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Association of IRF5 polymorphisms with activation of the interferon α pathway

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Abstract

Objective—The genetic association of interferon regulatory factor 5 (*IRF5*) with systemic lupus erythematosus (SLE) susceptibility has been convincingly established. To gain understanding of the effect of *IRF5* variation in individuals without SLE, a study was undertaken to examine whether such genetic variation predisposes to activation of the interferon α (IFN α) pathway.

Methods—Using a computer simulated approach, 14 single nucleotide polymorphisms (SNPs) and haplotypes of *IRF5* were tested for association with mRNA expression levels of *IRF5*, IFN α and IFN-inducible genes and chemokines in lymphoblastoid cell lines (LCLs) from individuals of European (CEU), Han Chinese (CHB), Japanese (JPT) and Yoruba Nigerian (YRI) backgrounds. IFN-inducible gene expression was assessed in LCLs from children with SLE in the presence and absence of IFN α stimulation.

Results—The major alleles of *IRF5* rs13242262 and rs2280714 were associated with increased *IRF5* mRNA expression levels in the CEU, CHB+JPT and YRI samples. The minor allele of *IRF5* rs10488631 was associated with increased *IRF5*, IFN α and IFN-inducible chemokine expression in CEU ($p_c=0.0005$, 0.01 and 0.04, respectively). A haplotype containing these risk alleles of rs13242262, rs10488631 and rs2280714 was associated with increased *IRF5*, IFN α and IFN-inducible chemokine expression in CEU LCLs. In vitro studies showed specific activation of IFN-inducible genes in LCLs by IFN α .

Conclusions—SNPs of *IRF5* in healthy individuals of a number of ethnic groups were associated with increased mRNA expression of *IRF5*. In European-derived individuals, an *IRF5* haplotype was associated with increased *IRF5*, IFN α and IFN-inducible chemokine expression. Identifying individuals genetically predisposed to increased IFN-inducible gene and chemokine expression may allow early detection of risk for SLE.

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INTRODUCTION

A major breakthrough in the pathogenesis of systemic lupus erythematosus (SLE) is the role of interferon α (IFN α) pathway activation,¹² a common heritable risk factor for SLE.³ IFN α , part of the innate immune response, functions in autoimmunity by promoting dendritic cell maturation, T cell survival and antibody and cytokine production.^{4–6} Variants of the IFN α pathway genes interferon regulatory factor 5 (*IRF5*), tyrosine kinase 2 (*TYK2*), *STAT4* and osteopontin (*OPN*) have been associated with SLE susceptibility in multiple ethnic groups,^{7–20} but the complete impact of genetic variation on pathway activation is not fully understood. *IRF5* has been repeatedly implicated in susceptibility to many other autoimmune diseases including rheumatoid arthritis,^{21–23} multiple sclerosis,²⁴ inflammatory bowel disease²⁵ and Sjogren's syndrome,²⁶ which suggests a critical and common function in the promotion of autoimmunity and a rationale for the focus of our study.

Increased IFN α -inducible gene expression is observed in peripheral blood cells from patients affected with a number of autoimmune diseases including polymyositis and dermatomyositis,^{27,28} suggesting a common role for the IFN pathway. Interestingly, high serum IFN α activity is not associated with ANA or anti-SSA/Ro antibody positivity in subjects without disease manifestations,^{29,30} suggesting these are independent variables in the development of SLE. The correlation between IFN-inducible gene expression in peripheral blood cells and lupus disease activity and fl are,^{31–36} however, implicates the IFN α pathway specifically in the development and exacerbation of SLE.

