Association of Polymorphisms in Interferon Regulatory Factor 5 Gene with Rheumatoid Arthritis: A Metaanalysis

SEUNG WOO HAN, WON KI LEE, KI TAE KWON, BYUNG KI LEE, EON JEONG NAM, and GUN WOO KIM

ABSTRACT. ***Objective.*** We investigated potential associations between rheumatoid arthritis (RA) and interferon regulatory factor 5 (*IRF5*) polymorphisms in a metaanalysis.

Methods. This metaanalysis included 5 case-control studies, which provided a total of 6582 RA cases and 5375 controls. Odds ratios (OR) were employed to evaluate the risk of RA according to the 4 single-nucleotide polymorphisms (SNP) in *IRF5* (rs729302, rs2004640, rs752637, and rs2280714) and data were analyzed in respect to association between alleles.

Results. Among 4 candidate SNP, rs729302, rs2004640, and rs2280714 were statistically significant; both allele C of rs729302 and allele G of rs2004640 within the promoter region of *IRF5* were associated with a protective effect [random-effects (RE) OR 0.889, 95% confidence interval (CI) 0.803–0.977, p = 0.015 for rs729302; and RE OR 0.905, 95% CI 0.848–0.965, p = 0.002 for rs2004640]. Similar results were also obtained in T allele of rs2280714 in the 3'-untranslated region (RE OR 0.927, 95% CI 0.866–0.992, p = 0.029). There was no evidence of publication bias from funnel-plot asymmetry and Egger's regression test.

Conclusion. Our metaanalysis supported the evidence of the significant role of *IRF5* polymorphisms in RA. (J Rheumatol First Release Feb 15 2009; doi:10.3899/jrheum.081054)

Key Indexing Terms:
INTERFERON REGULATORY FACTOR 5 RHEUMATOID ARTHRITIS
METAANALYSIS SINGLE-NUCLEOTIDE POLYMORPHISM

Rheumatoid arthritis (RA) is characterized by a chronic inflammatory process that targets the synovial lining of joints and is a classical example of a complex genetic disease[1,2]. The role of immunological perturbations in the arthritis process seems reasonably established, but the mechanisms involving the initiation of inflammation have remained largely elusive[1,3]. Recent advances in knowledge of the disease mechanism linking innate and adaptive immunity have led to reconsideration of the roles of the innate immune system in synovial inflammation and destruction of joint cartilage and bone[3]. Indeed, whether activation of the innate immune system is a cause or a consequence of inflammation, it constitutes a target for new treatment strategies.

The interferon regulatory factor (IRF), as a family of transcription factors with 9 members, was initially found to be involved in the induction of genes that encode the type I interferon (IFN) system[4]. Besides regulating the IFN system, the IRF family has gained much attention as essential regulators of the activation of immune cells with the discovery of pattern recognition receptors, which seem to function as a "platform" that links innate and adaptive immune responses[5,6]. Among 9 mammalian IRF, IRF5 has been known to have a crucial function in Toll-like receptor (TLR) mediated signaling. As ligands of TLR4, TLR5, TLR7, and TLR9, IRF5 activates the transcription of proinflammatory cytokine genes, such as tumor necrosis factor (TNF), interleukin 6 (IL-6), and IL-12p40, presumably in cooperation with nuclear factor-κB (NF-κB). In *IRF5* knockout mice, there was an increased resistance to endotoxic shock following exposure to CpG oligonucleotide or lipopolysaccharide, as well as an impaired induction of proinflammatory cytokine genes[7]. These observations indicate that *IRF5* involves the induction of inflammatory cytokines of the innate immune system and, therefore, the differential

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expression of *IRF5* target genes conferred by *IRF5* genotype may modify the immune response.

A recent genome-wide association study revealed that the susceptibility of RA had a marginal association with single-nucleotide polymorphism (SNP) rs3807306, which was the tagging SNP of the haplotype block containing *IRF5* gene located at chromosome 7q32[8]. In addition, the strong association of the SNP in the *IRF5* gene with systemic lupus erythematosus (SLE) has been reported by epidemiologic and molecular functional studies[9-11], which prompted the investigation of its possible role in RA susceptibility. However, the early association studies of *IRF5* in RA failed to show any significant difference in allele or genotypic frequencies of genetic variants of rs2004640 and rs2280714[12,13]. Sigurdsson, *et al*[14] were the first to show significant association of the SNP in the 5'-untranslated region (UTR) of *IRF5* with RA, especially in the anticyclic citrullinated peptide (CCP) antibody-negative group. Since then, 2 more studies have tested the association of genetic variants with RA. One reproduced the positive association in rs2004640[15], but the other study did not, instead showing a significant association with other SNP of rs729302[16]. Given all these conflicting results, it is necessary to perform a quantitative synthesis of the evidence to reassess the importance of *IRF5* SNP for RA susceptibility. We conducted a metaanalysis that maximizes the power to find associations between RA and the 4 *IRF5* SNP.

