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A redundant epistatic interaction between IRF5 and STAT4 of the type I interferon pathway in susceptibility to lupus and rheumatoid arthritis

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Objective: Two transcription factors in the type I interferon pathway, IRF5 and STAT4, have been genetically associated with susceptibility to both systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). This study aimed to determine whether these two genes interact with each other to affect the disease susceptibilities. Methods: The genetic interactions between IRF5 and STAT4 polymorphisms in SLE and RA susceptibility were examined using the epistasis options in PLINK software. This study analyzes the genetic data from 2558 unrelated Korean participants including 589 SLE patients, 987 RA patients, and 982 controls. Results: All 12 polymorphisms were individually associated with SLE susceptibility ($p = 2.49 \times 10^{-8}$ to $0.00360$). Among the three SLE-associated polymorphisms of IRF5, rs77571059, alternatively called CGGGG(3–4) indel, exhibited the lowest $p$ value ($2.49 \times 10^{-8}$) and accounted for the observed associations of the other two single-nucleotide polymorphisms (SNPs). Among the nine SLE-associated SNPs of STAT4, rs16833215 exhibited the lowest $p$ value ($2.49 \times 10^{-8}$) and accounted for all the other associations. These two polymorphisms, rs77571059 of IRF5 and rs16833215 of STAT4, interacted with each other for SLE susceptibility in a redundant manner ($\text{OR}_{\text{interaction}} = 0.77$, $\text{P}_{\text{epistasis}} = 0.040$). Furthermore, these two polymorphisms, which had been individually associated with RA susceptibility, also interacted for RA susceptibility in the same manner ($\text{OR}_{\text{interaction}} = 0.75$, $\text{P}_{\text{epistasis}} = 0.014$). Conclusions: A redundant interaction between IRF5 and STAT4 polymorphisms was found in susceptibility to the type I interferon pathway-associated rheumatic autoimmune diseases, SLE and RA, calling for further studies on confirmation of these findings.

Key words: Genetic polymorphism; epistatic interaction; systemic lupus erythematosus; rheumatoid arthritis; type I interferon

Introduction

Type I interferons (IFN), i.e. IFN-α and -β, are versatile cytokines that regulate immunity and cancer immune surveillance, and they are used for treatment of some autoimmune diseases and cancer. Activation of the type I IFN pathway has been observed in two autoimmune rheumatic diseases, systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), among others. Identifying the genetic modifications of critical molecules in the type I IFN pathway is a key to understanding their underlying connections with disease pathogenesis.

Two transcription factors, IFN regulatory factor 5 (IRF5) and signal transducer and activator of transcription 4 (STAT4), both individually regulate a large number of genes in the type I IFN pathway. Some target genes are shared by IRF5 and STAT4. In addition, these two transcription factors are also known to exert effects on distinct points. IRF5 is critical for producing the proinflammatory cytokines, tumor necrosis factor (TNF)-α and interleukin (IL)-6, upon activation of Toll-like receptors (TLRs) and is involved in
the synthesis of IFN-α in viral infection.⁴,⁵ It has been suggested that an antinucleic acid antibody in SLE patients forms an immune complex with the ligands and binds to Fc receptors on the surfaces of plasmacytoid dendritic cells.

The immune complexes are delivered to the endolysosomal compartments, where TLR7 and TLR9 bind the cognate ligands and relay downstream signals through IRF7, and most likely IRF5, to activate type I IFN.⁶ The secreted type I IFN amplifies its own production and triggers the induction of its target genes via IFN-α/β receptors (IFNAR) through both autocrine and paracrine mechanisms. In humans, STAT4 has been shown to bind to IFNAR and induce the transcription of IFN-α/β-inducible genes, increasing the sensitivity of cells to the type I IFN signal.⁷

Some variants of IRF5 and STAT4 have been associated with susceptibility to both SLE and RA.⁸—¹⁰ Some SNPs in IRF5 affect the trans-activation activity of IRF5 or the splicing of the IRF5 mRNA and are consequently associated with the blood levels of IFN-α in SLE patients.⁸,¹¹,¹² The expression levels of STAT4 variants parallel those of IFN-α-inducible genes in SLE patients.⁵,¹³ In this study, we discovered an interaction between the IRF5 and STAT4 polymorphisms in susceptibility to SLE and RA.

