Different Genetic Effects of Interferon Regulatory Factor 5 (*IRF5*) Polymorphisms on Systemic Lupus Erythematosus in a Korean Population

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ABSTRACT. ***Objective.*** In an effort to replicate additional associations of interferon regulatory factor 5 (*IRF5*) polymorphisms with systemic lupus erythematosus (SLE) in an Asian population, we examined those genetic effects in a Korean SLE cohort.

Methods. Each *IRF5* polymorphism was genotyped in 1565 subjects using the TaqMan method and examined to determine whether it could explain the association with SLE.

Results. Three single-nucleotide polymorphisms (*IRF5-15-1*, *rs2070197*, and *rs10488631*), which showed strong and/or independent association in Caucasian populations, were not polymorphic in our Korean population. Association analysis revealed different genetic effects in Koreans compared with Caucasian populations. In addition, conditional analysis suggested independent genetic effects of 3 variant groups in the Korean population.

Conclusion. We demonstrate different genetic effects of *IRF5* polymorphisms on the risk of SLE according to ethnicity. (First Release Oct 1 2008; J Rheumatol 2008;35:2148–51; doi:10.3899/jrheum.080124)

Key Indexing Terms:
INTERFERON REGULATORY FACTOR 5 POLYMORPHISM
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Recently, several studies have provided convincing evidences that interferon regulatory factor 5 [*IRF5* (MIM 607218)] polymorphisms are significantly associated with systemic lupus erythematosus [SLE (MIM 152700)][1-4]. We previously reconfirmed the genetic association of *IRF5* polymorphisms with the risk of SLE in a Korean population[3]. In a subsequent study of the same group from which *IRF5* association with SLE had been identified in Caucasian populations, 3 functional alleles of *IRF5* were found to be associated with SLE: the previously described exon 1B splice site variant; a 30-bp in-frame insertion/deletion variant of exon 6 that alters a proline-, glutamic acid-, serine-, and threonine-rich domain region; and a variant in a conserved polyA_signal sequence that alters the length of the 3' untranslated region (UTR) and stability of *IRF5* mRNA among Caucasian populations[5]. In addition, the *CGGGGindel* located in the promoter region of *IRF5*, only 64 bp upstream of exon 1a of *IRF5*, was reported as an additional candidate for a causal variant of *IRF5*[5].

In an effort to replicate the additional associations with SLE in an Asian population, we examined those genetic effects in our SLE cohort from the Korean population.

MATERIALS AND METHODS

A total of 593 Korean patients with SLE [mean age 32.36 yrs (6.99–70.7), male/female ratio 35/558] who fulfilled the 1997 American College of Rheumatology (ACR) criteria for SLE[6] were consecutively enrolled in our study between September 1998 and February 2005 from the Hospital for Rheumatic Diseases, Hanyang University, Seoul. The following clinical and laboratory data were obtained: sex, age, ages at first symptom onset and clinical diagnosis, ACR diagnosis, and Systemic Lupus International Collaborating Clinics (SLICC)/ACR damage index[7]. As a control group, we included 972 healthy ethnically matched subjects [mean age 37.2 yrs (range 16.6–78.6), male/female ratio 139/832]. Written informed consent was obtained from each subject, and the protocol was approved by the Institutional Review Board of Hanyang University Hospital.

Six polymorphisms (*CGGGGindel*, *IRF5-15-1*, *Exon6indel*, *rs2070197*, *rs10954213*, and *rs10488631*), which showed strong and/or independent association in Caucasian populations[5,8], were genotyped using TaqMan®[9] among our cases with SLE and controls from the Korean population.

Chi-squared analyses were used to evaluate the significance of differences in genotype and allele frequencies in the case-control samples using SAS. For conditional analysis of *IRF5* polymorphisms, the variants were subdivided into 3 groups according to linkage disequilibrium (LD) values

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among them (Table 1). Conditional p values were estimated and permutated using the WHAP software[10].

RESULTS

The genotype distributions of 10 markers, including 4 markers that had been reported in our previous study, are shown in Table 2. All polymorphisms except *rs729302* showed significant differences between Korean and Caucasian populations. Interestingly, 3 single-nucleotide polymorphisms (SNP; *IRF5-15-1*, *rs2070197*, and *rs10488631*) that showed strong and/or independent association in Caucasian populations were not polymorphic in the Korean population. By pairwise LD analysis using 7 polymorphic markers in the Korean population, 2 sets of tight linkages (*CGGGGindel: rs2004640: rs752637* and *Exon6indel: rs10954213: rs2280714*) (D' > 0.97) were observed (Table 1).