A focus of SLE research has been on downstream activation of IFN-inducible genes as representative of IFN α activity. In particular, interferon scores, derived from significantly increased IFN-inducible genes, have been associated with SLE subpopulations with increased disease activity.^{34,36} These interferon scores in a functional assay have been used to identify an *IRF5* SLE risk haplotype associated with high IFN α activation.²⁹ Similarly, chemokines that are upregulated by IFN α have been described as measures of IFN α pathway activation and increased SLE disease activity, including neuropsychiatric SLE.^{32,37}

This project expands current understanding of *IRF5* variations by studying basal gene transcript levels representing IFN α pathway activation. Important resources including genotyping data provided by Hapmap³⁸ and gene expression data from NCBI Gene Expression Omnibus (GEO) were used in the analysis. Our purpose is to examine whether genetic variation of *IRF5* predisposes individuals without autoimmune disease to IFN α pathway activation by analysing gene expression levels of IFN α and IFN-inducible genes and chemokines. Greater understanding of the complex genetics influencing the IFN α pathway may lead to future developments such as autoimmune risk prediction models.

MATERIALS AND METHODS

Sources of data

The Hapmap Project (www.hapmap.org) provided complete genotyping data of *IRF5* (± 5 kb) from all unrelated individuals including 60 individuals of European background (CEU), 45 Han Chinese (CHB), 45 Japanese (JPT) and 60 with a Yoruba Nigerian (YRI) background (phenotypic data unknown). Of the total 18 single nucleotide polymorphisms (SNPs), 14 with minor allele frequency (MAF) >0.025 were used for analyses.

IRF5, the 13 IFN α subtypes and specific IFN-inducible gene including chemokine expression levels from Epstein-Barr virus (EBV)-immortalised lymphoblastoid cell lines (LCLs) of Hapmap individuals obtained from the GEO repository (GSE5859 for CEU, CHB

and JPT; GSE7761 for YRI)³⁹⁴⁰ were used to generate and test association hypotheses. Only individuals whose gene expression was not available were excluded.

Interferon and chemokine scores, gene expression and haplotypes

For each individual, two interferon scores, one chemokine score and one total IFN α expression sum were calculated by summing normalised mean expression levels of relevant genes. The first IFN score (IFN score 1) consisted of summed gene expression of *LY6E*, *OASL*, *OAS1*, *MX-1* and *ISG15*, which were previously identified by our group as explaining 98% of variation in differential quantitative IFN-inducible gene expression between patients with SLE and controls.³⁴

The second IFN score (IFN score 2) consisted of summed gene expression of *PRKR*, *IFIT1* and *IFI44*, three genes that are preferentially induced by IFN α (but not IFN γ) in microarray analysis of patients with SLE versus controls.³⁵³⁶ The CMK score was calculated by summing gene expression of *CXCL10*, *CCL2* and *CCL9*, the three IFN-inducible chemokines that have highest correlation with SLE disease activity.³²⁴¹ Total IFN α expression was calculated by summing gene expression of the 13 IFN α subtypes.

Statistical inference of haplotypes from the unphased Hapmap SNP genotypes was required. Individual haplotypes were deduced with Haplotyper 1.0 using the Bayesian algorithm. Resolved haplotypes were divided into blocks (Haploviewer 4.0) using the approach of CI.

Lymphoblastoid cell line in vitro stimulation and mRNA quantification

EBV-transformed LCLs of children with SLE were suspended in AIM V (Invitrogen) and supplemented with 15% fetal bovine serum. LCL cells (2×10^6 cells/ml) were incubated (24 h) in complete medium with 600 units/ml of pure recombinant human IFN α (Invitrogen). An aliquot of 0.25 μ g/ml of recombinant B18R (eBioscience), a type I IFN receptor protein, was added to some wells as a neutralising agent.⁴² RNA was extracted (Trizol) and first-strand cDNA was synthesised from 2 μ g RNA template using Omniscript reverse transcriptase (Qiagen) and oligo(dT) 20 primers (Invitrogen). Transcript levels were quantified in each sample using the AB 7500 RT-PCR System under standard thermal cycling conditions. A mean threshold cycle for each sample in two replicates was used to calculate the relative expression of IFN-inducible genes compared with housekeeping gene human transcripts used as endogenous references.

