Studying the Association between *STAT4* Gene Polymorphism and Susceptibility to Rheumatoid Arthritis Disease: An Updated Meta-Analysis

Hamidreza Ebrahimiyan[1,2], Shayan Mostafaei[3], Saeed Aslani[1], Ahmadreza Jamshidi[1], Mahdi Mahmoudi[1*]

[1]Rheumatology Research Center, Tehran University of Medical Sciences, [2]Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, [3]Department of Community Medicine, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran

ABSTRACT

Background: STAT4 is a transcription factor that plays a role in various cytokine signaling pathways and in T cell subsets differentiation. Several studies have reported STAT4 gene polymorphism in association with various autoimmune diseases. **Objective:** To evaluated the association between STAT4 rs7574865 SNP and RA risk by meta-analysis. **Methods:** Two major databases, namely Scopus and PubMed, were searched to find studies investigating the STAT4 polymorphism and RA in different populations up to November 2017. Association between STAT4 polymorphism and RA were analyzed using pooled odds ratio (OR) and their corresponding 95% CI. **Results:** In this meta-analysis, 21 population studies (16 papers) comprising 15,732 cases and 15641 healthy subjects evaluating the STAT4 gene rs7574865 SNP were included based on inclusion criteria. Herein, we found a significant positive association between minor T allele as well as different genotypes with the risk of RA. **Conclusions:** In summary, this study revealed an association between STAT4 gene rs7574865 SNP and risk of RA.
Ebrahymian H, et al. Iran J Immunol. 2019; 16(1):71-83.

Keywords: Meta-Analysis, Rheumatoid Arthritis, Rs7574865, *STAT4*, Single Nucleotide

---

*Corresponding author: Dr. Mahdi Mahmoudi, Ph.D.; Rheumatology Research Center, Shariati Hospital, Kargar Ave., Tehran, Iran, e-mail: mahmoudim@tums.ac.ir

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune and auto inflammatory disorder of joints that leads to disability and destruction in RA patients (1,2). Both environmental factors (e.g., smoking and vitamin D) and genetic factors (e.g., human leukocyte antigen (HLA)) have roles in RA pathogenesis (3-12). Moreover, various cells such as lymphocytes, osteoclasts, dendritic cells (DCs), and synovial fibroblasts (SFs) are involved in RA pathogenesis. Both T and B cells have been isolated from inflamed synovium (13,14). Among the CD4+ T cell subsets, T helper (Th) 1 and Th17 play pathogenic roles in RA patients. Interleukin (IL)-17, which mainly is secreted from Th17 cells, increases expression of several inflammatory cytokines such as IL-1 and tumor necrosis factor (TNF)-α in cells, such as fibroblasts and macrophages. Consequently these cytokines trigger inflammation in joints of RA patients (15-19).
STAT 4 is a transcription factor that is involved in IL-12, IL-23, and type 1 interferon (IFN) signal transmission that is important in differentiation and proliferation of both Th1 and Th17 cells (20,21). IFN-γ, the main cytokine in Th1 mediated immune response, needs STAT4 signaling for its functions (22). Many researchers have reported Th1 and Th17 as two main lymphocytes that trigger autoimmune responses in autoimmune diseases such as RA (15,16,19). Considering its important role in Th1 and Th17 differentiation and proliferation, we designed a meta-analysis to evaluate the STAT4 polymorphism in RA pathogenesis. To date, the rs7574865 SNP in the STAT4 gene have been associated with increasing risk of various autoimmune diseases such as Sjogren's syndrome (SjS) (23-25),RA (26-36), Systemic lupus erythematosus (SLE) (29, 30, 34, 37-42), and Systemic sclerosis (SSc) (43-45).
Considering the mentioned points, this meta-analysis was conducted to evaluate and update the association between STAT4 gene rs7574865 SNP and RA risk in performed case-control studies worldwide.

MATERIALS AND METHODS

Searches and data sources. In the current meta-analysis, we searched two major databases, including Scopus and PubMed, to find any related case-control studies around STAT4 gene polymorphisms and RA risk up to November 2017. For this purpose, we used the keywords including ("STAT4" OR "signal transducer and activator of transcription 4") and ("Rheumatoid arthritis" OR "RA") and ("polymorphism" OR "polymorphisms" OR "variation" OR "single nucleotide" OR "SNP" OR "mutation"). Any related references in these studies were also reviewed. Herein, we included only English-language and human population studies. The title/abstracts of all relevant studies were reviewed to evaluate the relevancy of the study.
Inclusion and exclusion criteria. The following inclusion criteria were considered in this meta-analysis: 1) Case-control studies with evaluation of STAT4 gene rs7574865 polymorphisms and RA risk and 2) Only studies were included that contained allele or genotype frequency, which allowed calculation of odds ratio (OR) with 95% confidence interval (CI). The exclusion criteria were 1) any study with duplicated subjects and 2) other types of studies such as letter, review, and comment (Table 1, Figure 1).

Table 1. Characteristics of the studies included in the meta-analysis.

