

Single Nucleotide Polymorphism of *TYK2* Gene and Susceptibility to Rheumatoid Arthritis in Iranian Population

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Abstract

Background: Rheumatoid Arthritis (RA) is a debilitating disorder in which the immune system mainly targets the synovial tissue. Janus kinase family including tyrosine kinase 2 (TYK2) is one of the crucial mediators of the downstream signaling pathway of inflammatory cytokines that further contributes to RA pathogenesis. In this study, the association of *TYK2* gene rs34536443 polymorphism, which may affect the function of TYK protein and, hence, the inflammatory settings, with RA susceptibility was investigated. Moreover, its correlation with demographic and serological features of the patients was assessed.

Methods: In the present study, 700 RA patients and 700 sex, age and ethnicity-matched healthy individuals as the control group were included. MGB TaqMan real-time allelic discrimination method was used to determine the rs34536443 polymorphism. Rheumatoid factor, anti-cyclic citrullinated peptide antibody, erythrocyte sedimentation rate and C-reactive protein were also measured.

Results: The frequency of rs34536443 minor allele (C allele) was not different between patients and control group [1.7 vs. 2.61 percent, OR (95% CI)=1.35 (0.78-2.33);p=0.27]. There was not a statistically significant association between rs34536443 genotypes and RA susceptibility. Genotypes of rs34536443 polymorphism were associated nor with demographic neither with serological features of RA patients.

Conclusion: In the present study, there was not any association between *TYK2* gene rs34536443 polymorphism with either disease susceptibility, demographic and serological features of Iranian RA patients. These findings are not compatible with previous works from other ethnicities, further supporting the role of genetics in disease susceptibility.

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Introduction

Rheumatoid Arthritis (RA) is an auto-immune disease characterized by inflammatory cascades primarily happening in joints but the other organs, albeit less so, can be affected¹. According to the World Health Organization (WHO) regions classification, the prevalence of RA was reported in a range from 0.37% in Eastern Mediterranean region to 1.25% in American region². RA exhibits a strong gender predilection with the female to male ratio of 4.7:1 in low and middle-income countries². RA has the estimated disability-adjusted life years of 4.8 million in 2010 which was ranked as the 42nd contributor to the global disability³. Thus, according to its relatively high prevalence and morbidity, RA is one of the major fields of interests in Rheumatology and Immunology.

In RA, arthritis-associated antigens are presented by dendritic cells to naïve CD4+ T cells⁴. Following their activation and differentiation toward the T Helper (TH) 17 cells, the immune system orchestrates an inflammatory cascade with a high spectrum of cytokines production. These cytokines affect a wide range of receptors, which are linked to the signaling mediators from Janus Kinase (JAK) family⁵. JAK family has four major members; JAK1, JAK2, JAK3, and tyrosine kinase 2 (TYK2)⁶. They are non-receptor tyrosine kinases, which are activated after the binding of cytokines to their relative receptors. Following their activation, they make a docking site for signaling molecules, mostly members of the Signal Transducer and Activator of Transcription (STAT) family⁶. STAT molecules make

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a dimer after phosphorylation which then enter the nucleus and induce the expression of further molecules of the inflammatory cascade ⁵.

TYK2 is linked to the cytoplasmic domain of type I and II cytokine receptors, which are involved in the signaling pathway of interleukin (IL)-6, IL-12 and IL-23 ⁵. Its gene is located in 19p13.2 position and its polymorphisms have been linked to a variety of autoimmune diseases ⁷. It has been reported previously that TYK2 gene polymorphisms influenced the susceptibility to RA ⁸, Systemic Lupus Erythematosus (SLE) ⁹, systemic sclerosis ¹⁰, Inflammatory Bowel Disease (IBD) ¹¹ and Multiple Sclerosis (MS) ¹². The present study aimed to investigate the rs34536443 Single Nucleotide Polymorphism (SNP) of TYK2 gene in Iranian RA patients. Also, its association with demographic and serological features of the patients was described as well.

Materials and Methods

Study population

This study was conducted on 700 RA patients from the outpatient’s rheumatology clinic of Shariati Hospital who were diagnosed based on the American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) 2010 RA classification criteria ¹³. Control group of this study composed of 700 age, sex, and ethnicity matched healthy individuals. Written informed consent was obtained from all the participants. The present study was approved by the ethics committee of Yazd University of Medical Sciences.