Statistical analysis

CEU and YRI consisted of unrelated individuals and were analysed as independent samples. CHB and JPT data were combined for analysis as, in previous studies, expression levels of >99% of over 4000 genes have been shown to be similar between these two groups.⁴⁰

Two well correlated ($r=0.5-0.63$; $p<0.001$, Pearson test) microarray gene expression data sets were analysed using SAS Software (www.sas.com). Equality of variances was tested by the Folded F method. Genotypes of SNPs were assessed by the Student unpaired t test of dominant models (major allele homozygotes vs minor allele homozygotes plus heterozygotes) for association with IRF5 expression and total IFN α expression; other than SNP 2 (table 1), the studied SNPs had the same minor allele in all population samples. SNPs associated with IRF5 and IFN α expression were tested for association with IFN and CMK scores using the same methods. For data that did not meet the assumption of normality, the non-parametric Wilcoxon rank sum test was used. Only SNPs that withstood Bonferroni correction are reported.

Stratification for haplotype analysis was based on haplotypes containing risk alleles associated with increased gene expression. Haplotypes were tested for association by the Student unpaired *t* test with the gene's own expression, total IFN α expression, and IFN and CMK scores. Statistical significance of the expression in LCLs was tested by the Student *t* test.

RESULTS

Association of SNPs of IRF5 with increased IRF5 expression in multiple ethnic groups

Six of the 14 SNPs (figure 1A) are intronic; the others are located at the 5' upstream or 3' downstream regions. Introns and 5' and 3' regions often contain sequences that influence mRNA expression and are thus considered important for our hypothesis. There were seven tagSNPs in the CEU and the CHB+JPT samples, and eight in the YRI, with most r^2 values below 0.8 (figure 1B). Based on tagSNP genotyping availability, it is likely that a large fraction of genetic variation in the *IRF5* locus has been adequately covered by linkage disequilibrium (LD) and determined with a minimum of redundancy.

SNPs were individually tested for association with IRF5 mRNA expression. Eight of 11 (73%) in CEU individuals, 7 of 9 (78%) in CHB+JPT, but only 2 of 12 (17%) in YRI individuals were associated with increased IRF5 expression after Bonferroni correction for multiple testing (table 1).

Increased IRF5 mRNA expression was seen in the CEU sample with multiple SNPs, including SNPs 1, 2, 3, 4, 6, 10, 13 and 14 (table 1). Five of these were tagSNPs, spanning the length of the single 15 kb haplotype block (figure 1B). Similarly, seven SNPs (SNPs 3, 4, 6, 7, 10, 12 and 14; table 1) showed significant association with increased IRF5 expression in CHB+JPT. These SNPs included five tagSNPs that cover both CEU+JPT haplotype blocks (6 kb and 12 kb blocks; figure 1B). In the YRI sample the two SNPs (SNP 10 and 14) that were associated with IRF5 expression tagged block 3 (figure 1B) located in the 3' gene region; these two SNPs were also associated with increased IRF5 expression in the CEU and CHB+JPT samples (figure 2).

Association of IRF5 SNPS, IFN α expression and downstream IFN α pathway activation

SNPs that were significantly associated with IRF5 expression, after correction for multiple testing, were tested for association with total IFN α expression. These results showed two SNPs in the CEU sample (SNP 4, $p=0.03$; SNP 13, $p=0.003$) and two SNPs in the CHB+JPT sample (SNP 4, $p=0.02$; SNP 12, $p=0.02$) that were associated with increased IFN α expression ($p<0.05$); however, only SNP 13 (rs10488631) in the CEU sample withstood Bonferroni correction ($p_c=0.03$). Interestingly, SNP 13 is non-polymorphic in the YRI sample; neither of the two SNPs associated with increased IRF5 expression (SNPs 10 and 14) were associated with increased IFN α expression. However, these three SNPs (10, 13 and 14) are located in the 3' downstream region of the gene and form one haplotype block in the YRI sample.