First, genetic effects of the polymorphisms were individually analyzed. We found that 4 variants in the promoter region (*rs729302*, *CGGGGindel*, *rs2004640*, and *rs752637*) and 2 haplotypes (*ht2* and *ht3*) showed strong associations (p = 0.003–0.00002). However, the signals of 3 variants (*CGGGGindel*, *rs2004640*, and *rs752637*) might not be independent because of tight LD among them (D' > 0.97).

Next, we investigated whether each polymorphism could explain the association to SLE using conditional logistic regression. The variants were subdivided into 3 groups according to LD values among them (Table 1). The results of conditional analysis were as follows. (1) When condi-

Table 1. Linkage disequilibrium among *IRF5* polymorphisms (r^2 and |D'|).

			D'						
		rs729302A>C	*CGGGGindel*	*rs2004640G>T*	*rs752637T>C*	*Exon6indel*	*rs10954213G>A*	*rs2280714T>C*
	rs729302A>C	—	0.878	0.833	0.321	0.713	0.71	0.762
	CGGGGindel	0.063	—	0.981	0.978	0.666	0.668	0.943
	rs2004640G>T	0.160	0.348	—	0.984	0.36	0.358	0.937
r^2	*rs752637T>C*	0.032	0.256	0.714	—	0.467	0.466	0.937
	Exon6indel	0.223	0.083	0.068	0.155	—	1	0.996
	rs10954213G>A	0.222	0.085	0.068	0.155	1	—	0.996
	rs2280714T>C	0.168	0.114	0.312	0.419	0.663	0.661	—

Table 2. Frequencies of *IRF5* polymorphisms in Korean, Japanese, Chinese, and Caucasian populations.

rs	Region	Genotype			Allele	Frequency				p*
						Korean (n = 1565)	Caucasian (n = 934[a], 533[b], 1048[c])	Japanese[d] (n = 3540)	Chinese[e] (n = 190)	
rs729302	Promoter	A	AC	C	A	0.70	0.69[a]	0.73		0.6163
		763	650	144						
CGGGGindel	Promoter	Del	Indel	Ins	Ins	0.16	0.50 (0.44)[c]	0.12		4.062×10^{-141}
		1151	304	95						
rs2004640	Promoter	G	GT	T	T	0.345	0.57[a]	0.33	0.29	5.2×10^{-28}
		676	664	199						
rs752637	Promoter	T	CT	C	C	0.42	0.68[a]	0.41		3.7×10^{-36}
		537	724	290						
IRF5-15-1	Intron2	C	CT	T	T	0.00	0.18 (0.14)[c]			8.8312×10^{-136}
		1585	0	0						
Exon6indel	Exon6	Del	Indel	Ins	Ins	0.50	0.51[b]			0.5934
		396	797	392						
rs2070197	3'UTR	T	CT	C	C	0.00	0.13[b]		0.00	4.5×10^{-32}
		1586	0	0						
rs10954213	3'UTR	A	AG	G	A	0.50	0.63[b]		0.49	4.6×10^{-9}
		394	799	394						
rs10488631	3'down	T	CT	C	C	0.00	0.17 (0.12)[c]			3.112×10^{-129}
		1582	0	0						
rs2280714	3'down	T	CT	C	T	0.60	0.72[a]	0.56		1.6×10^{-11}
		566	747	252						

[a] *Graham, et al*[1]. Data of 4 SNP are from Caucasian parental chromosomes used in transmission disequilibrium test analysis. [b] *Kozyrev*[17]. Data of 4 SNP are from Argentinian, Spanish, and German combined subjects. [c] *Sigurdsson, et al*[4]. Data of 3 SNP are from Swedish subjects (frequency of 563 controls in parentheses). [d] *Shimane, et al*[18]. [e] *Siu, et al*[16]. * Chi-square comparison of ethnic difference between Korean and Caucasian populations.

tioned by *CGGGGindel*, which showed the strongest association among first-group variants (*CGGGGindel*, *rs2004640*, and *rs752637*), 2 variants in the third group now appeared to have associations (*Exon6indel* and *rs10954213*). This might suggest that the genetic effects of the third-group variants were hidden by first-group variants. (2) When conditioned by the second-group SNP (*rs729302*), the effects of the first-group variants remained significant (p = 0.03–0.003). (3) When conditioned by a third-group variant [*Exon6indel*, which absolutely linked (r^2 = 1) with *rs10954213*], the effects of first and second-group variants remained significant (p = 0.002–0.00002). (4) Although no significant p values were obtained when conditioned on any 2 variants among each variant group simultaneously, *CGGGGindel* still remained significant (p = 0.03; Table 3), possibly due to the strongest association.

