

Putative Role of Functional Interferon Regulatory Factor 5 (*IRF5*) Polymorphism in Rheumatoid Arthritis in a Korean Population

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ABSTRACT. Objective. Recent studies suggest that polymorphisms of interferon regulatory factor 5 (*IRF5*) are significantly associated with systemic lupus erythematosus in several populations. The effect of *IRF5* polymorphism on susceptibility to rheumatoid arthritis (RA) has been investigated, and the results were inconsistent. We analyzed the genetic effects of *IRF5* polymorphisms on RA in a Korean population.

Methods. Eight single-nucleotide polymorphisms (SNP) and 2 insertion-deletion polymorphisms in *IRF5* were genotyped in 2183 subjects (1204 RA cases and 979 controls) using the TaqMan® method. The genetic effects of SNP on the risk of RA were evaluated using chi-square tests and multivariate logistic regression, controlling for age, sex, and shared epitope (SE), and we then performed conditional analysis by SE status and anti-cyclic citrullinated peptide (anti-CCP) antibody (Ab) status. Data from a Mantel-Haenszel metaanalysis of odds ratios (OR) were subsequently combined in a separate analysis with the results of the association of *rs2004640* with RA from a previous study.

Results. Two of the *IRF5* polymorphisms, *CGGGGindel* (OR 1.38, 95% CI 1.09–1.76, $p_{\text{corr}} = 0.04$) and *rs2004640* (OR 1.36, 95% CI 1.09–1.68, $p_{\text{corr}} = 0.03$), and one haplotype, including the *rs2004640* and the *CGGGGindel*, *ht3 (A-Del-T-C-del-A-T)* (OR 1.39, 95% CI 1.09–1.79, $p_{\text{corr}} = 0.04$) were significantly associated with an increased risk of RA. After stratification according to anti-CCP Ab and SE status, *rs2004640* SNP was associated with the anti-CCP Ab-positive (OR 1.47, 95% CI 1.15–1.88, $p_{\text{corr}} = 0.01$) or SE-positive group (OR 1.54, 95% CI 1.14–2.09, $p_{\text{corr}} = 0.03$). A combined analysis including all 3 independent cohorts from the previous study revealed an association of the *rs2004640* with RA (pooled OR 1.21, 95% CI 1.07–1.38, pooled $p = 0.0031$ in dominant model).

Conclusion. Our results suggest that the *IRF5* polymorphism is associated with genetic susceptibility to RA at least in a Korean population, and that it may contribute to disease susceptibility in SE-positive or anti-CCP Ab-positive patients with RA. (First Release Oct 1 2008; J Rheumatol 2008;35:2106–12; doi:10.3899/jrheum.080114)

Key Indexing Terms:

INTERFERON REGULATORY FACTOR 5

RHEUMATOID ARTHRITIS

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The central roles of cytokines in autoimmune diseases are well established. Interferons (IFN) are considered among the most important cytokines due to their strong pleiotropic effects and a vast range of biologic functions that influence both innate and adaptive immune responses.

IFN regulatory factors (*IRF*) are transcriptional mediators of IFN-induced signaling pathways that have been shown to play notable roles in the intricate networks of the immune system. The *IRF* family comprises 9 members, of which the expression of IFN regulatory factor 5 (*IRF5*) is constitutive and inducible by type I IFN and Toll-like receptor (TLR) ligation¹.

There is substantial evidence of a role for IFN in systemic lupus erythematosus (SLE)², and recent studies have provided convincing evidence that *IRF5* polymorphisms (*CGGGGindel*, *IRF5-15-1*, *rs10488631*, *rs2004640*, *rs2280714*, *exon 6 insdel*, *rs2070197*, and *rs10954213*) are significantly associated with SLE³⁻⁷.

Rheumatoid arthritis (RA), a chronic systemic inflammatory disease characterized by synovial inflammation and joint destruction, affects 0.5%–1% of the population and represents a significant cause of disability. Numerous cytokines and multiple cell interactions contribute to bone erosion and the development of arthritis, and *IRF5* is involved in regulation and production of the cytokines that are pivotal to RA pathophysiology^{8,9}.

Three recent studies (2 case–control studies and one family-based study) examined the genetic contribution of *IRF5* polymorphisms to RA^{10–12}. Although investigations of 3 case-control cohorts from Spain, Sweden, and Argentina¹¹ and of 100 French trio families with RA¹² found no associations between *IRF5* polymorphism and the risk of RA, the other case–control study found that a polymorphism in *IRF5* (*rs2004640*) was associated with RA in a Swedish population¹⁰. To determine the association of *IRF5* polymorphisms with RA in Asian populations, we examined the genetic effects in a cohort of Korean patients with RA.