DNA preparation and polymorphism analysis

For all participants, peripheral blood was collected into tubes with EDTA as an anticoagulant and Erythrocyte Sedimentation Rate (ESR) was measured. Genomic DNA was extracted using standard proteinase K digestion- phenol/chloroform extraction method ¹⁴. The extracted DNA samples were stored at -20°C. Approximately, 20 ng of the genomic DNA of each sample was used for genotyping. Genotyping of TYK2 gene rs34536443 was performed using the MGB-TaqMan Allelic Discrimination method (Applied Biosystems, Foster City, CA, USA). Amplification was performed in 10 µl reaction volumes, containing 5 µl of the TaqMan Genotyping master mix, 0.25 µl of TaqMan Genotyping assay mix, 0.25 µl of distilled water, and 4.5 µl of genomic DNA.

Serologic analysis

The plasma samples were obtained after centrifuging the patients’ whole blood samples, which were drained previously into EDTA-containing test tubes. The Rheumatoid Factor (RF) and anti-Cyclic Citrullinated Peptide antibody (anti-CCP), and C-Reactive Protein (CRP) were measured by commercially available kits.

Statistical analysis

Control group was tested to be in the Hardy-Weinberg Equilibrium. The frequency of alleles and geno-

types were compared between RA patients and control group using chi-square test. The mean comparison of ESR level, RF and anti-CCP titers of different genotypes in case group was done by ANOVA. p-values less than 0.05 were considered statistically significant. All analyses were performed using SPSS package for Windows (Version 19.0, IBM SPSS Inc., USA).

Results

The demographic and clinical characteristics of our study population are shown in table 1. The mean age of the RA patients was 50.32±11.27 years whereas the mean age of control group was 48.44±12.37 years. The female to male ratio was 3.48:1 in patients group and 6.52:1 in healthy individuals. The mean ESR level was higher in patients (20.64±17.14 vs. 10.27±7.95), but the healthy individuals had a higher percentage of positive CRP (61.1 vs. 46%). The means of RF and anti-CCP titer were 85.5±7.63 and 138.7±18.96 international unit (IU)/ml, respectively among RA patients.

The allele and genotype frequencies of rs34536443 in RA patients and control group are shown in table 2. The frequency of minor allele (C allele) was not different among patients and healthy individuals (1.7 vs. 2.61%, OR (95% CI)=1.35 (0.78-2.33), p-value= 0.27). The frequencies of CC and GC genotypes were negligible; however, according to our analyses, there was not a statistically significant difference between the geno-typic distribution of the case and control groups. Moreover, the dominant model of CC+CG had no significant differences between patients and controls. Finally, the association of genotype frequencies with clinical characteristics of RA patients is shown in table 3. There was not any correlation between genotype frequencies and patients’ age and gender, ESR level, and the titer of RF and anti-CCP.

Discussion

In the present study, an attempt was made to investigate the association of TYK2 gene rs34536443 polymorphism with RA susceptibility. According to our

Table 1. Demographic and clinical data of RA patients and healthy controls

Characteristics	RA patients (n=700) n (%)	Healthy controls (n=700) n (%)
Gender		
Male	156 (22.2%)	93 (13.3%)
Female	544 (77.8%)	607 (86.4)
Smoking		
Positive	-	35 (5%)
Negative	-	665 (95%)
CRP		
Positive	322 (46%)	428 (61.1%)
Negative	378 (54%)	272 (38.9%)
ESR	20.64 ±17.14	10.27±7.95
Age (yr)	50.32 ± 11.27	48.44 ±12.37
RF	85.5 ± 7.63	-
Anti-CCP	138.7±18.96	-

Table 2. Allele and genotype distribution of *TYK2* gene rs34536443 SNP in RA patients and healthy controls

SNP	Allele/Genotype	RA(n=700) n (%)	Control (n=700) n (%)	OR (95% CI)	p-value
rs34536443	C	31 (1.70%)	23 (2.61%)	1.35 (0.78-2.33)	0.27
	G	1369 (98.30%)	1377 (97.39%)	Reference	
	CC	1 (0.14%)	0 (0%)	3 (0.12-73.98)	0.50
	CG	8 (1.14%)	7 (1%)	0.14 (0.41-3.17)	0.79
	CC+CG	9 (1.28%)	7 (1%)	1.28 (0.47-3.48)	0.61
	GG	691 (98.71%)	693 (99%)	Reference	
	HWE			p= 0.05	