We next tested only SNPs associated with increased IRF5 and IFN α expression for downstream IFN α pathway activation. Based on this criterion, only SNP 13 in the CEU sample was analysed. This SNP was not tested in the CHB+JPT or the YRI samples owing to unavailability of genotyping (CHB+JPT) or the SNP not being polymorphic (YRI). SNP 13 in the CEU sample was associated with increased IRF5 and IFN α expression and increased CMK score ($p_c=0.0005$, 0.01, 0.04, respectively; figure 3A); IFN scores 1 and 2 showed no significance. A recent report showed that a *STAT4* risk allele (rs7574865, T allele) in patients with SLE was dominant over the SLE *IRF5* risk allele of rs3807306 with regard to IFN pathway activity.⁴³ We analysed the co-occurrence of the *STAT4* and *IRF5*

risk alleles in our CEU sample but it did not account for our IFN score 1 and 2 results (data not shown).

Haplotypes of *IRF5* conferred risk of IFN α pathway activation

Haplotype-based analysis can be much more robust than single marker analysis. A stratified analysis was performed on the CEU haplotypes based on SNP 13 (rs10488631)—that is, individuals with at least one haplotype containing the SNP 13 risk allele were tested against those that did not. Three of the 15 CEU haplotypes (haplotypes 3 (H3), 4 (H4) and 15 (H15); figure 1C) contained the minor C allele of SNP 13. Haplotype 15 was not independently tested as it was found in only one heterozygote individual.

H3 and H4 differed by only the allele at SNP 10, one of two SNPs (the other was SNP 14) associated with increased *IRF5* expression in all three ethnic groups (figure 2). We therefore extended our haplotype analysis to include SNPs 10 and 14: H4 contains the three risk alleles of SNPs 10, 13 and 14; H1 contains none of the risk alleles. As shown in figure 3B, the risk haplotype H4 was associated with increased *IRF5* and IFN α expression and an increased CMK score compared with the non-risk haplotype H1. There was no significant difference in the two IFN scores using haplotype analysis.

Comparison of results with previous SLE studies

Eleven of the studied SNPs have been associated with SLE (all except SNPs 8, 9 and 11), predominantly in cohorts of mixed European descent.⁹¹¹¹⁸⁴⁴⁴⁵ We did not have genotyping of the four previously reported functional *IRF5* SNPs (rs2004640, rs10954213, the exon 6 indel and the CGGGG promoter indel)¹¹¹²¹⁶⁴⁴ for testing our hypothesis. However, in 379 British families with SLE, SNPs 3 and 14 are in high LD with, respectively, rs2004640, the exon 1B splice site ($r^2=0.91$) and rs10954213, the *IRF5* polyadenylation site ($r^2=0.99$).¹¹ The promoter indel and the exon 6 indel were shown to be in relatively high LD with SNPs 3 and 7 ($r^2=0.7$ and 0.61 , respectively) in a Swedish sample,⁴⁵ but these may not fully represent the functional SNPs in CEU samples. For example, although the CGGGG indel was associated with higher *IRF5* transcripts in a minigene reporter assay and peripheral blood cells in Swedish patients with SLE,⁴⁵ SNP 3 was not associated with IFN α pathway activation in this study.

In vitro confirmation of LCL stimulation by IFN α

To confirm the utility of our computer simulated model in vitro, we established EBV-transformed cell lines of seven children with SLE. LCLs were incubated with media alone, recombinant human IFN α and/or the IFN α neutralising agent recombinant B18R protein.⁴² Expression of IFN-inducible genes *MX-1*, *ISG15*, *OASL* and *OAS1*, part of IFN score 1, was inducible by IFN α in these LCLs ($p=0.0002$; figure 4). The addition of an IFN receptor competitive inhibitor, B18R, abolished this increased expression which was not observed with the non-IFN-inducible housekeeping gene, *RFLPO*.