MATERIALS AND METHODS

Patients and controls. This study investigated 1204 Korean patients who met the American College of Rheumatology 1987 classification criteria for RA¹³ and 979 Korean controls, very close to the same population as in our previous studies in SLE⁷, recruited from the Hospital for Rheumatic Diseases, Hanyang University, Seoul. Written informed consent was obtained from each participant. Patients with RA were aged 52.5 ± 12.3 (mean \pm SD) years with an age at disease onset of 40.9 ± 12.6 years; 88.4% were female, 80% were positive for rheumatoid factor (RF), and 68.1% were positive for the shared epitope (SE). Among 750 cases, 84.9% were positive for anti-cyclic citrullinated peptide (CCP) antibody (Ab). The controls were aged 37.3 ± 12.6 years.

Laboratory studies. Clinical data, including sex, current age, age at disease onset, age at the time of RA diagnosis, time from disease onset to initiation of therapy, and the dates of clinic visits, were obtained from medical records and from interviews at the time of enrollment. Serum levels of RF were measured nephelometrically. A positive RF titer was defined as the level present in < 5% of unaffected people (< 20 IU/ml). Anti-CCP Ab was examined by ELISA (second generation test). All subjects had been genotyped for HLA-DRB1 alleles by polymerase chain reaction (PCR) and sequence-specific oligonucleotide probe hybridization according to the reference protocol described by the 12th International Histocompatibility Workshop¹⁴, followed by direct DNA sequencing¹⁵. The SE was defined by the following alleles: DRB1*0101, *0102, *0401, *0404, *0405, *0408, *0410, *1001, *1402, and *1406¹⁶.

Genotyping of *IRF5* polymorphisms. Eight single-nucleotide polymorphisms (SNP) and 2 insertion/deletion polymorphisms in *IRF5* [*rs729302*(A>C), *CGGGGindel*, *rs2004640*(G>T), *rs752637*(T>C), *IRF5-15-1*(C>T), *exon 6 insdel*, *rs2070197*(T>C), *rs10954213*(G>A), *rs10488631*(T>C), and *rs2280714*(T>C)], which had showed significant or possible associations with a genetic predisposition to SLE in previous studies, were genotyped among our RA patients and controls using TaqMan[®] (Applied Biosystems, Foster City, CA, USA)¹⁷ with specifically designed amplifying primers and probes. Primer Express was used to design both the PCR primers and the MGB TaqMan probes. One allelic probe was labeled with the fluorescent 6-carboxyfluorescein dye and the other with fluorescent VIC[®] dye. PCR were run in TaqMan Universal Master mix without UNG (Applied Biosystems) with PCR primers at a concentration of 900 nM and TaqMan MGB probe at 200 nM. Reactions were performed in 384-well format in a total reaction volume of 5 μ l using 20 ng of genomic DNA.

The plates were placed in a thermal cycler (PE9700, Applied Biosystems) and heated at 50°C for 2 min and 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min, with a final soak at 25°C. The fluorescence intensity in each well of the TaqMan assay plates was then read (Prism 7900HT, Applied Biosystems), with fluorescence data from each plate analyzed using automated allele-calling software (SDS 2.1).

Statistical analysis. Logistic regression models were used for calculating odds ratios (OR), 95% confidential intervals (95% CI), and the corresponding p values for each SNP site and haplotype. Results were obtained for codominant, dominant, and recessive models. Age (continuous value), sex (male = 0, female = 1), and SE status (negative = 0, positive = 1) were adjusted by inclusion in the logistic analysis as covariates. In addition, we performed conditional analysis by SE status and anti-CCP Ab status. Pooled analyses were performed using the Mantel-Haenszel test. For assessing the homogeneity of effect size between studies, the Breslow-Day test was calculated. Haploview v3.2 (Broad Institute of Harvard and MIT, Cambridge, MA, USA) was used to determine haplotype frequencies. To optimally correct for multiple testing of SNP in linkage disequilibrium (LD) with each other, the effective number of independent marker loci (4.7285) in *IRF5* was calculated using SNPSpD software (<http://genepi.qimr.edu.au/general/daleN/SNPSpD/>) based on the spectral decomposition of matrices of pairwise LD between SNP.

RESULTS

The clinical characteristics of the study subjects are summarized in Table 1. Although the controls were younger, age was adjusted in our calculations, and the female to male ratio did not differ significantly between cases and controls.