RA: Rheumatoid Arthritis; HWE: Hardy-Weinberg Equilibrium

Table 3. Association of genotypes of *TYK2* gene rs34536443 SNP with characteristics of the RA patients

Clinical characteristics	CC*	CG	GG	p-value
Age	30*	54.25±13.27 ^S	50.27±11.15	0.12
ESR	10	27.62±34.55	20.43±16.28	0.42
RF	15.9	44.55±24.96	85.50 ±7.63	0.43
Anti-CCP	17	58.28±32.02	142.24±19.67	0.64
Gender				
Male	0 (0%)	6 (0.8%)	149 (21.3%)	0.785
Female	3 (0.4%)	19 (2.7%)	523 (74.7%)	

* The data was available for only one patient with CC genotype for *TYK2* gene rs34536443 SNP.

results in Iranian population, rs34536443 polymorphisms do not have any influence on RA development. Furthermore, neither demographic features nor RF and the anti-CCP titer was correlated with rs34536443 polymorphisms.

The importance of *TYK2* gene polymorphisms in autoimmune diseases was supported by a vast majority of the studies. The previously studied works on *TYK2* gene polymorphisms in RA are shown briefly in table 4. According to a meta-analysis published in 2011, the rs34536443 and rs2304256 SNPs were associated with auto-immune inflammatory disorders including RA, SLE, MS and IBD¹⁵. But, at that time, there was not any published evidence on rs34536443 polymorphism and RA susceptibility. In 2012, the Genome-Wide Association Study (GWAS) on European RA patients, have reported the association of rs34536443 with RA for the first time⁸. According to results from the Immunochip array, G allele of rs34536443 had a protective effect on RA development. After regrouping the patients based on anti-CCP status, the association remained significant only in seropositive individuals. These findings were also replicated for combined analysis of Immunochip results and imputed GWAS data from non-overlapping samples. In line with mentioned discoveries, Diogo *et al* showed that the three protein-coding variants in *TYK2* gene, including P1104A (rs-34536443) have a protective effect against RA in European patients¹⁶. Additionally, they showed that the same three variants also protect against SLE and IBD. Moreover, based on the phenome-wide association

study, they reported that the P1104A was not associated with non-autoimmune phenotypes. Their findings can support further the role of *TYK2* targeting as the possible promising treatment for these auto-immune diseases.

According to the analyses of the Immunochip array on 2816 European patients with Juvenile Idiopathic Arthritis (JIA), it was shown that G allele of rs-34536443 had a protective effect against oligoarticular and RF-negative polyarticular JIA¹⁷. In contrast to this study, no association was found between *TYK2* gene rs34536443 SNPs and JIA development either at an allelic or genotypic level among Greek patients¹⁸. Unexpectedly, in a study performed on Greece adult RA patients, Myrthianou *et al* revealed that the GC genotype of rs34536443 SNP had a protective role against RA with an OR of 0.33 (0.123-0.90)¹⁹.

In a functional point of view, it has been shown previously that the rs34536443 SNP, located in protein-coding region of *TYK2* gene (Assembly: GRCh37. p13, annotation release: 105, Chromosome: 19, Location: NC_000019.9), can influence the three-dimensional structure of *TYK2*, which can consequently affect its function¹⁸. Moreover, studies on MS patients revealed that the protective C allele of rs34536443 SNP mediates the divergence of T lymphocytes differentiation toward a TH2 phenotype²⁰. They showed that the downstream pathways of *TYK2*, which were activated by interferon (IFN)- β , IL-6 and IL-10 were less pronounced in carriers of GC genotype compared to GG carriers. In addition, they reported that the *GATA3* expression was higher in GC genotype carriers, which can favor the TH2 modulation pathway. Besides, the secretion level of IL-5 and IL-13 was also higher in GC carrier T cells upon activation by CD3/CD28 signaling. Nonetheless, no correlation between rs34536443 polymorphisms and M1/M2 balance of macrophages phenotype was identified in their work. Although these data are governed by MS, according to the crucial role of TH1 and TH17 in RA pathophysiology²¹, this scenario might also be implicated in RA. Confusingly, a certain piece of evidence described that despite the catalytic impairment of *TYK2* P1104A, it has a comparable IFN- α signaling potency relative to the wild-type protein²².