DISCUSSION

Using publicly available databases, we have shown that the major alleles of two correlated *IRF5* 3' SNPs, rs13242262 and rs2280714 (SNPs 10 and 14), are associated with increased *IRF5* expression in unstimulated LCLs from unrelated individuals from three ethnic groups. Results from recent publications showed increased *IRF5* expression in CEU LCLs containing the risk allele of SNP 14,¹² and in CEU, CHB and JPT LCLs containing the risk allele of rs10954213,¹¹¹⁴⁴⁴ for which SNP 10 is a proxy. In our study, SNPs spanning *IRF5* were associated with increased *IRF5* transcripts in the CEU and CHB+JPT samples, but only SNPs tagging for the 3' block (figure 1B) were associated with increased mRNA in the

YRI sample. One SNP from the 3' region (rs10488631) in the CEU sample showed increased IFN α levels by multiple testing criteria; this was not seen in the other populations. *IRF5* studies of SLE in East Asians indicate that the 3' region may be less important to SLE susceptibility than in individuals of European descent.¹⁴⁴⁶ Whether one region of *IRF5* is more important in the regulation of gene expression to certain ethnic groups awaits further testing in larger samples.

We report that the minor allele of rs10488631 (SNP 13) was associated with increased *IRF5*, total IFN α and IFN-inducible chemokine transcripts in unstimulated CEU LCLs. Along with a CGGGG promoter indel, rs10488631 has accounted for the strongest association signals observed with *IRF5* in Swedish SLE studies.⁴⁵ rs10488631 can be considered a marker for an SLE risk haplotype, as described in two large-scale studies of diverse European ancestries, which contains the three other functional *IRF5* risk alleles: rs2004640, rs10954213 and the exon 6 indel.⁹⁴⁴ An *IRF5* SLE risk haplotype has been associated with increased IFN α pathway activation in European and Hispanic Americans with SLE;²⁹ the same has not been reported in healthy individuals. In our study, a CEU haplotype defined by the risk alleles of rs13242262, rs10488631 and rs2280714 was associated with increased *IRF5*, total IFN α and IFN-inducible chemokine transcripts. The CGGGG indel is in LD with the risk allele of rs10488631 ($r^2=0.61$), which has been associated with SLE in European and African American cohorts¹¹¹⁵ and is present in our risk haplotype. This SNP was not associated with increased *IRF5* expression in the YRI sample but, as the YRI sample does not reflect the genetic admixture seen in African Americans, this again shows that different *IRF5* variations may impact on the IFN α pathway among ethnic groups.

The representation of SNPs across *IRF5* in our haplotypes allowed for a straightforward comparison with previously reported SLE risk haplotypes. In 555 families of diverse European descent, SNPs from three groups (rs2004640, the exon 6 indel and rs10954213) are contained in a risk haplotype for SLE.⁴⁴ Risk alleles of SNPs representing rs2004640 and rs10954213 (as described in the Results section) are present in our CEU risk haplotype. In another report, two over-transmitted haplotypes made up of six SNPs were found by transmission disequilibrium test analysis of UK SLE trios.¹¹ Of these, SNPs 2, 3, 13 and 14 overlapped with our analysis and the alleles present in our CEU risk haplotype were the same as in the SLE study. Finally, a large combined study from eight European countries identified the minor allele of rs10488631 as defining a susceptibility haplotype in European patients with SLE.⁹ As we reported, the minor allele of this SNP is contained in our CEU risk haplotype. Although *IRF5* polymorphisms, including rs2004640, have been associated with SLE in East Asians,¹⁴⁴⁶⁴⁷ the results in these populations do not report identical SLE risk haplotypes as those in individuals of European descent. Specifically, haplotypes containing the three European risk alleles of rs2004640, the exon 6 indel and rs10954213 are not associated with an increased risk of SLE in Japanese and Korean samples.¹⁴⁴⁶ Our study did not identify an association between *IRF5* variants and IFN α pathway activation in CHB +JPT or YRI individuals. It remains to be seen whether other variants not included in this study may be responsible for pathway activation in non-European populations, or whether other genes involved in IFN α regulation may play a more important role.