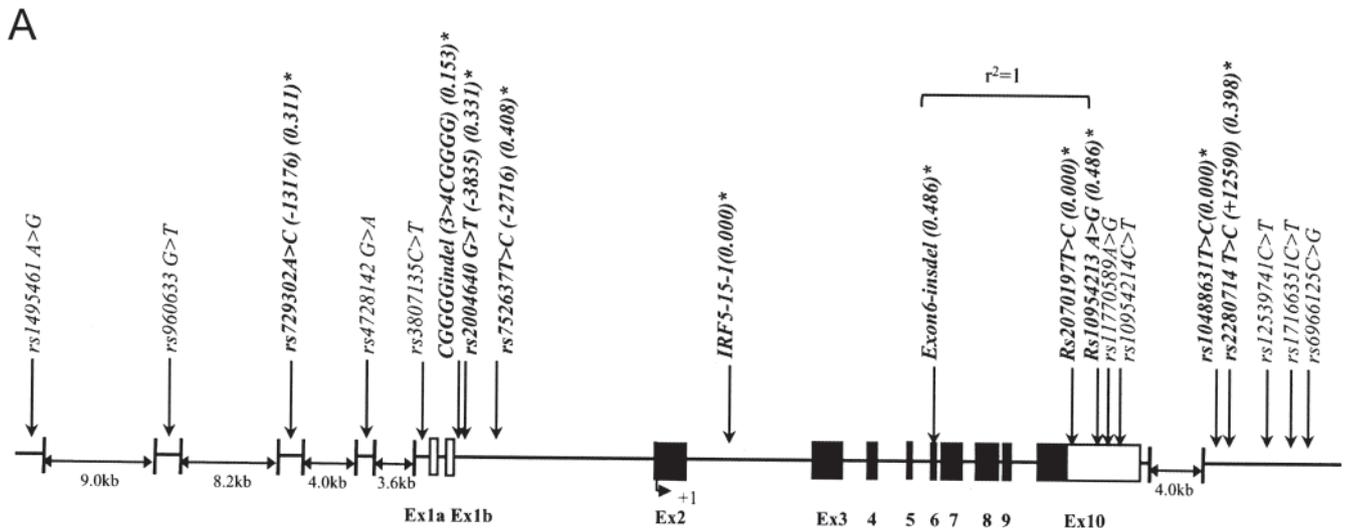
Figure 1 displays the positions of polymorphisms analyzed on *IRF5*, the haplotypes of *IRF5*, and the pairwise LD values among SNP.

We observed significant differences in the genotype distribution of *IRF5 rs2004640* (OR 1.36, 95% CI 1.09–1.68, $P_{\text{corr}} = 0.03$) and *CGGGGindel* (OR 1.38, 95% CI 1.09–1.76, $P_{\text{corr}} = 0.04$), and one haplotype, including the *rs2004640* and the *CGGGGindel*, *ht3* (A-Del-T-C-del-A-T) (OR 1.39, 95% CI 1.09–1.79, $P_{\text{corr}} = 0.04$), was significantly associated with an increased risk of RA in the dominant model (Table 2). In the analysis stratified by anti-CCP Ab and SE status, *rs2004640* showed significant association with susceptibility to RA only in the anti-CCP Ab-positive (OR 1.47, 95% CI 1.15–1.88, $P_{\text{corr}} = 0.01$) or SE-positive group (OR 1.54, 95% CI 1.14–2.09, $P_{\text{corr}} = 0.03$) compared with the control group (Table 3). We performed a combined analysis of *rs2004640* comprising 3 independent cohorts (from Sweden, Spain, and Argentina) in a previous study¹¹ and our cohort. The Breslow-Day test for homogeneity between studies showed no significant heterogeneity ($p = 0.12$). Another previous Swedish cohort could not be included in the combined analysis since only the allele frequency was stated in that report¹⁰. Although no significant associations had been detected in these Caucasian populations (Swedish, Spanish, and Argentinian), the same direction of genetic effects was observed in all populations, including ours, and a more significant association was observed in the combined analysis (pooled OR 1.21, 95% CI 1.07–1.38, pooled $p = 0.0031$ in dominant model; Table 4).

Table 1. Characteristics of Korean RA cases and controls.

| | Cases, n = 1204 | Controls, n = 979 |
|---|---------------------|---------------------|
| Age, yrs, mean ± SEM (range) | 52.5 ± 12.3 (20–82) | 37.4 ± 12.6 (16–79) |
| Sex, female/male (approximate ratio) | 1065/139 (8:1) | 838/141 (6:1) |
| Age at onset, yrs, mean ± SEM (range) | 40.9 ± 12.6 (6–78) | — |
| Disease duration, yrs, mean ± SEM (range) | 11.7 ± 8.5 (0–50) | — |
| Treatment duration, yrs, mean ± SEM (range) | 9.5 ± 7.2 (0–46) | — |
| RF-positive, % | 80.0 | — |
| Anti-CCP antibody, % | 84.9 | — |
| Shared epitope-positive*, % | 68.1 | — |

* SE was defined by the following alleles: DRB1*0101, *0102, *0401, *0404, *0405, *0408, *0410, *1001, *1402, and *1406. CCP: cyclic citrullinated peptide.