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Table 4. Association of TYK2 gene polymorphism with RA susceptibility in different populations

Ethnicity	Number of cases	Allele	Odds ratio	p-value	Reference
European	11,475	G	0.62	2.3×10^{-14}	(8)
European (US, UK, Germany)	2816	G	0.56 (0.47-0.67)	1×10^{-10}	(17) (JIA)
Greek	125	C	2.45 (0.53-11.55)	0.34	(18) (JIA)
Greek	392	C	0.60 (0.27-1.35)	0.25	(19)
Iranian	700	C	1.35 (0.78-2.33)	0.27	Present study

JIA: Juvenile Inflammatory Arthritis

According to the intimate contribution of JAK family proteins in inflammatory pathways, there was a great interest to target them for the treatment of inflammatory disorders. Tofacitinib, a monoclonal antibody, is a JAK inhibitor approved for RA treatment with long-term safety and efficacy²³. Nevertheless, Tofacitinib targets more specifically the JAK3 and JAK1 with less affinity for TYK2²⁴. Moreover, it was described that the Tofacitinib could bind to the adenosine triphosphate-binding cavity of TYK2, which can further diminish its potential to activate the STAT molecules²⁵. Recent studies have indicated promising outcomes about the efficacy of TYK2 inhibitors in SLE and IBD treatment *via* blocking the IL-12, IL-23, and IFN- γ pathways in animal models with short-term safety profile in human^{26,27}.

Conclusion

In general, JAK family has a key role in mediating the inflammatory responses in auto-immune diseases. TYK2 is one of the family members that its gene polymorphisms were associated with RA susceptibility in different populations (Table 4). However, in the present work, no association was found between the rs-34536443 polymorphism and RA. These findings can further support the determinative role of ethnicity in the etiopathogenesis of such diseases.

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Conflict of Interest

None.

References

- Grassi W, De Angelis R, Lamanna G, Cervini C. The clinical features of rheumatoid arthritis. *Eur J Radiol* 1998; 27(Suppl 1):S18-24.
- Rudan I, Sidhu S, Papan A, Meng SJ, Xin-Wei Y, Wang W, et al. Prevalence of rheumatoid arthritis in low- and middle-income countries: A systematic review and analysis. *J Glob Health* 2015;5(1):010409.
- Cross M, Smith E, Hoy D, Carmona L, Wolfe F, Vos T, et al. The global burden of rheumatoid arthritis: estimates from the global burden of disease 2010 study. *Ann Rheum Dis* 2014;73(7):1316-1322.
- Choy E. Understanding the dynamics: pathways involved in the pathogenesis of rheumatoid arthritis. *Rheumatology* (Oxford) 2012;51(Suppl 5):v3-11.
- Schwartz DM, Kanno Y, Villarino A, Ward M, Gadina M, O'Shea JJ. JAK inhibition as a therapeutic strategy for immune and inflammatory diseases. *Nat Rev Drug Discov* 2017;16(12):843-862.
- Yamaoka K, Saharinen P, Pesu M, Holt VET, Silvenoinen O, O'Shea JJ. The Janus kinases (Jaks). *Genome Biol* 2004;5(12):253.
- Krolewski JJ, Lee R, Eddy R, Shows TB, Dalla-Favera R. Identification and chromosomal mapping of new human tyrosine kinase genes. *Oncogene* 1990;5(3):277-282.
- Eyre S, Bowes J, Diogo D, Lee A, Barton A, Martin P, et al. High density genetic mapping identifies new susceptibility loci for rheumatoid arthritis. *Nat Genet* 2012; 44(12):1336-1340.
- Lee YH, Choi SJ, Ji JD, Song GG. Associations between PTK and TYK2 polymorphisms and systemic lupus erythematosus: a meta-analysis. *Inflamm Res* 2012;61(9): 949-954.
- Lopez-Isac E, Campillo-Davo D, Bossini-Castillo L, Guerra SG, Assassi S, Simeon CP, et al. Influence of TYK2 in systemic sclerosis susceptibility: a new locus in the IL-12 pathway. *Ann Rheum Dis* 2016;75(8):1521-1526.
- Sato K, Shiota M, Fukuda S, Iwamoto E, Machida H, Inamine T, et al. Strong evidence of a combination polymorphism of the tyrosine kinase 2 gene and the signal transducer and activator of transcription 3 gene as a DNA-based biomarker for susceptibility to Crohn's disease in the Japanese population. *J Clin Immunol* 2009;29 (6):815-825.
- Ban M, Goris A, Lorentzen ÅR, Baker A, Mihalova T, Ingram G, et al. Replication analysis identifies TYK2 as a multiple sclerosis susceptibility factor. *Eur J Hum Genet* 2009;17(10):1309-1313.
- Kay J, Upchurch KS. ACR/EULAR 2010 rheumatoid arthritis classification criteria. *Rheumatology* 2012;51 (Suppl 6):vi5-vi9.
- Kochl S, Niederstatter H, Parson W. DNA extraction and quantitation of forensic samples using the phenol-chloroform method and real-time PCR. *Methods Mol Biol* 2005;297:13-30.
- Tao JH, Zou YF, Feng XL, Li J, Wang F, Pan FM, Ye DQ. Meta-analysis of TYK2 gene polymorphisms asso-

