regulates the cytokine cascade during the pathogenesis of RA. TNF-α stimulates monocytes/macrophages, fibroblasts, and endothelial cells to produce IL-1 and IL-6 or chemokines (CXCL8, CCL2), which are heavily involved in the pathogenesis of RA including leukocytes infiltration and tissue destruction. Inflammation induced by IL-1 and TNF-α can lead to the increased expression of the matrix metalloproteinase, collagenase, and elastase during the pathogenesis of RA, which is responsible for cartilage degradation and bone resorption (6, 7).

TNF-α affects the target cell via two receptors, TNF-RI (TNFRSF1A) and TNF-RII (TNFRSF1B). Although the homology of the extracellular domains of the TNF-α receptor is very close, their intracellular domains are different (8). TNF binding to TNF-RI triggers apoptosis while its binding to
TNF-RII triggers cell survival. Both TNF-RI and TNF-RII are expressed in the synovial tissue in patients with RA (7, 9). The TNF-RI and TNF-RII genes are located on chromosomes 12p13 and 1p36, respectively (10).

Genome-wide association studies have identified that 1p36 locus is associated with RA. Interestingly, TNF-RII is located within this gene area with 10 exons (11). Reports indicate that one of the most important single nucleotide polymorphisms is located in exon 6. This missense mutation results in methionine to arginine substitution (ATG→AGG) polymorphism (rs1061622) that is within the fourth extracellular domain of TNF-RII (12).

Based on the biological and molecular analysis, the TNF-RII gene is considered as one of the best candidates among RA-susceptibility genes. SNP analyses at 196 (rs1061622) indicate that the 196R mutant allele has a different performance than the 196M wild-type allele (13). As reported, there appears to be an association between the exons 6 polymorphism (T676G) and susceptibility to RA (14).

Genetic markers may differ among different populations. These differences may affect the TNF-RII association with RA disease in different populations, leading to contradictory results. Previous studies have reported an association between TNF-RII 196R allele polymorphisms and RA in other populations (15, 16). Therefore, we need to evaluate the genotype and allele frequency in 196 (rs1061622) the TNF-RII gene and investigate the genetic risk of this polymorphism in our population.

3.2. DNA Extraction

Using QIAamp® DNA Mini extracting kit, genomic DNA was extracted from 500 µL of blood lymphocytes; the extracted DNA was stored at -20°C until use for genotyping.

3.3. SNP Genotyping

The real-time PCR method was used for the determination of M196R allele polymorphism in the TNF-RII gen. All PCR tests were performed in a volume of 50 µL, containing TaqMan Universal PCR Master Mix, specific TaqMan SNP genotyping assays, life technologies, assay ID (C886123220, part number 4351379, USA) and genomic DNA.

Thermal cycling conditions were 5 min at 95°C, followed by 40 cycles of 95°C for 15 s, and annealing and extension were performed at 60°C for one minute. After PCR, to measure the allele-specific fluorescence, the genotyping of rs061622 SNPs was performed by TaqMan allelic discrimination with a thermocycler 7900 ABI system (17).

In this study, two TaqMan probes labeled with different fluorescent dyes were used for each sample in an allelic discrimination assay. The probes labeled with fluorescent dye FAM had a perfect match with the wild-type allele. The probes were labeled with fluorescent dye VIC because it was perfectly matched with the mutated allele (Figure 1).

3.4. Statistical Analysis

We used SPSS version 22 software for statistical analyses. The t-test was used to compare the mean age of cases and controls. In addition, Hardy-Weinberg equilibrium (HWE) was investigated in case and control groups of RA. The chi-square test was used to compare genotype frequencies and allele and genotype distributions in patients and controls. The associations between genotypes of the TNF-RII gene and RA were assessed by computing odds ratios (ORs) and 95% confidence intervals (95% CIs) using logistic regression analysis. The significance level was set at P < 0.05.

4. Results

Our study aimed to investigate the association between the functional 196R polymorphism of TNF-RII and RA. The mean age (± SD) of the participants was 48.3 ± 13.1 years, ranging from 23 to 77 years. The mean age (± SD) of patients was 48.1 ± 12.6 years. Table 1 presents the characteristics of RA patients. The differences between normal and patient groups were not significant in terms of age and sex (P > 0.05, Table 1).