B

| Hap. | rs729302(A>C) | CGGGGindel(3>4CGGGG) | rs2004640(G>T) | rs752637(T>C) | Exon6_insdel(Ins>del) | rs10954213(G>A) | rs2280714(T>C) | Freq. |
|---------|---------------|----------------------|----------------|---------------|-----------------------|-----------------|----------------|-------|
| ht1 | A | Ins | G | T | Ins | G | C | 0.358 |
| ht2 | C | Ins | G | T | Del | A | T | 0.198 |
| ht3 | A | Del | T | C | Del | A | T | 0.128 |
| ht4 | A | Ins | T | C | Del | A | T | 0.105 |
| ht5 | C | Ins | G | C | Del | A | T | 0.068 |
| ht6 | A | Ins | T | C | Ins | G | T | 0.063 |
| Others. | . | . | . | . | . | . | . | 0.079 |

C. LDs among *IRF5* polymorphisms

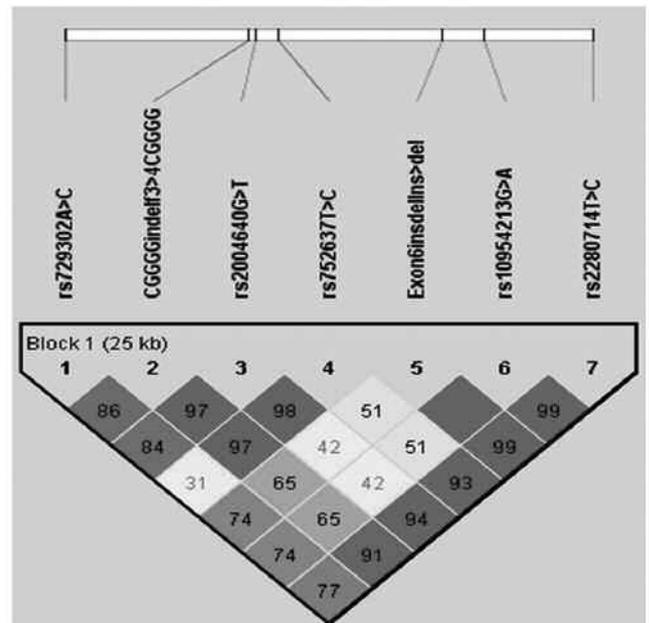


Figure 1. Gene map and haplotypes of the *IRF5* gene on 7q32 (about 12 kb). A. Polymorphisms of *IRF5*⁴. Coding exons are marked by black blocks, and 5' and 3' untranslated regions by white blocks. *Single-nucleotide polymorphisms (SNP) that were genotyped in the Korean population. B. Haplotypes of *IRF5* in the Korean population. Only those with frequencies > 0.05 are shown. C. Linkage disequilibrium coefficients (LD) among selected SNP based on the genotypes of all subjects in this study.

Table 2. Logistic analysis of RA susceptibility with *IRF5* polymorphism and haplotype, controlling for age, sex, and shared epitope (SE). Logistic regression models were used for calculating OR, 95% CI, and corresponding p values for each SNP site and haplotype. Results of codominant, dominant, and recessive models are also given. Age (continuous value), sex (male = 0, female = 1), and SE (negative = 0, positive = 1) were adjusted by inclusion in logistic analysis as covariates.