Author	Published Year	Country/Race	Detection Technique	RA Patients	Healthy Controls
Lee (49)	2007	Korea/ Asian	PCR	1032	908
Barton-1 (50)	2008	UK/ European	PCR	1858	2934
Barton-2 (50)	2008	UK/ European	PCR	3399	3024
Kobayashi-1 (51)	2008	Japan/ Asian	TaqMan	1481	745
Kobayashi-2 (51)	2008	Japan/ Asian	TaqMan	1109	938
Kobayashi-3 (51)	2008	Japan/ Asian	TaqMan	941	500
Martinez (52)	2008	Spain/ European	TaqMan	559	716
Orozco-1 (53)	2008	Spain/ European	TaqMan	923	1296
Orozco-2 (53)	2008	Sweden/ European	TaqMan	273	285
Orozco-3 (53)	2008	Netherlands/ European	TaqMan	876	893
Palomino-Morales (54)	2008	Colombia/ Colombian	TaqMan	257	410
Stark(55)	2009	Slovakia/European	TaqMan assay	518	300
Liang(56)	2011	China/Asian	PCR-DHPLC	208	312
Mohamed(57)	2012	Egypt/African	PCR-RFLP	172	160
Shen (58)	2013	China/ Asian	Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS)	518	520
Zhao(59)	2013	China/Asian	Direct sequencing	640	662
Fodil(60)	2014	Algeria/African	TaqMan assay	110	197
Settin(61)	2014	Egypt/ African	PCR-RFLP	112	122
Ramírez(62)	2016	Mexico City/ Mexican	TaqMan	415	326
Ciccacci(63)	2016	Italy/ European	TaqMan	191	243
de JesúsDurán-Avelar(64)	2016	Mexico City/ Mexican	TaqMan	140	150

Data extraction and quality assessment. Data were extracted according to the inclusion and exclusion criteria. Last name of the first author, publication year, detection method, ethnicity of participants, and the number of cases and controls with minor T allele of rs7574865 were extracted. The Newcastle-Ottawa Scale (NOS) was included to evaluate the methodological quality (46). NOS was used to score the quality of studies as 0-3 for low quality, 4-6 for moderate, and 7-9 for high-quality studies. Two independent investigators reviewed the selected studies for eligibility with regard to the criteria and resolved any discrepancies.

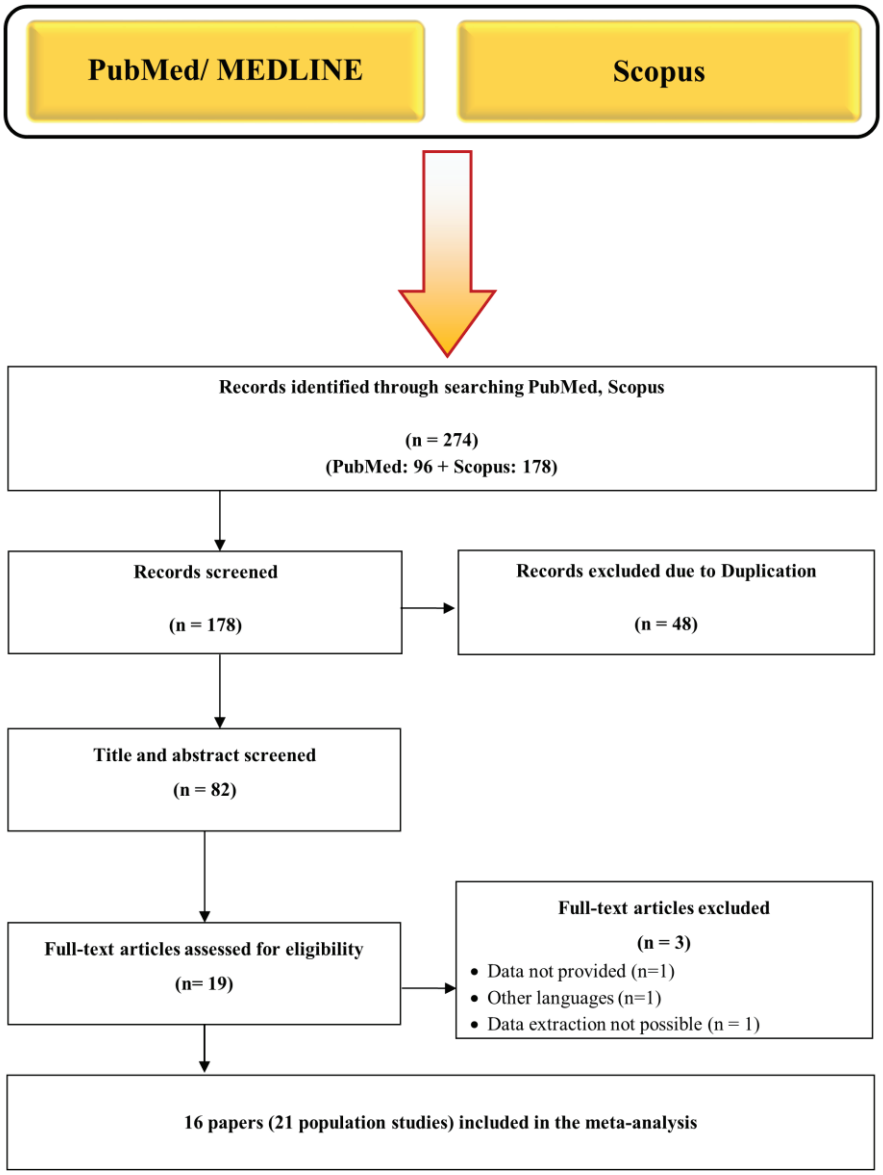

Figure 1. Flow-chart of procedure for the literature search and study selection. In this meta-analysis, 21 case-control studies comprising 15732 cases and 15641 healthy subjects evaluating rs7574865 SNP were included in the final analysis.