- ciation with susceptibility to autoimmune and inflammatory diseases. *Mol Biol Rep* 2011;38(7):4663-4672.
16. Diogo D, Bastarache L, Liao KP, Graham RR, Fulton RS, Greenberg JD, et al. TYK2 protein-coding variants protect against rheumatoid arthritis and autoimmunity, with no evidence of major pleiotropic effects on non-autoimmune complex traits. *PLoS One* 2015;10(4):e0122271.
 17. Hinks A, Cobb J, Marion MC, Prahalad S, Sudman M, Bowes J, et al. Dense genotyping of immune-related disease regions identifies 14 new susceptibility loci for juvenile idiopathic arthritis. *Nat Genet* 2013;45(6):664-669.
 18. Zervou MI, Dimopoulou DG, Eliopoulos E, Trachana M, Pratsidou-Gkertsis P, Andreou A, et al. The genetics of juvenile idiopathic arthritis: Searching for new susceptibility loci. *Mol Med Rep* 2017;16(6):8793-8798.
 19. Myrthianou E, Zervou MI, Budu-Aggrey A, Eliopoulos E, Kardassis D, Boumpas DT, et al. Investigation of the genetic overlap between rheumatoid arthritis and psoriatic arthritis in a Greek population. *Scand J Rheumatol* 2017;46(3):180-186.
 20. Couturier N, Bucciarelli F, Nurtdinov RN, Debouverie M, Lebrun-Frenay C, Defer G, et al. Tyrosine kinase 2 variant influences T lymphocyte polarization and multiple sclerosis susceptibility. *Brain* 2011;134(3):693-703.
 21. Smolen JS, Aletaha D, Redlich K. The pathogenesis of rheumatoid arthritis: new insights from old clinical data? *Nat Rev Rheumatol* 2012;8(4):235-243.
 22. Lazzari E, Jefferies CA. IRF5-mediated signaling and implications for SLE. *Clin Immunol* 2014;153(2):343-352.
 23. Cohen SB, Tanaka Y, Mariette X, Curtis JR, Lee EB, Nash P, et al. Long-term safety of tofacitinib for the treatment of rheumatoid arthritis up to 8.5 years: integrated analysis of data from the global clinical trials. *Annals of the Rheumatic Diseases Published Online First*: 31 January 2017.
 24. Cutolo M. The kinase inhibitor tofacitinib in patients with rheumatoid arthritis: latest findings and clinical potential. *Ther Adv Musculoskelet Dis* 2013;5(1):3-11.
 25. Cutolo M, Meroni M. Clinical utility of the oral JAK inhibitor tofacitinib in the treatment of rheumatoid arthritis. *J Inflamm Res* 2013;6:129-137.
 26. Catlett I, Aras U, Liu Y, Bei D, Girgis I, Murthy B, et al. SAT0226 A first-in-human, study of BMS-986165, a selective, potent, allosteric small molecule inhibitor of tyrosine kinase 2. *Annals Rheumatic Dis* 2017;76(Suppl 2):859.
 27. Gillooly K, Zhang Y, Yang X, Zupa-Fernandez A, Cheng L, Strnad J, et al. BMS-986165 is a highly potent and selective allosteric inhibitor of Tyk2, blocks IL-12, IL-23 and type I interferon signaling and provides for robust efficacy in preclinical models of systemic lupus erythematosus and inflammatory bowel disease [abstract]. *Arthritis Rheumatol* 2016;68(suppl 10).