MATERIALS AND METHODS

Identification of relevant studies and data extraction. We carried out a literature search in Medline and Embase (last updated in August 2008) and included all studies that examined the association of *IRF5* polymorphisms with RA. The search strategy was based on combinations of the terms "IRF5," "interferon regulatory factor," and "rheumatoid arthritis," according to the medical subject headings (MeSH) browser and "related links." Reference lists in retrieved articles were also screened without any language restriction. We could find only 5 published reports about genetic associations of IRF5 in RA[12-16]. Two investigators (SH and GK) independently extracted data, discussed disagreements, and reached consensus on all items. We also checked whether matching had been used, whether the genotyping method used had been validated, and whether genotype frequencies in control groups were in Hardy-Weinberg equilibrium. Among the SNP treated in the 5 studies[12-16], we focused on the 4 major SNP that were studied in more than 3 published articles: rs729302, rs2004640, rs752637, and rs2280714. Only allele frequency was tested, because we avoided multiple comparisons inflating type I error and most studies did not reveal the genotype frequency, but only minor allele frequencies[14-16].

Metaanalysis. Heterogeneity among studies was formally assessed using Cochran's Q statistic and considered significant at $p < 0.10$. We also report I^2 metrics, which quantify heterogeneity irrespective of the number of studies. Large heterogeneity is claimed for I^2 values of ≥ 75%. Data were combined using both fixed-effects (FE) (Mantel-Haenszel) and random-effects (RE) (DerSimonian and Laird) models, among which random effects are more appropriate when heterogeneity is present. Unless stated, therefore, RE estimates are reported here. We used inverted funnel plots and Egger's regression publication bias diagnostics to evaluate whether the magnitudes of the observed associations were related to the variance of each study. Analyses were conducted in Comprehensive Meta Analysis version 2.0 (http://www.meta-analysis.com). All p values presented are 2-tailed.

RESULTS

Study characteristics. The 5 studies eligible for analysis included a total of 6582 cases with RA and 5375 controls, which were available for analysis of the rs2004640[12-16]. Both rs729302 and rs752637 SNP were investigated in 3 other studies that included a total of 5197 cases and 3982 controls[14-16]. In the analysis of rs752637, however, allele frequency data were available for 4810 cases and 3801 controls, because Sigurdsson, *et al*[14] provided data only of the Swedish population. Finally, the SNP rs2280714 located 3'-UTR of *IRF5* was analyzed using 4570 cases and 4238 controls in 3 case-control studies[12,15,16]. Most studies were carried out in Caucasians except the one Asian study of Shimane, *et al*[16]. Characteristics of studies included in our metaanalysis are presented in Table 1.

Rs729302 and rs2004640 variants in promoter region of IRF5 associated with RA. The pooled frequency of the C allele in rs729302 was 29.0% and 31.1% among RA cases and control subjects, respectively. For 3 studies with 6 subgroups, significant between-study heterogeneity was observed ($p = 0.060$ for heterogeneity; $I^2 = 53\%$). Under a RE model, the summary odds ratio (OR) suggested a 0.88-fold decrease in susceptibility to RA among persons with the C allele, which was statistically significant ($z = –2.553$, $p = 0.011$; Table 2, Figure 1A). The distribution of the OR in the funnel plot was symmetrical, suggesting a low probability of publication bias (Egger's regression test, $p = 0.27$).

Another target SNP rs2004640 in the promoter region of *IRF5* had no significant heterogeneity ($p = 0.168$), in which 30% of the heterogeneity was explained by the between-study variance. Analysis of the allele G of rs2004640 also showed a pooled RE OR = 0.904 (95% CI 0.857-0.952), indicating that the presence of allele G at rs2004640 may exert a weak protective effect against the development of RA (Table 2, Figure 1B). No evidence for publication bias was detected using Egger's regression test (intercept –0.508, 95% CI –3.64-2.63, $p = 0.718$) as well as funnel plot inspection (Figure 2).