Statistical methods. Pooled odds ratio (OR) and their corresponding 95% CI for minor alleles were included to calculate STAT4 gene polymorphisms and risk of RA. Phenotypic frequency (pf%) for each RA was calculated using the percentage of positive numbers between all samples. We used the formula gf = 1– (1 – pf) ½ for genotypic frequency (gf) calculation among all specimen. The heterogeneity and the variation in the pooled estimations were analyzed with Cochran's Q test and I-squared test. $p<0.1$ level was considered as statistically significant (47). Random effect model was used with a significant Cochran's Q test ($p<0.10$), which means heterogeneity

existed between the individuals. On the other hand, the fixed effects model was used in individuals with no heterogeneity. The forest plot presents a series of ORs as central values and their confidence intervals in order to calculate pooled OR and its 95% CI. In other words, in the forest plot, each study and the summary effect (OR) are depicted as a point estimate bounded by its CI. The funnel plot is a graphical test to check for the existence of publication bias, it is as a mechanism for displaying the relationship between study size and the effect size (OR). Traditionally, the funnel plot was plotted with ORs on the X-axis and the variance on the Y-axis. Sensitivity analysis was performed by the influence plot. A sensitivity analysis, as a statistical test, is an important part of the meta-analysis that is applied to determine the robustness of the observed outcomes/overall OR to the assumptions made in performing the analysis. Influence plot was plotted by omitting any groups or individual studies to assess the robustness of the overall OR against omitted individual in the studies. The publication bias ($p<0.05$) calculated with Egger's test and Begg's test and funnel plots also were measured (48). We used the STATA statistical software (version 11.0; Stata Corporation, College Station, TX) and R software (v.3.4.0) for data analysis.

RESULTS

Characteristics of eligible studies. Using the criteria mentioned above, 21 case-control studies comprising 15732 cases and 15641 healthy subjects for rs7574865 SNP were included in the final meta-analysis (49-64). Among the 21 investigated case-control studies, 8 case-control studies were on European people, 7 in Asians, 3 in African, and the remaining 3 studies in Mexican and Colombian people. Publication year of these studies was ranged from 2007 to 2016. According to the NOS criteria, all studies were scored between 7 and 9 (Table 1). The key characteristics and the allele frequencies of the included studies in this meta-analysis are presented in Table 2.

Main results, subgroup, and sensitivity analysis. Via the overall analysis (Table 2), we found a significant positive association between STAT4 gene rs7574865 SNP and RA predisposition in patients. The pooled OR was 1.26 (95% CI: 1.22-1.31, $p<0.001$) for the minor T allele of STAT4 rs7574865 SNP. The TT genotype was more frequently found in RA patients and significantly increased the RA risk (OR=1.56, CI: 1.42-1.71, $p<0.001$). In addition, GT genotype had a higher frequency in the RA patients in comparison to the controls and was significantly associated with raised risk of RA (OR=1.27, CI: 1.21-1.34, $p<0.001$). The dominant and recessive genetic models of TT+GT vs. GG and TT vs. GT+GG both were associated with higher RA risk (OR=1.32, CI: 1.26-1.38, $p<0.001$ and OR=1.40, CI: 1.28-1.53, $p<0.001$, respectively).

Sensitivity analysis. We evaluated the stability of meta-analysis. The pooled ORs were not altered when we omitted any groups or individual in studies.

Heterogeneity and publication bias. Heterogeneity of the studies was analyzed based on Cochran's Q test and I^2 test. The $I^2\%>50\%$ and P Heterogeneity<0.10 were considered as statistically significant. Heterogeneity was observed in T genotype and TT+GT dominant genetic model ($I^2\%= 55.8\%$, P Heterogeneity<0.001 and $I^2\%= 55\%$, P Heterogeneity<0.001, respectively). Then, the fixed and random effect models were used to pool the results. Funnel plot and Egger's and Begg's tests were used for calculating the publication bias (Figures 2 and 3). No publication bias was found in any of the analyses (Table 2).

Table 2. Meta-analysis of the pooled association between *STAT4* gene rs7574865 polymorphism and RA disease.

Variation	Number of Studies	Frequency		Association Test			Heterogeneity Test (I^2%, *P-value*)	Publication Bias (Begg's Test, P-value; Egger's test, P-value)	Effect Model
		Case	Control	P value	Pooled OR	(95 % CI)			
rs7574865 (G>T)									
G	21	21788 (69.3%)	23431 (74.9%)						
T	21	9676 (30.7%)	7851 (25.1%)						
GG	21	7625 (48.5%)	8845 (56.6%)						
GT	21	6538 (41.5%)	5741 (36.7%)						
TT	21	1569 (10%)	1055 (0.7%)						
T *vs.* G	21	-	-	**<0.001**	1.26	(1.22-1.31)	(55.8%, 0.001)	(Begg's Test, 0.001; Egger's test, 0.001)	Random
TT *vs.* GG	21	-	-	**<0.001**	1.56	(1.42-1.71)	(24.3%, 0.15)	(Begg's Test, 0.006; Egger's test, 0.001)	Fixed
GT *vs.* GG	21	-	-	**<0.001**	1.27	(1.21-1.34)	(46.9%, 0.01)	(Begg's Test, 0.001; Egger's test, <0.001)	Random
TT+GT *vs.* GG	21	-	-	**<0.001**	1.32	(1.26-1.38)	(55%, 0.001)	(Begg's Test, <0.001; Egger's test, <0.001)	Random
TT *vs.* GT+GG	21	-	-	**<0.001**	1.40	(1.28-1.53)	(7.4%, 0.36)	(Begg's Test, 0.03; Egger's test, 0.01)	Fixed

Figure 2. Forest plot. The plot shows results of pooled OR for A: T vs. G, B:GT vs. GG, C:TT vs. GG, D: TT vs. GG, and E: TT +GT vs. GG patterns. In this meta-analysis, the fixed and random effect models were used to pool the results and Egger's and Begg's tests were used to calculate the publication bias.