| rs | Position | Frequency | | Codominant | | | Dominant | | | Recessive | | |
|--------------------------------|----------|-----------|----------|------------------|------|--------------------------------|------------------|-------|--------------------------------|-------------------|------|--------------------------------|
| | | Cases | Controls | OR (95% CI) | p | P _{corr} [*] | OR (95% CI) | p | P _{corr} [*] | OR (95% CI) | p | P _{corr} [*] |
| <i>rs729302 (A>C)</i> | Promoter | 0.305 | 0.320 | 0.92 (0.78–1.08) | 0.29 | 1 | 0.94 (0.76–1.17) | 0.59 | 1 | 0.77 (0.54–1.10) | 0.16 | 0.73 |
| <i>CGGGGindel</i> | Promoter | 0.164 | 0.138 | 1.19 (0.99–1.44) | 0.07 | 0.32 | 1.38 (1.09–1.76) | 0.009 | 0.04 | 0.86 (0.53–1.40) | 0.54 | 1 |
| <i>rs2004640 (G/T)</i> | Promoter | 0.340 | 0.322 | 1.13 (0.96–1.33) | 0.14 | 0.66 | 1.36 (1.09–1.68) | 0.006 | 0.03 | 0.80 (0.56–1.12) | 0.19 | 0.90 |
| <i>rs752637 (T>C)</i> | Promoter | 0.416 | 0.399 | 1.07 (0.92–1.25) | 0.36 | 1 | 1.29 (1.03–1.61) | 0.03 | 0.12 | 0.85 (0.64–1.13) | 0.26 | 1 |
| <i>IRF5-15-1(C>T)</i> | Intron2 | 0.000 | 0.000 | — | — | — | — | — | — | — | — | — |
| <i>Exon6_insel</i> | Exon6 | 0.478 | 0.495 | 0.94 (0.81–1.09) | 0.43 | 1 | 0.99 (0.78–1.26) | 0.95 | 1 | 0.85 (0.66–1.10) | 0.21 | 1 |
| <i>rs2070197 (T>C)</i> | 3'UTR | 0.000 | 0.000 | — | — | — | — | — | — | — | — | — |
| <i>rs10954213 (G>A)</i> | 3'UTR | 0.477 | 0.495 | 0.93 (0.80–1.08) | 0.34 | 1 | 0.97 (0.76–1.23) | 0.80 | 1 | 0.85 (0.66–1.09) | 0.19 | 0.91 |
| <i>rs10488631 (T>C)</i> | 3'down | 0.000 | 0.000 | — | — | — | — | — | — | — | — | — |
| <i>rs2280714 (T>C)</i> | 3'down | 0.395 | 0.401 | 0.97 (0.83–1.13) | 0.67 | 1 | 1.04 (0.84–1.30) | 0.72 | 1 | 0.83 (0.62–1.11) | 0.20 | 0.95 |
| <i>ht1 (A-Ins-G-T-ins-G-C)</i> | — | 0.354 | 0.360 | 0.97 (0.83–1.13) | 0.71 | 1 | 1.09 (0.88–1.35) | 0.42 | 1 | 0.74 (0.54–1.01) | 0.05 | 0.25 |
| <i>ht2 (C-Ins-G-T-del-A-T)</i> | — | 0.195 | 0.204 | 0.94 (0.78–1.13) | 0.48 | 1 | 0.96 (0.77–1.19) | 0.69 | 1 | 0.74 (0.42–1.29) | 0.29 | 1 |
| <i>ht3 (A-Del-T-C-del-A-T)</i> | — | 0.140 | 0.115 | 1.31 (1.05–1.64) | 0.02 | 0.08 | 1.39 (1.09–1.79) | 0.009 | 0.04 | 1.01 (0.45–2.26) | 0.98 | 1 |
| <i>ht4 (A-Ins-T-C-del-A-T)</i> | — | 0.106 | 0.104 | 1.05 (0.82–1.35) | 0.68 | 1 | 1.01 (0.78–1.31) | 0.92 | 1 | 3.44 (0.78–15.18) | 0.10 | 0.49 |
| <i>ht5 (C-Ins-G-C-del-A-T)</i> | — | 0.069 | 0.067 | 0.98 (0.73–1.31) | 0.89 | 1 | 0.97 (0.71–1.32) | 0.84 | 1 | 1.29 (0.25–6.54) | 0.76 | 1 |
| <i>ht6 (A-Ins-T-C-ins-G-T)</i> | — | 0.062 | 0.066 | 0.94 (0.69–1.28) | 0.68 | 1 | 0.95 (0.69–1.31) | 0.77 | 1 | 0.19 (0.01–4.92) | 0.32 | 1 |

* To optimally correct for multiple testing of SNP in linkage disequilibrium (LD) with each other, the effective number of independent marker loci (4.7285) in *IRF5* was calculated using the software SNPSpD based on the spectral decomposition of matrices of pairwise LD between SNP.

DISCUSSION

Despite the *IRF5* polymorphism not being associated with RA in 2 of the 3 previous studies of Caucasian populations, we found that it was associated with susceptibility to RA in our Korean population.

Genetic predisposition and environmental factors are considered to play important roles in the development of RA. Genetic susceptibility to RA has been studied extensively, with genetic factors estimated to account for 60% of the disease risk¹⁸. Several recent studies have suggested that the *IRF5* gene is significantly associated with SLE^{4–7}. *IRF5* is constitutively expressed mainly in the cells of the immune system¹⁹, and the expression of *IRF5* can be enhanced by type I IFN^{1,20}. *IRF5* expression can also be increased by TLR ligation, contributing to the gene induction of proinflammatory cytokines such as interleukin 6 (IL-6) and tumor necrosis factor- α (TNF- α)⁸. In addition, *IRF5* modulates the expression of several factors involved in cell-cycle regulation and apoptosis, and has antiproliferative properties^{21,22}. These findings suggest that polymorphisms within the *IRF5* gene are important genetic risk factors for development of autoimmune diseases, and this has actually been identified in SLE.