Given the heterogeneity and incomplete penetrance of SLE, and given the relatively small role that B cells play in the IFN response, it is remarkable to observe an association of *IRF5* variants with unstimulated mRNA levels of IFN α and IFN-inducible chemokines, however small, in LCLs from Hapmap individuals. The lack of association of *IRF5* variants with IFN-inducible genes in these individuals may be explained by a number of factors. It is plausible that other transcription factors such as *IRF7*, *IRF3*, *OPN* or *STAT4* may be involved in activation of the IFN α pathway,⁴⁸ or that this association may only be present after stimulation by autoantibodies or IFN α . A recent study showed that some patients with

SLE had raised levels of IFN-inducible genes and chemokines whereas others only had raised levels of IFN-inducible chemokines,⁴¹ suggesting a non-linear pattern of modulation of IFN-inducible genes and downstream chemokines. Based on the results from our computer simulated study, we are investigating whether genetic variants of the IFN α signalling pathway regulate its threshold of activation, potentially contributing to the development of SLE and other autoimmune diseases in unaffected individuals of different populations.

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Figure 1.

Interferon regulatory factor 5 (*IRF5*) gene structure and haplotypic architecture

(A) Translated exons are shown as boxes. Single nucleotide polymorphisms (SNPs) used in the analysis have been encoded as: rs4728142 (SNP 1), rs752637 (SNP 2), rs3807306 (SNP 3), rs11761199 (SNP 4), rs6975315 (SNP 5), rs7808907 (SNP 6), rs1874328 (SNP 7), rs6970960 (SNP 8), rs6951628 (SNP 9), rs13242262 (SNP 10), rs1727172 (SNP 11), rs10488630 (SNP 12), rs10488631 (SNP 13) and rs2280714 (SNP 14). (B) Linkage disequilibrium (LD) r^2 chart summarises the LD pattern in European (CEU), Han Chinese + Japanese (CHB+JPT) and Yoruba Nigerian (YRI) individuals as a plot. Darker grey represents regions of high pairwise r^2 and white represents regions of low pairwise r^2 . The numbers in the boxes are the pairwise r^2 values. TagSNPs are noted by asterisks. (C) The haplotypes across *IRF5* constructed from 58 unrelated CEU individuals. The haplotypes are numbered on the left of each haplotype and haplotype frequencies are shown to the right of each haplotype. The SNP numbers across the top of the haplotypes correspond to those in the gene diagram above. The grey-boxed haplotype is the risk haplotype, defined by the risk alleles of SNPs 10, 13 and 14. The black-boxed haplotype is the non-risk haplotype.

Figure 2.

Association of interferon regulatory factor 5 (IRF5) single nucleotide polymorphisms (SNPs) with increased IRF5 expression in three samples. IRF5 expression in European, Chinese/Japanese and Yoruba Nigerian individuals with or without risk alleles (homozygotes only) of (A) rs13242262 (SNP 10) and (B) rs2280714 (SNP 14). All p values withstand the Bonferroni correction method. SNP 14 is a proxy for the known functional SNP rs10954213, the polyadenylation variant site.

Figure 3.

Mean mRNA expression ($\pm$ SEM) of interferon regulatory factor 5 (IRF5) and interferon α (IFN α), and mean IFN or CMK scores ($\pm$ SEM) of (A) individuals of European background (CEU) with or without the systemic lupus erythematosus (SLE)-associated risk allele of single nucleotide polymorphism (SNP) 13 (rs10488631) or (B) CEU individuals with the risk haplotype (H4, black) or the non-risk haplotype (H1, white). The risk haplotype is defined by the risk alleles of SNPs 10, 13 and 14 which are all located in the 3' downstream region. All haplotypes, including haplotype 4 (H4, AAGGCTTACA) were previously deduced using Haplotyper 1.0 and are shown in figure 1C. p Values shown are those which remained significant after application of the Bonferroni correction.

Figure 4.