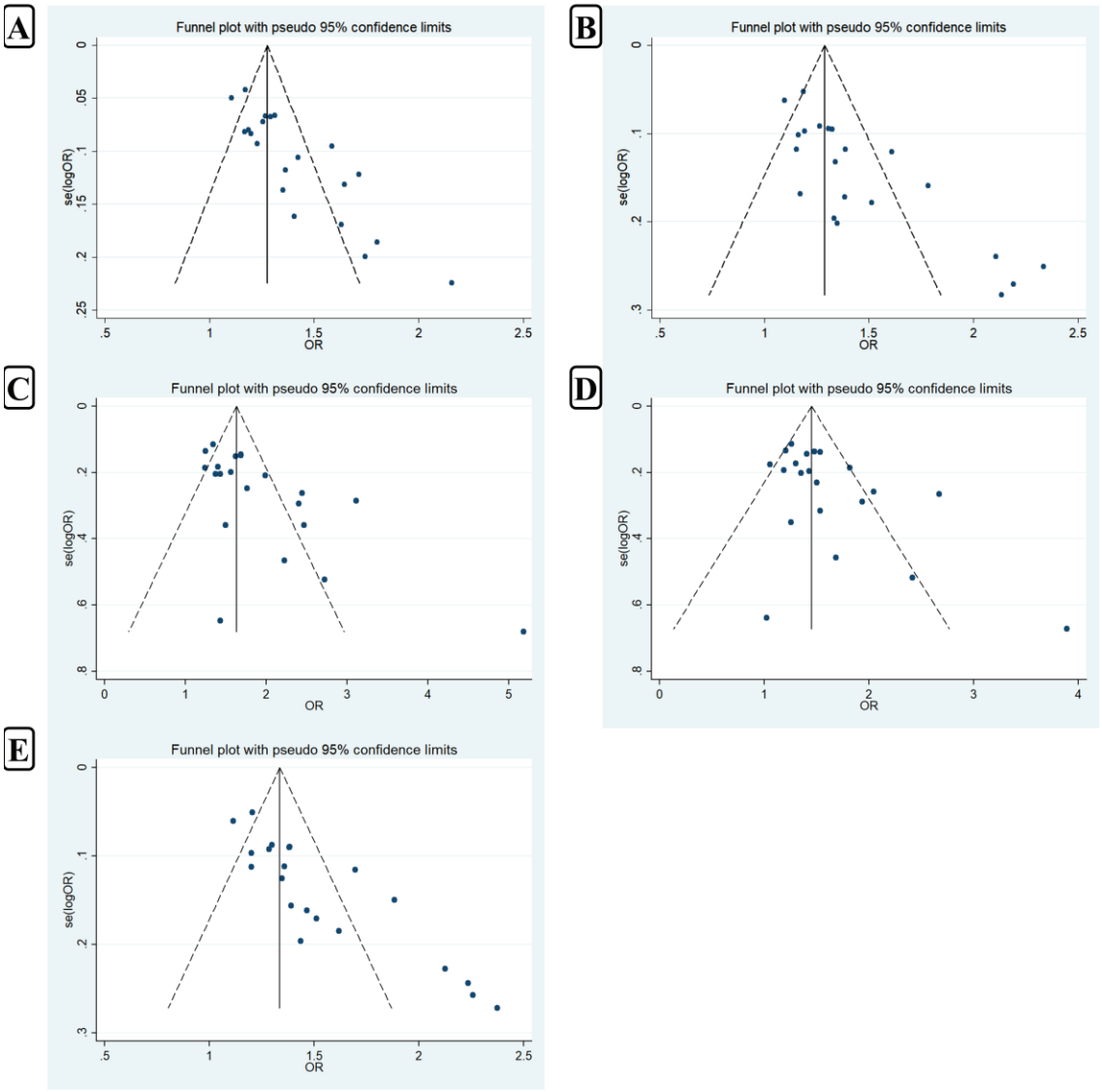

Figure 3. Funnel plot. The plot depicts publication bias and heterogeneity results between studies for A) T vs. G, B) GT vs. GG, C) TT vs. GG, D) TT vs. GT + GG, and E) TT + GT vs. GG patterns. Egger's and Begg's tests were used to calculate the publication bias and evaluate the heterogeneity between the studies.