Methods

Study subjects

The study participants were recruited at the Hanyang University Hospital for Rheumatic Diseases (Seoul, Korea) with the approval of the Institutional Review Board of the hospital. The case-control cohort for SLE and RA consisted of 2558 unrelated Korean participants, including 589 patients with SLE (31.7 ± 11.0 years; 93.9% female), 987 with RA (52.7 ± 12.1 years; 88.6% female) and 982 healthy controls (36.8 ± 12.5 years; 85.9% female). The control subjects were used for statistical comparisons with SLE and RA patients.

Genotype data of IRF5 and STAT4 polymorphisms

The genotype data for nine SNPs in STAT4 (rs925847, rs3024861, rs3024877, rs6434435, rs16833215, rs10168266, rs10174238, rs13407419 and rs16833239) and three polymorphisms in IRF5 (rs2004640, rs77571059 and rs752637) have been analyzed in our four previous studies⁴—¹⁷ and were used for epistatic interaction analyses in this study.

Statistical analyses

Each polymorphism was tested individually for association with SLE susceptibility using χ² test in allelic models or using logistic regression in genotypic models. Crude odds ratio (OR), 95% confidence interval (CI) and p values were calculated using 2 × 2 contingency tables. The independently associated SNPs were identified using conditional multivariate logistic regression analysis. Gene-gene interactions in SLE or RA susceptibility were examined¹⁸ using the “-epistasis” and “-fast-epistasis” options of PLINK v1.07. The -epistasis option performs logistic regression analysis for interaction where the dependent case/control grouping is expressed as a function of two polymorphisms and their interaction term according to allelic genetic models. The -fast-epistasis option calculates the association OR between two polymorphisms and its standard error separately in cases and controls, and the test for epistasis is based on a standard score (Z score) for difference in the OR between cases and controls. All statistical analyses were performed using SPSS v11.5 or PLINK v1.07.

Results

SLE-associated polymorphisms in IRF5 and STAT4

Our previous studies had already shown the genetic association of IRF5 and STAT4 with SLE susceptibility using dense-SNP and comprehensive approaches by genotyping unrelated Korean SLE patients and controls.¹⁴,¹⁵ Three polymorphisms in IRF5 (rs2004640, rs77571059 and rs752637) and nine SNPs in STAT4 (rs925847, rs3024861, rs3024877, rs6434435, rs16833215, rs10168266, rs10174238, rs13407419 and rs16833239) were associated with susceptibility to SLE. To examine epistatic (gene-gene) interactions in this study, we retrieved the genotype data for the subjects who had been genotyped for the associated IRF5 and STAT4 polymorphisms and compared 589 SLE cases (31.7 ± 11.0 years; 93.9% female) with 982 healthy controls (36.8 ± 12.5 years; 85.9% female). Each of the 12 polymorphisms was tested for association with SLE using χ² tests, and all tested polymorphisms were significantly associated with susceptibility to SLE (p = 2.49 × 10⁻⁸ to 0.00360; Table 1) at the significance level of Bonferroni correction for multiple testing (α = 0.05/12 = 0.00417).
The epistatic interaction between \textit{IRF5} susceptibility in SLE susceptibility was examined using the redundant morphism (Table 1). Similarly, the association of this SNP (rs16833215) was no longer significant when adjusted for the genotypes of the other eight SNPs with SLE. The overall effect size of OR for both SNPs (538 SLE patients and 943 controls) was less than expected (2.07 \( \times \) 1.52). The interactions between rs77571059 and rs16833215 in SLE and RA susceptibilities were tested with the overall combined effect (1.59 \( \times \) 1.52). This analysis revealed that the interaction of these alleles significantly affected SLE susceptibility, which was represented by rs77571059 and rs16833215 in SLE and RA susceptibilities. The risk-associated alleles, which were more frequent in cases than controls, are marked in bold. Single-nucleotide polymorphisms (SNPs) are listed in ascending order of the minor allele frequency (MAF).