There is considerable evidence that type I IFN play a pivotal role in the development and activation of the disease

process in SLE^{2,23,24}. Although there is no strong association between type I IFN and the pathophysiology of RA, there is evidence for the cross-regulation of TNF and type I IFN²⁵, and *IRF5* is implicated in the production of cytokines that are central to the pathophysiology of RA, such as TNF- α , IL-6, and IL-12^{1,8}.

In addition, recent genetic studies have found that SLE shares with RA one genetic factor, PTPN22, and there is evidence that other common genetic factors are involved in the development of autoimmunity²⁶.

Three recent studies that investigated the association between *IRF5* polymorphisms and RA in different Caucasian populations^{10–12} produced conflicting results. One case-control study of Spanish, Swedish, and Argentinian cohorts and one family-based study of a French population suggested that *IRF5* polymorphisms did not contribute to the predisposition to RA. However, the most recent study, involving another Swedish cohort, found that *IRF5* polymorphisms contribute to the predisposition to RA, although the association was observed only in anti-CCP Ab-negative patients.

The previous 3 studies involved only Caucasian populations, but their results were inconsistent. In an additional effort to find the association with RA in an Asian population, we analyzed *IRF5* polymorphisms in a Korean popula-

Table 3. Logistic analysis of RA susceptibility with *IRF5* polymorphisms according to shared epitope (SE) and anti-cyclic citrullinated peptide antibody (anti-CCP) status.