DISCUSSION

RA is an autoimmune systemic disorder, prevalent in about ~1% of people around the world. The disease is defined with chronic inflammation, destruction, and deformity in the synovial joints, leading to pain and reduced quality of life in affected individuals (65). Genetic and environmental factors have been reported to be important in RA pathogenesis. Studies have shown that genetic factors are up to 50-60% responsible for RA pathogenesis (66). Several genes have been observed to be involved in the pathogenesis of the disease (3-6). The variety of cells such as lymphocytes, SFs, DCs,

and osteoclasts exert an important role in RA pathogenesis. Lymphocytes are one of the important cells in inflamed synovium (13,14). STAT4 is one of the important genes that have been associated with RA pathogenesis (26-36) and other autoimmune diseases such as systemic lupus erythematosus (29,30,34,37-42), Sjogren's syndrome (23-25), and Systemic sclerosis (43-45). STAT4 gene, which is located on chromosome 2q32.3, encodes a transcription factor, which is activated by IL-12 and IL-23 and is important in signaling of both T cells' subsets (67-71). Therefore, to emerge a normal immune system, STAT4 participates actively and importantly in immune mechanobiology.

Due to the important role of T-cell subsets in RA development, we decided to analyze all studies evaluating the STAT4 polymorphism. In this study, we meta-analyzed the association of the STAT4 gene rs7574865 polymorphism with RA risk in all case-control studies with available data. Previous studies in this regard have reported increased susceptibility to RA by G allele (OR=1.63, CI: 1.17-2.27, $p< 0.05$) (64) or T allele (OR=1.59, CI: 1.31-1.92, $p<0.05$) (52) of STAT4 gene rs7574865 polymorphism. Furthermore, the previous meta-analysis in 2015 reported a significant association between genetic models including TT vs. GT+GG and GT+TT vs. GG with RA risk (72). Our results confirmed the already reported association, as an allele, genotype, and genetic models of STAT4 gene rs7574865 polymorphism and were all associated with RA risk.

By the use of random or fixed effect models, our meta-analysis results indicated a positive association between rs7574865 SNP and RA risk. We analyzed the available alleles and genotypes of previous studies to detect any association with susceptibility to RA. Genotypes, dominant and recessive models, and minor T allele significantly increased the risk of RA. However, there are some limitations in the present meta-analysis. Insufficient original data such as clinical properties and treatment details, ethnicity, gene, and environment interaction limited us to track more risk factor in these patients. In the current study, we only included English original studies. Thus, our work may be subjected to language bias. Nevertheless, only one meta-analysis of case-control studies, which was directly used for combining the studies of similar design, indicated reliable results. Fortunately, the data were not sparse in this study; otherwise, these meta-analysis models would suffer further limitation.

In summary, we found a positive association between minor T allele of STAT4 gene rs7574865 polymorphism and RA susceptibility. Moreover, TT and GT genotypes, as well as the dominant and recessive models, had positive associations with RA risk. Overall, STAT4 gene rs7574865 was associated with the RA risk.

ACKNOWLEDGEMENTS

This study was supported by a grant from Deputy of Research, Tehran University of Medical Sciences (Grant No. 94-03-41-30267).

REFERENCES

1. Huber LC, Distler O, Tarner I, Gay RE, Gay S, Pap T. Synovial fibroblasts: key players in rheumatoid arthritis. Rheumatology (Oxford). 2006;45:669-75.