Altogether, conditional analysis in the Korean population suggested independent genetic effects of the 3 variant groups, although the causal polymorphism in each group could not be clearly identified.

DISCUSSION

IRF5 was originally identified as a regulator of type I interferon gene expression[11]. Transcription factors of the IRF family play several roles in viral infection and immunostimulation, as well as cell growth regulation. *IRF5* is regulated by type I interferon, which indicates an important regulatory pathway for the controlled induction of multiple immunomodulatory genes. *IRF5* is phosphorylated in cells upon viral infections and translocates to the nucleus, which results in activation of a spectrum of interferon genes[12]. Thus, polymorphism within the *IRF5* gene may affect several cellular functions of importance for the development of an autoimmune disease such as SLE.

Genetic association studies provide a potentially powerful tool for identifying genetic variations that influence susceptibility to common diseases. However, there are many cases of associations that are not replicated afterward, which has led to skepticism about genetic epidemiology studies of complex diseases[13-15]. It is well known that ethnicity is one of the most important factors for evaluating genetic effects on common complex traits. Here, we demonstrate the different genetic effects of *IRF5* polymorphisms on the risk of SLE according to ethnicity.

In our study, different genetic effects of *IRF5* polymorphisms on SLE were observed in a Korean population compared with those in Caucasian populations. Three SNP (*IRF5-15-1*, *rs2070197*, and *rs10488631*), which showed strong/independent associations in Caucasian populations, had no effects (monomorphic) in the Korean population. Similar results were also reported in a Chinese population[16]. In addition, we have shown that the 3 groups of *IRF5* variants have independent genetic effects on the risk of SLE in a Korean population, which were different from those in Caucasian populations[4,5].

Association analysis of *IRF5* polymorphisms in a Korean population revealed different genetic effects of *IRF5* polymorphisms on SLE compared with those in Caucasian populations. These results indicate different genetic backgrounds of *IRF5* polymorphisms and different genetic effects on the risk of SLE according to ethnicity. The difference among ethnic groups should be considered for the clinical application of SNP.

Table 3. Conditional analysis of *IRF5* polymorphisms in Koreans with SLE and healthy controls (con).

Loci	Allele	Conditional Analysis Group	Frequency SLE	Frequency Con	p	Conditional p Values *CGGGGindel*	*rs729302*	*Exon6indel*	*CGGGGindel* and *rs729302*	*CGGGGindel* and *Exon6indel*	*rs729302* and *Exon6indel*
rs729302	C	Group 2	0.270	0.319	**0.003**	0.07	—	**0.002**	—	0.66	—
*CGGGGindel**	Ins	Group 1	0.197	0.138	**0.00006**	—	**0.003**	**0.00002**	—	—	**0.03**
*rs2004640**	T	Group 1	0.386	0.321	**0.0002**	0.16	**0.03**	**0.0003**	0.28	0.17	0.44
rs752637	C	Group 1	0.457	0.399	**0.0008**	0.28	**0.03**	**0.002**	0.52	0.10	0.33
Exon6indel	Ins	Group 3	0.503	0.495	0.73	**0.02**	0.13	—	0.35	—	—
*rs10954213**	G	Group 3	0.505	0.496	0.72	**0.02**	0.19	NA	0.38	NA	NA
rs2280714	C	Group 3	0.395	0.402	0.68	0.27	0.10	0.24	0.55	0.20	0.47
Haplotype†											
ht1 (A-Ins-G-T-Ins-G-C)			0.349	0.357	0.59						
ht2 (C-Ins-G-T-Del_A-T)			0.157	0.205	**0.0007**						
ht3 (A-Del-T-C-Del-A-T)			0.170	0.116	**0.00002**						
ht4 (A-Ins-T-C-Del-A-T)			0.094	0.103	0.43						
ht5 (A-Ins-T-C-Ins-G-T)			0.077	0.066	0.37						
ht6 (C-Ins-G-C-Del-A-T)			0.064	0.067	0.76						

Conditional p values were estimated and permutated using WHAP software[10]. * Markers reported to have strong and/or independent effects on risk of SLE in Caucasian populations. † Haplotype consisting of 7 polymorphic markers. NA: No p values were available in conditional analysis because *Exon6indel* and *rs10954213* were absolutely linked (r^2 = 1).

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