### Table 1: Association of \textit{IRF5} and \textit{STAT4} polymorphisms with SLE susceptibility

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Controls BB:Bb:bb (MAF)</th>
<th>SLE patients BB:Bb:bb (MAF)</th>
<th>Minor allele</th>
<th>OR (95% CI)</th>
<th>( p ) value</th>
<th>( p )-conditional</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{IRF5}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs77571059 A &gt; G</td>
<td>747:165:50 (0.138)</td>
<td>391:131:44 (0.194)</td>
<td>A</td>
<td>1.50 (1.23, 1.83)</td>
<td>4.60 ( \times ) 10^{-3}</td>
<td>Adjusted</td>
</tr>
<tr>
<td>rs2004640 C &gt; T</td>
<td>450:379:112 (0.320)</td>
<td>215:278:82 (0.384)</td>
<td>T</td>
<td>1.32 (1.14, 1.54)</td>
<td>3.25 ( \times ) 10^{-4}</td>
<td>0.0871</td>
</tr>
<tr>
<td>rs752637 C &gt; T</td>
<td>361:425:166 (0.398)</td>
<td>170:291:117 (0.454)</td>
<td>T</td>
<td>1.26 (1.09, 1.46)</td>
<td>2.11 ( \times ) 10^{-3}</td>
<td>0.211</td>
</tr>
<tr>
<td>\textit{STAT4}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs16833215 A &gt; G</td>
<td>294:481:187 (0.444)</td>
<td>112:282:167 (0.549)</td>
<td>G</td>
<td>1.52 (1.31, 1.77)</td>
<td>2.49 ( \times ) 10^{-8}</td>
<td>Adjusted</td>
</tr>
<tr>
<td>rs10168266 A &gt; G</td>
<td>328:276:59 (0.297)</td>
<td>198:273:82 (0.395)</td>
<td>A</td>
<td>1.55 (1.31, 1.83)</td>
<td>3.91 ( \times ) 10^{-7}</td>
<td>0.0118</td>
</tr>
<tr>
<td>rs10174238 A &gt; G</td>
<td>313:293:57 (0.307)</td>
<td>206:256:91 (0.396)</td>
<td>A</td>
<td>1.48 (1.25, 1.75)</td>
<td>4.34 ( \times ) 10^{-8}</td>
<td>0.00934</td>
</tr>
<tr>
<td>rs925847 A &gt; G</td>
<td>163:335:143 (0.484)</td>
<td>105:255:166 (0.558)</td>
<td>G</td>
<td>1.34 (1.14, 1.58)</td>
<td>4.00 ( \times ) 10^{-4}</td>
<td>0.189</td>
</tr>
<tr>
<td>rs16833239 G &gt; A</td>
<td>476:171:16 (0.153)</td>
<td>439:110:4 (0.107)</td>
<td>A</td>
<td>0.66 (0.52, 0.84)</td>
<td>7.62 ( \times ) 10^{-4}</td>
<td>0.0594</td>
</tr>
<tr>
<td>rs3024877 G &gt; A</td>
<td>196:340:73 (0.399)</td>
<td>106:294:76 (0.409)</td>
<td>A</td>
<td>1.33 (1.12, 1.58)</td>
<td>1.18 ( \times ) 10^{-3}</td>
<td>0.432</td>
</tr>
<tr>
<td>rs6434435 A &gt; C</td>
<td>480:167:16 (0.150)</td>
<td>440:108:5 (0.107)</td>
<td>C</td>
<td>0.68 (0.53, 0.86)</td>
<td>1.55 ( \times ) 10^{-3}</td>
<td>0.0944</td>
</tr>
<tr>
<td>rs13407419 A &gt; C</td>
<td>521:131:11 (0.115)</td>
<td>470:80:3 (0.078)</td>
<td>A</td>
<td>0.65 (0.49, 0.85)</td>
<td>1.91 ( \times ) 10^{-3}</td>
<td>0.131</td>
</tr>
<tr>
<td>rs3024861 A &gt; T</td>
<td>175:364:124 (0.462)</td>
<td>127:275:150 (0.521)</td>
<td>T</td>
<td>1.27 (1.08, 1.49)</td>
<td>3.60 ( \times ) 10^{-3}</td>
<td>0.358</td>
</tr>
</tbody>
</table>

SLE: systemic lupus erythematosus; MAF: minor allele frequency; OR: odds ratio; CI: confidence interval.