2. Bartok B, Firestein GS. Fibroblast-like synoviocytes: key effector cells in rheumatoid arthritis. Immunol Rev. 2010;233:233-55.
3. Scott DL, Wolfe F, Huizinga TW. Rheumatoid arthritis. Lancet. 2010;376:1094-108.
4. Albano SA, Santana-Sahagun E, Weisman MH. Cigarette smoking and rheumatoid arthritis. Seminars in arthritis and rheumatism. 2001;31:146-59.
5. Wen H, Baker JF. Vitamin D, immunoregulation, and rheumatoid arthritis. J Clin Rheumatol 2011;17:102-7.
6. Plenge RM, Seielstad M, Padyukov L, Lee AT, Remmers EF, Ding B, et al. TRAF1-C5 as a risk locus for rheumatoid arthritis--a genomewide study. N Engl J Med. 2007;357:1199-209.
7. Aslani S, Mahmoudi M, Salmaninejad A, Poursani S, Ziaee V, Rezaei N. Lack of Association between STAT4 Single Nucleotide Polymorphisms and Iranian Juvenile Rheumatoid Arthritis Patients. Fetal Pediatr Pathol. 2017;36:177-83.
8. Mahmoudi M, Hamzeh E, Aslani S, Ziaee V, Poursani S, Rezaei N. Single nucleotide polymorphism of Methyl-CpG-binding protein 2 gene associates with juvenile idiopathic arthritis. Clin Rheumatol. 2017:1-7.
9. Shamsian E, et al. PADI4 polymorphisms in Iranian patients with rheumatoid arthritis. Acta Reumatol Port. 2016;41:338-343.
10. Nazari M, Mahmoudi M, Rahmani F, Akhlaghi M, Beigy M, Azarian M, et al. Association of killer cell immunoglobulin-like receptor genes in iranian patients with rheumatoid arthritis. PloS one. 2015;10:e0143757.
11. Malekshahi Z, Mahmoudi M, Akhlaghi M, Garshasbi M, Jamshidi A, Poursani S, et al. Evaluation of the association of single nucleotide polymorphisms in DDP4 and CDK5RAP2 genes with rheumatoid arthritis susceptibility in Iranian population. Egyptian Journal of Medical Human Genetics. 2017.
12. Almasi S, Aslani S, Poormoghim H, Jamshidi A, Poursani S, Mahmoudi M. Gene Expression Profiling of Toll-Like Receptor 4 and 5 in Peripheral Blood Mononuclear Cells in Rheumatic Disorders: Ankylosing Spondylitis and Rheumatoid Arthritis. Iran J Allergy Asthma Immunol. 2016;15:87.
13. Schroder AE, Greiner A, Seyfert C, Berek C. Differentiation of B cells in the nonlymphoid tissue of the synovial membrane of patients with rheumatoid arthritis. Proc Natl Acad Sci U S A. 1996;93:221-5.
14. Randen I, Mellbye OJ, Forre O, Natvig JB. The identification of germinal centres and follicular dendritic cell networks in rheumatoid synovial tissue. Scand J Immunol. 1995;41:481-6.
15. Gaffen SL. The role of interleukin-17 in the pathogenesis of rheumatoid arthritis. Curr Rheumatol Rep. 2009;11:365-70.
16. Sarkar S, Fox DA. Targeting IL-17 and Th17 cells in rheumatoid arthritis. Rheum Dis Clin North Am. 2010;36:345-66.
17. Koenders MI, Joosten LA, van den Berg WB. Potential new targets in arthritis therapy: interleukin (IL)-17 and its relation to tumour necrosis factor and IL-1 in experimental arthritis. Ann Rheum Dis. 2006;65 Suppl 3:iii29-33.
18. Stamp LK, James MJ, Cleland LG. Interleukin-17: the missing link between T-cell accumulation and effector cell actions in rheumatoid arthritis? Immunol Cell Biol. 2004;82:1-9.
19. Miltenburg AM, van Laar JM, de Kuiper R, Daha MR, Breedveld FC. T cells cloned from human rheumatoid synovial membrane functionally represent the Th1 subset. Scand J Immunol.. 1992;35:603-10.
20. Watford WT, Hissong BD, Bream JH, Kanno Y, Muul L, O'Shea JJ. Signaling by IL-12 and IL-23 and the immunoregulatory roles of STAT4. Immunol Rev. 2004;202:139-56.
21. Mathur AN, Chang HC, Zisoulis DG, Stritesky GL, Yu Q, O'Malley JT, et al. Stat3 and Stat4 direct development of IL-17-secreting Th cells. J Immunol. 2007;178:4901-7.
22. Thierfelder WE, van Deursen JM, Yamamoto K, Tripp RA, Sarawar SR, Carson RT, et al. Requirement for Stat4 in interleukin-12-mediated responses of natural killer and T cells. Nature. 1996;382:171-4.
23. Palomino-Morales RJ, Diaz-Gallo LM, Witte T, Anaya JM, Martin J. Influence of STAT4 polymorphism in primary Sjogren's syndrome. J Rheumatol.. 2010;37:1016-9.
24. Nordmark G, Kristjansdottir G, Theander E, Eriksson P, Brun JG, Wang C, et al. Additive effects of the major risk alleles of IRF5 and STAT4 in primary Sjogren's syndrome. Genes Immun. 2009;10:68-76.