| rs | Subgroup | Codominant | | Dominant | | Recessive | |
|------------------|--------------|------------------|---------------------------------|------------------|---------------------------------|------------------|---------------------------------|
| | | OR (95% CI) | p/p _{corr} vs Controls | OR (95% CI) | p/p _{corr} vs Controls | OR (95% CI) | p/p _{corr} vs Controls |
| rs729302 (A>C) | SE-pos | 0.89 (0.70–1.13) | 0.34/1 | 0.97 (0.72–1.31) | 0.83/1 | 0.60 (0.35–1.03) | 0.07/0.31 |
| | SE-neg | 0.94 (0.75–1.17) | 0.58/1 | 0.92 (0.68–1.24) | 0.58/1 | 0.93 (0.58–1.49) | 0.76/1 |
| | Anti-CCP-pos | 0.89 (0.74–1.08) | 0.24/1 | 0.91 (0.72–1.16) | 0.46/1 | 0.75 (0.49–1.14) | 0.17/0.83 |
| | Anti-CCP-neg | 1.17 (0.88–1.55) | 0.29/1 | 1.20 (0.81–1.77) | 0.37/1 | 1.28 (0.72–2.30) | 0.40/1 |
| CGGGGindel | SE-pos | 1.10 (0.84–1.44) | 0.48/1 | 1.28 (0.91–1.81) | 0.15/0.73 | 0.70 (0.36–1.36) | 0.29/1 |
| | SE-neg | 1.29 (0.99–1.69) | 0.06/0.29 | 1.48 (1.05–2.08) | 0.02/0.11 | 1.08 (0.54–2.18) | 0.83/1 |
| | Anti-CCP-pos | 1.14 (0.92–1.42) | 0.24/1 | 1.33 (1.01–1.76) | 0.05/0.22 | 0.74 (0.41–1.31) | 0.30/1 |
| | Anti-CCP-Neg | 1.22 (0.88–1.70) | 0.23/1 | 1.41 (0.91–2.18) | 0.13/0.6 | 1.05 (0.45–2.44) | 0.91/1 |
| rs2004640 (G>T) | SE-pos | 1.19 (0.94–1.51) | 0.14/0.67 | 1.54 (1.14–2.09) | 0.005/0.03 | 0.68 (0.41–1.12) | 0.13/0.61 |
| | SE-neg | 1.07 (0.86–1.34) | 0.53/1 | 1.19 (0.88–1.62) | 0.26/1 | 0.91 (0.57–1.44) | 0.68/1 |
| | Anti-CCP-pos | 1.19 (0.99–1.43) | 0.06/0.3 | 1.47 (1.15–1.88) | 0.002/0.01 | 0.83 (0.56–1.24) | 0.36/1 |
| | Anti-CCP-Neg | 1.01 (0.76–1.34) | 0.93/1 | 1.21 (0.82–1.79) | 0.34/1 | 0.65 (0.34–1.25) | 0.20/0.94 |
| rs752637 (T>C) | SE-pos | 1.13 (0.91–1.40) | 0.28/1 | 1.41 (1.03–1.93) | 0.03/0.14 | 0.85 (0.57–1.27) | 0.44/1 |
| | SE-neg | 1.03 (0.83–1.28) | 0.80/1 | 1.18 (0.86–1.62) | 0.31/1 | 0.85 (0.57–1.27) | 0.42/1 |
| | Anti-CCP-pos | 1.08 (0.91–1.29) | 0.40/1 | 1.30 (1.01–1.68) | 0.04/0.2 | 0.84 (0.60–1.17) | 0.29/1 |
| | Anti-CCP-Neg | 1.08 (0.83–1.42) | 0.56/1 | 1.35 (0.90–2.05) | 0.15/0.71 | 0.82 (0.49–1.39) | 0.46/1 |
| Exon6indel | SE-pos | 0.88 (0.71–1.09) | 0.23/1 | 0.87 (0.61–1.24) | 0.45/1 | 0.81 (0.57–1.14) | 0.23/1 |
| | SE-neg | 1.01 (0.82–1.26) | 0.91/1 | 1.12 (0.80–1.56) | 0.52/1 | 0.91 (0.63–1.31) | 0.62/1 |
| | Anti-CCP-pos | 0.98 (0.83–1.17) | 0.84/1 | 0.98 (0.74–1.29) | 0.88/1 | 0.97 (0.73–1.29) | 0.86/1 |
| | Anti-CCP-Neg | 0.74 (0.56–0.98) | 0.04/0.17 | 0.73 (0.48–1.11) | 0.14/0.67 | 0.60 (0.36–1.00) | 0.05/0.23 |
| rs10954213 (G>A) | SE-pos | 0.87 (0.70–1.08) | 0.20/0.96 | 0.86 (0.60–1.21) | 0.38/1 | 0.81 (0.58–1.14) | 0.23/1 |
| | SE-neg | 1.00 (0.81–1.24) | 0.98/1 | 1.09 (0.78–1.52) | 0.62/1 | 0.90 (0.62–1.29) | 0.56/1 |
| | Anti-CCP-pos | 0.97 (0.82–1.16) | 0.76/1 | 0.96 (0.73–1.27) | 0.77/1 | 0.97 (0.73–1.29) | 0.84/1 |
| | Anti-CCP-Neg | 0.74 (0.56–0.98) | 0.04/0.17 | 0.73 (0.48–1.11) | 0.14/0.66 | 0.61 (0.37–1.00) | 0.05/0.24 |
| rs2280714 (T>C) | SE-pos | 0.92 (0.74–1.14) | 0.44/1 | 0.96 (0.70–1.31) | 0.78/1 | 0.81 (0.55–1.20) | 0.29/1 |
| | SE-neg | 1.02 (0.82–1.27) | 0.86/1 | 0.13 (0.83–1.54) | 0.43/1 | 0.86 (0.56–1.31) | 0.47/1 |
| | Anti-CCP-pos | 0.99 (0.83–1.18) | 0.83/1 | 1.03 (0.80–1.33) | 0.83/1 | 0.93 (0.67–1.28) | 0.65/1 |
| | Anti-CCP-Neg | 0.77 (0.58–1.03) | 0.08/0.36 | 0.77 (0.52–1.15) | 0.20/0.94 | 0.61 (0.34–1.09) | 0.10/0.46 |

tion, with our results providing evidence that the *IRF5* gene influences RA, especially in anti-CCP Ab-positive or SE-positive subsets of patients.

In the present study, 2 polymorphisms in *IRF5*, *rs2004640* and *CGGGGindel*, showed significant association, and functional relevance for these polymorphisms was clarified in previous studies. The *rs2004640* was known to influence expression of multiple *IRF5* isoforms by creating a 5' donor-splice site in an alternate exon 1 of *IRF5*⁶.

Another polymorphism, *CGGGGindel*, contains 3 or 4 repeats of the sequence CGGGG. The longer allele contains an additional SP1 binding site and is associated with increased expression of *IRF5* messenger RNA³.

The expressed multiple isoforms of *IRF5* could influence the function of *IRF5*, including induction of proinflammatory cytokines and involvement of cell-cycle regulation or apoptosis, and encoding the *IRF5* target gene, and thus may contribute to the susceptibility of autoimmune diseases. Besides increasing the risk for SLE, associations between these polymorphisms and other autoimmune diseases such as inflammatory bowel disease, multiple sclerosis, and Sjögren's syndrome have been also reported^{27–29}. Including our study, the role of *IRF5* as a common genetic factor among various autoimmune diseases is suggested.