- The major and minor alleles are denoted as \( B \) and \( b \), respectively.
- The alleles were risk-associated in both single-nucleotide polymorphisms (SNPs).
- The risk-associated alleles, which were more frequent in cases than controls, are marked in bold.
- Single-nucleotide polymorphisms (SNPs) are listed in ascending order of the minor allele frequency (MAF).
- The associations of individual SNPs of interferon regulatory factor 5 (\textit{IRF5}) and signal transducer and activator of transcription 4 (\textit{STAT4}) with SLE susceptibility were tested (\( p \)-conditional) with adjustment for the genotypes of rs77571059 and rs16833215, which showed the lowest \( p \) values of all the SNPs for each gene.
- rs77571059 is a tandem repeat of three or four copies of CGGGG.

### Table 2: Genetic interactions between \textit{IRF5} rs77571059 and \textit{STAT4} rs16833215 in SLE and RA susceptibilities

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>SLE patients</td>
<td>71:196:105</td>
<td>26:57:41</td>
<td>11:18:13</td>
<td>0.77</td>
<td>0.040</td>
<td>0.035</td>
<td>–</td>
</tr>
<tr>
<td>RA patients</td>
<td>182:356:160</td>
<td>66:128:48</td>
<td>11:25:11</td>
<td>0.75</td>
<td>0.014</td>
<td>0.0099</td>
<td>–</td>
</tr>
</tbody>
</table>

SLE: systemic lupus erythematosus; RA: rheumatoid arthritis; OR: odds ratio. Major and minor alleles of interferon regulatory factor 5 (\textit{IRF5}) rs77571059 are denoted as \( B \) and \( m \), respectively, and those of signal transducer and activator of transcription 4 (\textit{STAT4}) rs16833215 are denoted as \( A \) and \( b \), respectively. The minor alleles were risk-associated in both single-nucleotide polymorphisms (SNPs).

- Only individuals who were genotyped for both rs77571059 of \textit{IRF5} and rs16833215 of \textit{STAT4} were included in these interaction analyses: 943 controls, 538 SLE patients, and 987 RA patients.

According to conditional logistic regression analysis results, the association of \textit{IRF5} with SLE could be represented by rs77571059 alone; the associations of the other two SNPs disappeared (\( p = 0.0871 \) or 0.211) when adjusted for the genotypes of this polymorphism (Table 1). Similarly, the \textit{STAT4} association could be represented by rs16833215 alone; the association of the other eight SNPs with SLE was no longer significant when adjusted for the genotypes of this SNP (\( p \geq 0.00593 \)).

**A redundant \textit{IRF5}-\textit{STAT4} interaction in SLE susceptibility**

The epistatic interaction between \textit{IRF5} and \textit{STAT4} in SLE susceptibility was examined using the -epistasis and -fast-epistasis analysis for the interaction between rs77571059 and rs16833215 in the study subjects whose genotype calling was successful for both SNPs (538 SLE patients and 943 controls). This analysis revealed that the interaction of these alleles significantly affected SLE susceptibility (\( P_{\text{epistasis}} = 0.040 \) in the -epistasis option; \( P_{\text{fast-epistasis}} = 0.035 \) in the -fast-epistasis option; Table 2). The effect size of interaction (\( OR_{\text{interaction}} \)) was 0.77, meaning that the observed overall combined effect (1.59 \( \times \) 1.36 \( \times \) 0.77) was less than expected (2.07 \( \times \) 1.36 \( \times \) 1.52). The overall effect size of OR was 1.59 in the presence of both alleles was not different from the effect of one risk allele alone in rs77571059 (OR = 1.36, 95% CI = 1.14–1.63; calculated from Table 2 using
a logistic regression analysis) or rs16833215 (OR = 1.52, 95% CI = 1.31–1.78), as 1.59 fell within the 95% CIs. Therefore, the interactions were considered to be redundant rather than synergistic or additive, based on a previously described classification of interactions between disease risk factors, where redundant interaction indicates the combination of factors produces no increased effect size in risk and synergistic interaction indicates there is an effect size larger than a simple additive effect.