The analysis of rs752637, the last target polymorphism in the promoter of *IRF5*, however, failed to show a significant association between this SNP and RA; under a RE model, subjects with A allele had a pooled RE OR of 0.915 (95% CI 0.824-1.016, $p = 0.096$), and significant heterogeneity was observed ($p = 0.033$, $I^2 = 61\%$; Table 2, Figure 1C).

Rs2280714 in 3'-UTR of IRF5 associated with RA. In terms of the SNP rs2280714, there was no heterogeneity among the 7 group of 3 studies ($p = 0.317$, $I^2 = 14.847$). When we tested the quantitative effect, we found a significant protective effect of rs2280714 T allele to RA; the OR under RE and FE models were 0.927 (95% CI 0.866-0.992) and 0.926 (0.871-0.986), respectively (Table 2, Figure 1D). A funnel plot did not indicate the presence of publication bias in these studies (Egger's test, $p = 0.851$).

Table 1. Characteristics of studies included in this metaanalysis of polymorphisms in the *IRF5* gene and susceptibility to RA. All were case-control studies.

Study	Year	Population	Subgroup	No. of cases	No. of Controls
Rueda[12]	2006	Caucasians	Spanish	724	542
			Swedish	281	472
			Argentinean	285	284
Garnier[13]	2007	Caucasians (France)	None	95	95
Sigurdsson[14]	2007	Caucasians	Swedish	1530	861
			Dutch	387	181
Dieguez-Gonzalez[15]	2008	Caucasians (Spain)	Exploratory	516	503
			Replication	822	839
Shimane[16]	2008	Asians (Japan)	First	830	658
			Second	1112	940
			Total	6582	5375

Table 2. Summary of association and heterogeneity of the *IRF5* SNP in RA.

	Model	No. of Studies (subgroup)	Effect Size (95% CI)	Test of Null (2-tail) Z-value	p	Heterogeneity Q-value	df (Q)	p	I^2
rs729302	Fixed-effects	3 (6)	0.892 (0.836–0.952)	–3.449	0.001	10.591	5	0.060	52.788
	Random-effects	3 (6)	0.881 (0.799–0.971)	–2.553	0.011				
rs2004640	Fixed-effects	5 (10)	0.904 (0.857–0.952)	–3.770	0.000	12.886	9	0.168	30.157
	Random-effects	5 (10)	0.901 (0.844–0.962)	–3.122	0.002				
rs752637	Fixed-effects	3 (5)	0.921 (0.864–0.982)	–2.532	0.011	10.484	4	0.033	61.845
	Random-effects	3 (5)	0.915 (0.824–1.016)	–1.663	0.096				
rs2280714	Fixed-effects	3 (7)	0.926 (0.871–0.986)	–2.421	0.015	7.046	6	0.317	14.847
	Random-effects	3 (7)	0.927 (0.866–0.992)	–2.188	0.029				

Q: Cochran's test; I^2: Higgins' test; df: degrees of freedom.

DISCUSSION

IRF5 is a transcription factor responsible for regulating TNF-α, IL-6, IL-12, and type 1 IFN, which play an important role in the pathophysiology of RA[5,7]. Recently, the strong association of SNP in the *IRF5* gene with SLE has been reported and it has prompted the investigation of a possible role of IRF5 in RA[9,11,17-19]. However, both positive and negative associations with RA have been reported for SNP located in the *IRF5* genes[12-16]. Overall, this meta-analysis provides summarized evidence for a statistically significant association of *IRF5* SNP with susceptibility to RA. Both rs729302 and rs2004640 in the promoter region and rs2280714 in the 3'-UTR of *IRF5* showed a modest association with RA.

The minor allele of rs729302 located about 9 kb upstream from the *IRF5* gene was associated with the protective effect to RA. As for the linkage disequilibrium (LD) and haplotype structure for the *IRF5* gene, the 3 polymorphisms investigated in this study, rs2004640, rs752637, and rs2280714, are located in one block, while rs729302 as tag SNP is in another block, according to HapMap data[18]. This SNP has already been studied in patients with SLE, which also has a protective effect[19]. However, the functional relevance of this SNP has not been identified. When we predicted the changes in transcription factor binding by the TRANSFAC version 6.0 database (www.cbil.upenn.edu/cgi-bin/tess/tess), rs729302 A allele contains putative GR, LEF-1, and TCF-1 transcription factor binding sites, whereas C allele does not. The change of transcriptional activity by genetic variants in the *IRF5* promoter region would contribute to susceptibility to RA. However, the functional characterization of regulatory polymorphisms has been made more difficult by the many potential confounders including the wide spectrum of cis-acting regulatory mechanisms, the inconsistent effects of regulatory variants in different tissues, and the linkage disequilibrium with many other variants[20].