Although the significant association of *IRF5* polymorphisms in the entire RA patient group was similar to the study with the Swedish population¹⁰, the results stratified by anti-CCP Ab status showed different findings between the 2 studies.

The clinical course of RA according to anti-CCP Ab status is quite different, and thus it has been considered that genetic and environmental risk factors contributing to each status are also different. Current findings suggest that the SE will predispose to the development of anti-CCP Ab and is associated with production of anti-CCP Ab³⁰. Although the interaction of tobacco smoking and the SE is thought to be associated with citrullination³¹, the pathogenesis between the SE and anti-CCP Ab remains unclear. In our studies, the *rs2004640* SNP showed significant association in the anti-CCP Ab-positive group or the SE-positive group, thus *IRF5* polymorphism may be one candidate gene involved in pathogenesis between the SE and citrullination in RA.

Our results suggest that *IRF5* polymorphisms contribute to the risk of susceptibility to RA only in the anti-CCP Ab-positive or SE-positive subsets of RA patients in a Korean population. The association of the *IRF5* polymorphism with subgroups stratified by anti-CCP Ab and SE status remains unresolved. The different results support the need for further

Table 4. Genotypic association of the rs2004640 (G>T) IRF5 SNP with RA.

| Study | Genotype | Cases | Frequency | Controls | Frequency | | OR (95% CI) | Chi-square | p* |
|---------------------------|----------|------------|-----------|----------|-------------|------------------|------------------------|------------|----------|
| Korean | | N** = 1193 | | N = 950 | | | | | |
| | GG | 507 | 0.425 | 454 | 0.478 | | | | |
| | GT | 561 | 0.470 | 383 | 0.403 | GT vs GG | 1.31 (1.09–1.57) | 8.60 | 0.0034 |
| | TT | 125 | 0.105 | 113 | 0.119 | TT vs GG | 0.99 (0.75–1.32) | 0.00 | 0.9478 |
| | | | | | | TT vs GT+GG | 0.87 (0.66–1.14) | 1.08 | 0.2997 |
| Spanish ¹¹ | | N = 724 | | N = 542 | | | | | |
| | GG | 125 | 0.173 | 112 | 0.207 | | | | |
| | GT | 375 | 0.518 | 269 | 0.496 | GT vs GG | 1.25 (0.93–1.69) | 2.13 | 0.1449 |
| | TT | 224 | 0.309 | 161 | 0.207 | TT vs GG | 1.25 (0.90–1.73) | 1.76 | 0.1843 |
| | | | | | | TT vs GT+GG | 1.06 (0.83–1.35) | 0.22 | 0.6366 |
| Swedish ¹¹ | | N = 281 | | N = 472 | | | | | |
| | GG | 57 | 0.203 | 102 | 0.216 | | | | |
| | GT | 142 | 0.505 | 224 | 0.475 | GT vs GG | 1.13 (0.77–1.67) | 0.41 | 0.5222 |
| | TT | 82 | 0.292 | 146 | 0.309 | TT vs GG | 1.00 (0.66–1.53) | 0.00 | 0.9814 |
| | | | | | | TT vs GT+GG | 0.92 (0.67–1.27) | 0.26 | 0.6131 |
| Argentinian ¹¹ | | N = 285 | | N = 284 | | | | | |
| | GG | 76 | 0.267 | 85 | 0.3 | | | | |
| | GT | 123 | 0.431 | 135 | 0.475 | GT vs GG | 1.02 (0.69–1.51) | 0.01 | 0.9254 |
| | TT | 86 | 0.302 | 64 | 0.225 | TT vs GG | 1.50 (0.96–2.35) | 3.19 | 0.074 |
| | | | | | | TT vs GT+GG | 1.49 (1.02–2.16) | 4.28 | 0.0386 |
| All | | N = 2483 | | N = 2248 | | | | | |
| | GG | 765 | 0.504 | 753 | 0.496 | | Pooled OR [†] | | Pooled p |
| | GT | 1201 | 0.543 | 1011 | 0.457 | GT vs GG | 1.24 (1.08–1.42) | 9.61 | 0.0019 |
| | TT | 517 | 0.516 | 484 | 0.484 | TT vs GG | 1.13 (0.95–1.35) | 1.92 | 0.1653 |
| | | | | | | TT vs GT+GG | 1.02 (0.89–1.18) | 0.09 | 0.7607 |
| | | | | | GT+TT vs GG | 1.21 (1.07–1.38) | 8.76 | 0.0031 | 0.92 |

* Uncorrected value for multiple tests, 1 degree of freedom. ** Mantel-Haenszel test of pooled OR and 95% CI. † Breslow-Day test for homogeneity was not significant (p = 0.12).

studies, involving diverse populations, into the effects of the *IRF5* gene on subgroups of RA.

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