25. Korman BD, Alba MI, Le JM, Alevizos I, Smith JA, Nikolov NP, et al. Variant form of STAT4 is associated with primary Sjogren's syndrome. Genes Immun. 2008;9:267-70.
26. Zervou MI, Sidiropoulos P, Petraki E, Vazgiourakis V, Krasoudaki E, Raptopoulou A, et al. Association of a TRAF1 and a STAT4 gene polymorphism with increased risk for rheumatoid arthritis in a genetically homogeneous population. Hum immunol. 2008;69:567-71.
27. Suarez-Gestal M, Calaza M, Dieguez-Gonzalez R, Perez-Pampin E, Pablos JL, Navarro F, et al. Rheumatoid arthritis does not share most of the newly identified systemic lupus erythematosus genetic factors. Arthritis Rheum. 2009;60:2558-64.
28. Stark K, Rovensky J, Blazickova S, Grosse-Wilde H, Ferencik S, Hengstenberg C, et al. Association of common polymorphisms in known susceptibility genes with rheumatoid arthritis in a Slovak population using osteoarthritis patients as controls. Arthritis Res Ther. 2009;11:R70.
29. Remmers EF, Plenge RM, Lee AT, Graham RR, Hom G, Behrens TW, et al. STAT4 and the risk of rheumatoid arthritis and systemic lupus erythematosus. N Engl J Med. 2007;357:977-86.
30. Palomino-Morales RJ, Rojas-Villarraga A, Gonzalez CI, Ramirez G, Anaya JM, Martin J. STAT4 but not TRAF1/C5 variants influence the risk of developing rheumatoid arthritis and systemic lupus erythematosus in Colombians. Genes Immun. 2008;9:379-82.
31. Orozco G, Alizadeh BZ, Delgado-Vega AM, Gonzalez-Gay MA, Balsa A, Pascual-Salcedo D, et al. Association of STAT4 with rheumatoid arthritis: a replication study in three European populations. Arthritis Rheum. 2008;58:1974-80.
32. Martinez A, Varade J, Marquez A, Cenit MC, Espino L, Perdigones N, et al. Association of the STAT4 gene with increased susceptibility for some immune-mediated diseases. Arthritis Rheum. 2008;58:2598-602.
33. Lee HS, Remmers EF, Le JM, Kastner DL, Bae SC, Gregersen PK. Association of STAT4 with rheumatoid arthritis in the Korean population. Mol Med. 2007;13:455-60.
34. Kobayashi S, Ikari K, Kaneko H, Kochi Y, Yamamoto K, Shimane K, et al. Association of STAT4 with susceptibility to rheumatoid arthritis and systemic lupus erythematosus in the Japanese population. Arthritis Rheum. 2008;58:1940-6.
35. Daha NA, Kurreeman FA, Marques RB, Stoeken-Rijsbergen G, Verduijn W, Huizinga TW, et al. Confirmation of STAT4, IL2/IL21, and CTLA4 polymorphisms in rheumatoid arthritis. Arthritis Rheum. 2009;60:1255-60.
36. Barton A, Thomson W, Ke X, Eyre S, Hinks A, Bowes J, et al. Re-evaluation of putative rheumatoid arthritis susceptibility genes in the post-genome wide association study era and hypothesis of a key pathway underlying susceptibility. Hum Mol Genet. 2008;17:2274-9.
37. Yang W, Ng P, Zhao M, Hirankarn N, Lau CS, Mok CC, et al. Population differences in SLE susceptibility genes: STAT4 and BLK, but not PXK, are associated with systemic lupus erythematosus in Hong Kong Chinese. Genes Immun. 2009;10:219-26.
38. Suarez-Gestal M, Calaza M, Endreffy E, Pullmann R, Ordi-Ros J, Sebastiani GD, et al. Replication of recently identified systemic lupus erythematosus genetic associations: a case-control study. Arthritis Res Ther. 2009;11:R69.
39. Kiyohara C, Washio M, Horiuchi T, Tada Y, Asami T, Ide S, et al. Cigarette smoking, STAT4 and TNFRSF1B polymorphisms, and systemic lupus erythematosus in a Japanese population. J Rheumatol. 2009;36:2195-203.
40. Kawasaki A, Ito I, Hikami K, Ohashi J, Hayashi T, Goto D, et al. Role of STAT4 polymorphisms in systemic lupus erythematosus in a Japanese population: a case-control association study of the STAT1-STAT4 region. Arthritis Res Ther. 2008;10:R113.
41. Harley JB, Alarcon-Riquelme ME, Criswell LA, Jacob CO, Kimberly RP, Moser KL, et al. Genome-wide association scan in women with systemic lupus erythematosus identifies susceptibility variants in ITGAM, PXK, KIAA1542 and other loci. Nat Genet. 2008;40:204-10.
42. Abelson AK, Delgado-Vega AM, Kozyrev SV, Sanchez E, Velazquez-Cruz R, Eriksson N, et al. STAT4 associates with systemic lupus erythematosus through two independent effects that correlate with gene expression and act additively with IRF5 to increase risk. Ann Rheum Dis. 2009;68:1746-53.
43. Tsuchiya N, Kawasaki A, Hasegawa M, Fujimoto M, Takehara K, Kawaguchi Y, et al. Association of STAT4 polymorphism with systemic sclerosis in a Japanese population. Ann Rheum Dis. 2009;68:1375-6.