Among *IRF5* genetic variants, SNP rs2004640 has been most actively investigated in RA[12-16]. Our study showed quantified evidence of a protective effect of rs2004640 G allele to RA, and this has been observed in another meta-analysis incorporating 4 individual studies[15]. However, the first 2 published studies, with relatively small sample sizes, reported negative association of rs2004640 with RA. Generally, the statistical uncertainty in the initial results of association may have originated from either sampling bias or lack of power to detect the differences[21,22]. We found evidence of lack of power in early studies; the study by Rueda, *et al*[12] showed a power of 82% in 724 Spanish RA cases and 40%–50% in other populations with 400 patients, assuming

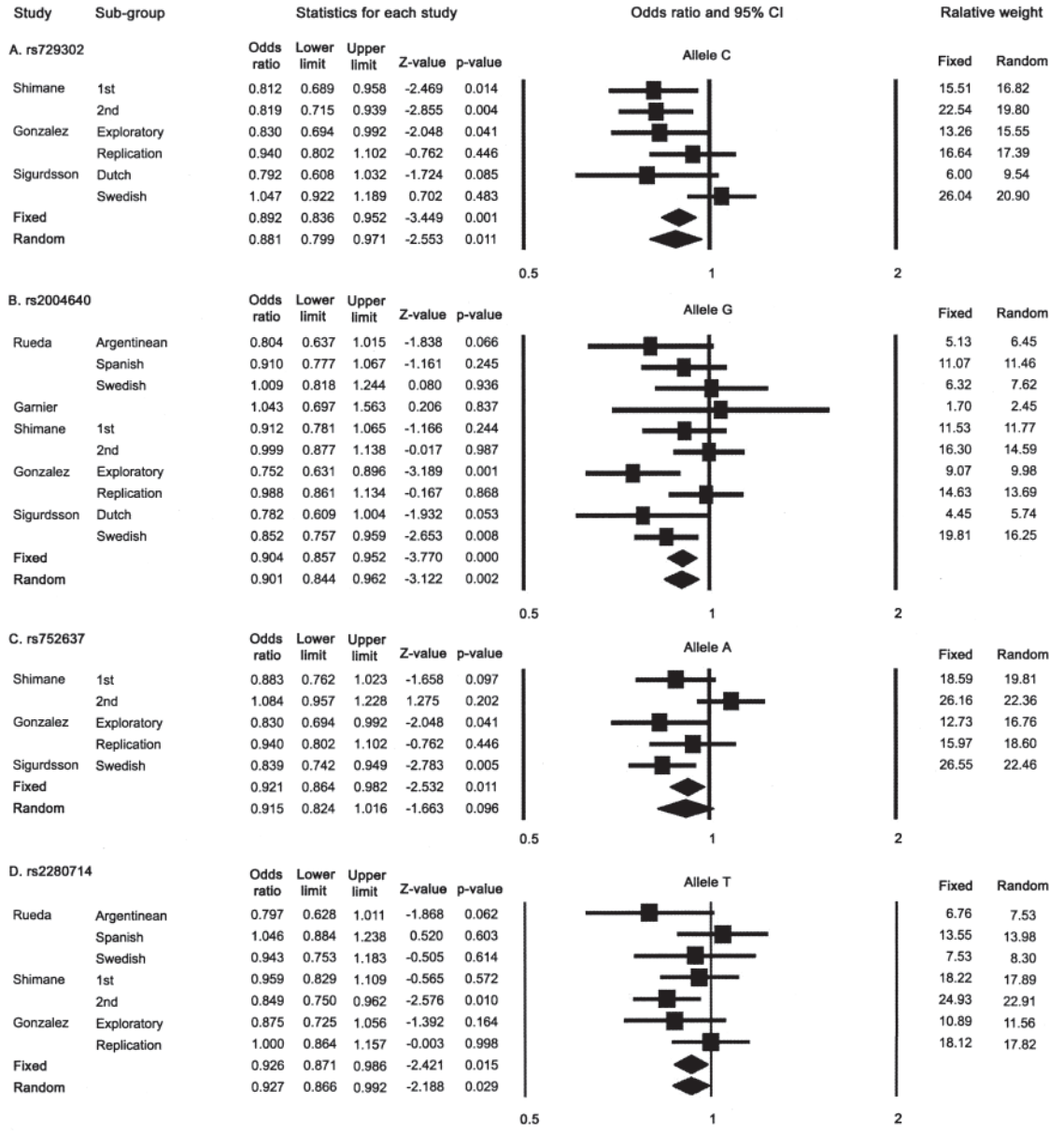

Figure 1. Metaanalysis for the *IRF5* gene polymorphisms using minor allele frequency. Forest plot shows the summarized results of individual studies (quadrangles) and the fixed and random-effects odds ratio (OR) from this metaanalysis (diamonds). OR for the association of rs729302 (A), rs2004640 (B), rs752637 (C), and rs2280714 (D) with RA are represented; horizontal lines show the 95% CI.

OR as 1.4. And Garnier, *et al*[13] included only 99 patients and controls, respectively. Since most associations refer to small relative risk (1.20–1.50)[21] and both allele frequency and genetic model cannot be controlled, a sample size of several thousand is necessary to address these genetic risk factors.

Mechanisms that link the *IRF5* SNP rs2004640 and RA risk are not fully understood, although the transcriptional difference by genetic variants is the most common candidate. *IRF5* transcripts are initiated at one of 3 promoters, giving rise to transcripts containing exon 1A, exon 1B, or exon 1C[18,23]. The SNP rs2004640 is located in a splice junction of an alternative exon 1B of *IRF5*. Interestingly, recent studies have shown that the major allele (T) of this SNP creates a splice-donor