Table 2 presents the genotype and allele frequencies of non-synonymous polymorphism rs1061622 of the TNF-RII gene in RA patients and controls. Significant differences were observed between genotype frequencies of the two groups in terms of 196R polymorphism. Moreover, the
rs1061622 variant increased the risk of RA. The GG genotype was more common in RA patients than in normal subjects (OR for GG genotype: 2.6, 95% CI: 1.135 - 5.6, P = 0.035) (Table 2). Moreover, the G allele frequency in RA patients the rs1061622 was significantly higher than in the control group (OR for G phenotype: 2.30, 95% CI: 1.1 - 5.1, P = 0.04) (Table 2).

Of a total of 135 (32.5%) alleles in patients with RA, 65 cases had 196R alleles, whereas of a total of 200 (26%) alleles in healthy individuals, 52 cases had 196R alleles. In addition, the G allele increased the risk of RA.

5. Discussion

In this case-control study, we investigated the relationship between TNF-RII rs1061622 polymorphism and
arginine changes the pathway for TNF-RII apoptosis that is 
Till et al. showed that the change of methionine to 
creases IL-6 production in the cells carrying the 196R allele 
exon 6 TNF-RII changes methionine to arginine which in-
Horiuchi et al. showed that a polymorphism in codon 196 
and apoptosis after TNF-α gene tends to characterize increased cytokine production 
diographic severity and diagnosis of RA (21, 23). 
and 196R allele (19, 20). However, Goeb et al. study revealed 
significant association between the severity of RA disease 
and East Asian population. In addition, in previous studies, 
a significant association was found between TNF-RII 196R 
polymorphism and lupus disease in the Japanese population 
(26). As noted, this association between RA and TNF-RII 
polymorphism was studied in the Japanese population. A similar study in France showed the same results (15). 
In conclusion, we investigated the association between 
TNF-RII gene (rs1061622) polymorphisms and RA in a sample of the Iranian population for the first time. Our results supported a significant association between the missense mutation, which involves a single base substitution at codon 196 (ATG → AGG) in exon 6 of the TNF-RII gene, and susceptibility to RA. 
Association studies may be limited by the heterogeneity of the population, small sample sizes, and the statistically significant differences between experimental and control groups. The difference in results could be due to different patient population genetic background in different studies. To confirm our findings, it is necessary to conduct further association studies in different ethnicities. 

Footnotes

Authors’ Contribution: Concept and design: Fateme Pak, Parviz Kokhaei, Mohammad Hassan Aminikhoo, and Mehdi Fasihi. Acquisition of data: Mehdi Barati and Mohammad Hassan Aminikhoo. Primary analysis and interpretation: Kazem Ahmadi. Drafting manuscript: Mohammad Hassan Aminikhoo, Mehdi Barati, and Zahra Rasouli Nejad. Supervision and critical revision: Parviz Kokhaei and Fateme Pak.

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### Table 2. Genotype and Allele Frequency Distribution of TNF-RII rs1061622 Polymorphism in Rheumatoid Arthritis (RA) Patients and Healthy Subjects

| Genotype | RA Patients | Control | OR | Confidence Interval | P  |
|----------|-------------|---------|----|---------------------|----|
| 196 MM   | 47 (47)     | 53 (53) | 0.9| (0.5 - 1.8)         | 0.74|
| 196 MR   | 41 (41)     | 42 (42) | 2.6| (1.35 - 5.6)        | 0.035|
| 196 RR   | 12 (12)     | 5 (5)   | 0.04| (1.1 - 5.1)         | 0.04|
| Allele 196 M | 47 (47) | 53 (53) | 0.74 | (0.5 - 1.8) | 0.74|
| 196 R    | 65          | 52      |    |                     |    |

Abbreviations: CI, 95% confidence interval; OR: odds ratio; RA; rheumatoid arthritis; TNF-RII, tumor necrosis factor receptor II. 

*Values are expressed as No. (%).
sonal consultants or promoters for companies or other organizations with financial interests for the promotion of particular health care products and services.

Ethical Approval: All human interventions performed in this study were conducted following the standards of the Ethics Committee of the Semnan University of Medical Sciences and the Helsinki Declaration and its later amendments. After obtaining the consent form, we enrolled individuals in this study.

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Informed Consent: Informed consent was obtained from all individual participants. The ethical approval number was 92/372650 at the Semnan University of Medical Sciences.

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