Increased relative expression of interferon-inducible genes, but not housekeeping genes, was seen in lymphoblastoid cells (LCLs) after interferon α (IFN α) stimulation. Relative expression of genes in LCLs incubated with IFN α was measured based on values of RFLPO, a non-IFN-inducible housekeeping gene and compared with expression of cells incubated with IFN α plus recombinant B18R protein, an established inhibitor of IFN activation. (A) Relative expression of GAPDH, a non-IFN-inducible gene, after stimulation of cells with IFN α . (B) The relative expression of each IFN-inducible gene (*ISG15*, *OAS1*, *OASL* or *MX1*), with or without incubation with IFN α , was combined from all seven cell lines. A summed total representing IFN score 1 is also presented in (B). Data points were connected by lines to illustrate the gene activation of a given gene. In both panels, data are expressed as mean $\pm$ SD (SD is not shown in (B) due to overlap between data sets). Each condition was done in duplicate and results shown were one of the two representative experiments.

* $p < 0.05$ (IFN score 1, $p = 0.0002$; OASL, $p = 0.006$; MX-1, $p = 0.03$); $^{\dagger}p < 0.1$ (ISG15, $p = 0.09$; OAS1, $p = 0.06$).

Table 1

Association of IRF5 SNPs with IRF6 expression in multiple ethnic groups

SNP	dbSNP marker	Risk allele*	Genotype [†]	CEU		CHB+JPT		YRI	
				RE	p Value	RE	p Value	RE	p Value
1	rs4728142	A	AA+AG/GG	1.5	0.0004 [‡]	1.2	0.006	1.1	NS
2	rs752637	G	GG/AG+AA	1.6	<0.0001 [‡]	1.2	0.07	1.2	0.02
3	rs3807306	A	AA+AC/CC	1.8	<0.0001 [‡]	1.2	0.0004 [‡]	1.1	NS
4	rs11761199	G	GG+AG/AA	1.6	<0.0001 [‡]	1.2	0.004 [‡]	1	NS
5	rs6975315	G	GG/AG+AA	0.9	NS	ND		1.1	NS
6	rs7808907	C	CC+CT/TT	1.6	<0.0001 [‡]	1.2	0.007 [‡]	0.9	NS
7	rs1874328	T	TT/CT+CC	0.8	0.02	1.2	0.002 [‡]	1	NS
8	rs6970960		GG+GT/TT	NP		NP		1.1	NS
9	rs6951628		TT+GT/GG	NP		NP		1.1	NS
10	rs13242262	T	TT/AT+AA	1.6	<0.0001 [‡]	1.3	0.004 [‡]	1.3	<0.0001 [‡]
11	rs1727172		GG+AG/AA	NP		NP		1.1	NS
12	rs10488630	G	GG+AG/AA	1.2	0.03	1.2	0.004 [‡]	NP	
13	rs10488631	C	CC+CT/TT	1.5	<0.0001 [‡]	ND		NP	
14	rs2280714	A	AA/AG+GG	1.7	<0.0001 [‡]	1.3	0.001 [‡]	1.2	0.004 [‡]

p denotes the uncorrected p value for the parameter, for Bonferroni correction n=11 (CEU), n=9 (CHB+JPT) and n=12 (YRI).

* Risk allele refers to alleles previously studied in association with SLE.

[†] Genotype analysis was performed with major allele homozygotes versus minor allele homozygotes plus heterozygotes; the risk allele (whether major or minor) is listed first to demonstrate the ratio based on the risk allele. All minor alleles are the same in each population except for SNP 2; the analysis for CHB+JPT and YRI for SNP 2 was GG+AG/AA.

[‡] SNPs that withstood Bonferroni correction: p<0.0045 in CEU; p<0.0056 in CHB+JPT; p<0.0042 in YRI.

CEU, European; CHB, Han Chinese; IRF, interferon regulatory factor; JPT, Japanese; ND, data not determined by Hapmap; NP, when the SNP is not polymorphic in the given sample; NS, not significant (p>0.1); RE, relative expression ratio of the gene expression as determined by the risk genotype; SLE, systemic lupus erythematosus; SNP, single nucleotide polymorphism; YRI, Yoruba Nigerian.