44. Gourh P, Agarwal SK, Divecha D, Assassi S, Paz G, Arora-Singh RK, et al. Polymorphisms in TBX21 and STAT4 increase the risk of systemic sclerosis: evidence of possible gene-gene interaction and alterations in Th1/Th2 cytokines. Arthritis Rheum. 2009;60:3794-806.
45. Dieude P, Guedj M, Wipff J, Ruiz B, Hachulla E, Diot E, et al. STAT4 is a genetic risk factor for systemic sclerosis having additive effects with IRF5 on disease susceptibility and related pulmonary fibrosis. Arthritis Rheum. 2009;60:2472-9.
46. Zeng X, Zhang Y, Kwong JS, Zhang C, Li S, Sun F, et al. The methodological quality assessment tools for preclinical and clinical studies, systematic review and meta analysis, and clinical practice guideline: a systematic review. J Evid Based Med. 2015;8:2-10.
47. Huedo-Medina TB, Sánchez-Meca J, Marín-Martínez F, Botella J. Assessing heterogeneity in meta-analysis: Q statistic or I² index? Psychol methods. 2006;11:193.
48. Egger M, Smith GD, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. Bmj. 1997;315:629-34.
49. Lee H-S, Remmers EF, Le JM, Kastner DL, Bae S-C, Gregersen PK. Association of STAT4 with rheumatoid arthritis in the Korean population. Mol Med. 2007;13:455.
50. Barton A, Thomson W, Ke X, Eyre S, Hinks A, Bowes J, et al. Re-evaluation of putative rheumatoid arthritis susceptibility genes in the post-genome wide association study era and hypothesis of a key pathway underlying susceptibility. Hum Mol Genet. 2008;17:2274-9.
51. Kobayashi S, Ikari K, Kaneko H, Kochi Y, Yamamoto K, Shimane K, et al. Association of STAT4 with susceptibility to rheumatoid arthritis and systemic lupus erythematosus in the Japanese population. Arthritis Rheum. 2008;58(7):1940-6.
52. Martinez A, Varade J, Marquez A, Cenit M, Espino L, Perdigones N, et al. Association of the STAT4 gene with increased susceptibility for some immune mediated diseases. Arthritis Rheum. 2008;58:2598-602.
53. Orozco G, Alizadeh BZ, Delgado Vega AM, González-Gay MÁ, Balsa A, Pascual Salcedo D, et al. Association of STAT4 with rheumatoid arthritis: a replication study in three European populations. Arthritis Rheum. 2008;58:1974-80.
54. Palomino-Morales R, Rojas-Villarraga A, Gonzalez C, Ramirez G, Anaya J, Martin J. STAT4 but not TRAF1/C5 variants influence the risk of developing rheumatoid arthritis and systemic lupus erythematosus in Colombians. Genes Immun. 2008;9:379-82.
55. Stark K, Rovenský J, Blažičková S, Grosse-Wilde H, Ferencik S, Hengstenberg C, et al. Association of common polymorphisms in known susceptibility genes with rheumatoid arthritis in a Slovak population using osteoarthritis patients as controls. Arthritis Res Ther. 2009;11:R70.
56. Liang Y-l, Wu H, Li P-q, Xie X-d, Shen X, Yang X-q, et al. Signal transducer and activator of transcription 4 gene polymorphisms associated with rheumatoid arthritis in Northwestern Chinese Han population. Life sci. 2011;89:171-5.
57. Mohamed RH, Pasha HF, El-Shahawy EE. Influence of TRAF1/C5 and STAT4 genes polymorphisms on susceptibility and severity of rheumatoid arthritis in Egyptian population. Cell Immunol. 2012;273:67-72.
58. Shen L, Liu R, Zhang H, Huang Y, Sun R, Tang P. Replication study of STAT4 rs7574865 G/T polymorphism and risk of rheumatoid arthritis in a Chinese population. Gene. 2013;526:259-64.
59. Zhao Y, Liu X, Liu X, Su Y, Li Y, Zhang X, et al. Association of STAT4 gene polymorphism with increased susceptibility of rheumatoid arthritis in a northern Chinese Han subpopulation. Int J Rheum Dis. 2013;16:178-84.
60. Fodil M, Benzaoui A, Zemani-Fodil F, Aberkane M, Boughrara W, Saidi-Mehtar N, et al. Association of PTPN22 (rs2476601) and STAT4 (rs7574865) polymorphisms with Rheumatoid Arthritis in the Western Algerian population. Acta Reumatol Port. 2015; 40:56-62.
61. Settin A, Salama A, Elshazli R. Signal transducer and activator of transcription 4 (STAT4) G> T gene polymorphism in Egyptian cases with rheumatoid arthritis. Hum immunol. 2014;75:863-6.
62. Ramírez OB, Rincón JM, Cobos RB, Ávila IA, Bello JR. STAT4 confers risk for rheumatoid arthritis and systemic lupus erythematosus in Mexican patients. Immunol Lett. 2016;175:40-3.
63. Ciccacci C, Conigliaro P, Perricone C, Rufini S, Triggianese P, Politi C, et al. Polymorphisms in STAT-4, IL-10, PSORS1C1, PTPN2 and MIR146A genes are associated differently with

prognostic factors in Italian patients affected by rheumatoid arthritis. Clin Exp Immunol. 2016;186:157-63.
64. de Jesús Durán-Avelar M, Vibanco-Pérez N, Hernández-Pacheco RR, del Carmen Castro-Zambrano A, Ortiz-Martínez L, Zambrano-Zaragoza JF. STAT4 rs7574865 G/T polymorphism is associated with rheumatoid arthritis and disease activity, but not with anti-CCP antibody levels in a Mexican population. Clin rheumatol. 2016;35:2909-14.
65. Ahmadloo S, Taghizadeh M, Akhiani M, Salimzadeh A, Keramatipour M. Single Nucleotide Polymorphism rs 2476601 of PTPN22 Gene and Susceptibility to Rheumatoid Arthritis in Iranian Population. Iran J Allergy Asthma Immunol. 2015;14:437-42.
66. Goeb V, Dieude P, Daveau R, Thomas-L'otellier M, Jouen F, Hau F, et al. Contribution of PTPN22 1858T, TNFRII 196R and HLA-shared epitope alleles with rheumatoid factor and anti-citrullinated protein antibodies to very early rheumatoid arthritis diagnosis. Rheumatology (Oxford). 2008;47(8):1208-12.
67. O'Malley JT, Eri RD, Stritesky GL, Mathur AN, Chang HC, Hogenesch H, et al. STAT4 isoforms differentially regulate Th1 cytokine production and the severity of inflammatory bowel disease. J Immunol. 2008;181:5062-70.
68. Morinobu A, Gadina M, Strober W, Visconti R, Fornace A, Montagna C, et al. STAT4 serine phosphorylation is critical for IL-12-induced IFN-gamma production but not for cell proliferation. Proc Natl Acad Sci U S A. 2002;99:12281-6.
69. Lund RJ, Chen Z, Scheinin J, Lahesmaa R. Early target genes of IL-12 and STAT4 signaling in th cells. J immunol. 2004;172:6775-82.
70. Kaplan MH. STAT4: a critical regulator of inflammation in vivo. Immunologic research. 2005;31:231-42.
71. Farrar JD, Smith JD, Murphy TL, Murphy KM. Recruitment of Stat4 to the human interferon-alpha/beta receptor requires activated Stat2. J Biol Chem. 2000;275:2693-7.
72. Gu E, et al. Rs7574865 polymorphism in signal transducers and activators oftranscription 4 gene and rheumatoid arthritis: an updatedmeta-analysis of28 case-control comparisons. Int J Rheum Dis. 2015;18:3-16.