A redundant IRF5-STAT4 interaction in RA susceptibility

Because the p value of the interaction in SLE susceptibility was just below the cut-off of 0.05, we next investigated whether the same pair of polymorphisms affected the susceptibility to another type I IFN-associated autoimmune disease, RA. The genotype data of Korean patients with RA retrieved from our previous studies were used to assess the interaction between IRF5 rs77571059 and STAT4 rs16833215, showing the genetic associations with RA susceptibility individually (p = 0.014 for rs77571059 and p = 0.026 for rs16833215 by logistic regression analyses).

When 987 RA cases (52.7 ± 12.1 years; 88.6% female) were compared with the 943 controls (Table 2), the IRF5 and STAT4 SNPs showed a redundant interaction with an ORinteraction = 0.75 for RA susceptibility ($P_{epistasis} = 0.014$ in the -epistasis option and $P_{fast-epistasis} = 0.0099$ in the -fast-epistasis option; Table 2), similar to the value (ORinteraction = 0.77) for SLE susceptibility. The observed overall combined effect (1.06) was smaller than an expected size (1.42). The overall effect size of 1.06 in the presence of both alleles was not different from the effect of a risk allele alone in rs77571059 (OR = 1.22, 95% CI = 1.04–1.44) or rs16833215 (OR = 1.16, 95% CI = 1.02–1.32), as 1.06 fell within the 95% CIs. Accordingly, the direction and effect size of interaction between the IRF5 and STAT4 polymorphisms were similar for SLE and RA.

Discussion

This is the first report showing an epistatic interaction between IRF5 and STAT4 in SLE or RA susceptibility. The effects of IRF5 and STAT4 polymorphisms on SLE or RA susceptibility were not additive to each other but instead demonstrated a redundancy of the individual polymorphism effects. As the combined effect of both risk alleles in the IRF5 and STAT4 genes was smaller than the estimated sum of individual effects (OR_{IRF5-STAT4} = OR_{IRF5} × OR_{STAT4}), there was redundant interaction between the two genes in lupus and RA susceptibilities. Thus, the two polymorphisms do not affect susceptibilities independently from each other, but the two gene products participate in a common biological pathway. In fact, these two transcription factors, IRF5 and STAT4, act sequentially in the IFN-α induction and signaling pathway.

A negative feedback loop may explain the redundant epistatic interaction in disease susceptibility. For example, the activation of molecules downstream of the type I IFN pathway may inhibit the activity or gene expression of an upstream protein activator for the pathway. Similarly, it is possible that this interaction results from interference between nonshared STAT4 and IRF5 signaling pathways.

Another hypothesis is that the redundant interaction in the type I IFN pathway is due to a certain threshold for the activation of the pathway. If the sensitivity to IFN-α is high enough because of risk alleles of STAT4, the elevated production/secretion of IFN-α due to the effect of IRF5 risk alleles would have only a small or null effect on disease susceptibility, and vice versa.

It is also worth noting that IRF5 and STAT4 share 3190 common target genes, mostly because they bind closely to each other in the promoter regions, according to a recent chromatin immunoprecipitation-sequencing study. Given that two transcription factors may control the same targets, it is also possible that these two factors activate gene expression in a redundant manner.

Two previous studies of SLE susceptibility showed no evidence of an epistatic interaction between these genes in a Swedish population (485 cases and 563 controls), and a mixed population (390 Spanish cases and 620 controls, 247 German cases and 220 controls, 221 Italian patients and 207 controls, 171 Argentinean patients and 171 controls, and 231 Mexican cases and 250 controls). These previous results showing null interaction could be due to the small sample sizes used or to genetic heterogeneity within the samples. In contrast, the cohort for our study was larger (589 SLE patients and 982 controls) than the Swedish population and ethnically more homogeneous than the mixed population.

In summary, a redundant interaction is evident between IRF5 and STAT4 polymorphisms in both SLE and RA susceptibilities, suggesting that there
is a negative feedback loop or activation threshold for the type I IFN pathway in the etiology of these autoimmune diseases, although this hypothesis should be confirmed in other populations and reinforced by mechanistic studies. Furthermore, the same interaction is shared by the two distinct rheumatic diseases, SLE and RA, suggesting that these two diseases share a common underlying mechanism.

**Funding**

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**Conflict of interest**

None declared.

**References**


