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

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**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.


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

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

**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.
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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 $gf^2 = 1 - (1 - pf)^2$ 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 I² test. The I²%>50% and P Heterogeneity<0.10 were considered as statistically significant. Heterogeneity was observed in T genotype and TT+GT dominant genetic model (I²%= 55.8%, P Heterogeneity<0.001 and I²%= 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.

<table>
<thead>
<tr>
<th>Variation</th>
<th>Number of Studies</th>
<th>Frequency</th>
<th>Case</th>
<th>Control</th>
<th>Pooled OR (95% CI)</th>
<th>Test</th>
<th>P-value</th>
<th>Test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G vs T</td>
<td>100'0&gt;</td>
<td>149</td>
<td>1.40</td>
<td>1.32</td>
<td>1.32 (1.28-1.36)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T vs G</td>
<td>100'0&gt;</td>
<td>21788</td>
<td>69.3%</td>
<td>74.9%</td>
<td>1.26 (1.22-1.31)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG vs TT</td>
<td>100'0&gt;</td>
<td>9676</td>
<td>30.7%</td>
<td>25.1%</td>
<td>1.56 (1.42-1.71)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GT vs GG</td>
<td>100'0&gt;</td>
<td>6538</td>
<td>41.5%</td>
<td>36.7%</td>
<td>1.27 (1.21-1.34)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT vs GG</td>
<td>21</td>
<td>1569 (10%)</td>
<td>10%</td>
<td>0.7%</td>
<td>1.40 (1.28-1.53)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT vs GC</td>
<td>21</td>
<td>100'0&gt;</td>
<td>1.32</td>
<td>1.27</td>
<td>1.27 (1.22-1.33)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Publication Bias (Begg's Test, P-value; Egger's test, P-value)
- Random
  - TT vs GC
  - TT vs GC
  - G vs T
  - TT vs GG
  - TT vs GG
  - TT vs GG
  - TT vs GC
  - TT vs GG

Effect Model
- Random
  - TT vs GG
  - TT vs GG
  - TT vs GG
  - TT vs GG
  - TT vs GG
  - TT vs GG
  - TT vs GG
  - TT vs GG

Association of the pooled association between STAT4 gene rs7574865 polymorphism and RA disease.
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.
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**

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