site for exon 1B, indicating that *IRF5* isoforms initiated at exon 1B may influence the function of *IRF5* or the transcriptional profile of *IRF5* target genes[18,23]. The results of our metaanalysis support the evidence that common SNP in the promoter region of the *IRF5* gene modulate the expression of IRF5 and contribute to susceptibility to RA[9,18,19].

Our current pooled data suggest that rs2280714 located at the 3'-UTR of *IRF5* gene also has a genetic association with RA. In most published studies, however, rs2280714

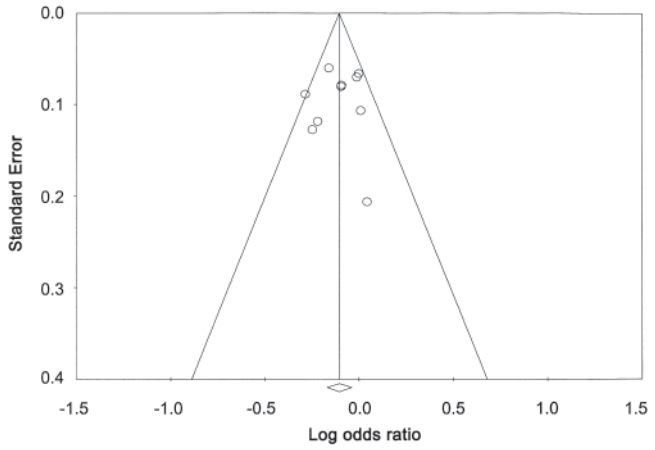

Figure 2. Begg's funnel plot of studies investigating the effect of the rs2004640 on the risk of RA.

failed to show any significant association with RA, except one subgroup analysis by Shimane, *et al*[16], which showed mild association of OR 1.19[16]. A recent functional study showed that *IRF5* SNP rs2280714 T allele was a good predictor of IRF5 overexpression as a cis-acting variant controlling expression[18]. In addition, several additional SNP in the 3' end of the *IRF5* gene, such as rs10954213 and rs10488631, have been found to be associated with the expression levels of IRF5[11,19]. Whether the positive association in RA is caused by the cis-regulatory action of rs2280714 itself or the strong linkage disequilibrium with other possible cis-acting determinants, the interaction between the genetic variant in the promoter and 3'-UTR seems to have an important role in the expression of IRF5[18].

The findings of our metaanalysis suggest that genetic variations in the promoter and 3'-UTR of *IRF5* might contribute to the susceptibility to RA. The functional effects of the *IRF5* gene have not been defined and need to be the focus of future research. In addition, the action mechanisms of IRF5 that interact with other transcription factors such as NF-κB, NFAT, Ets, and Stat families suggest the need for further studies about gene-gene interaction of *IRF5* gene[6].

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