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IRF5 haplotypes demonstrate diverse serological associations which predict serum interferon alpha activity and explain the majority of the genetic association with systemic lupus erythematosus

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Abstract

Objective—High serum interferon α (IFN α) activity is a heritable risk factor for systemic lupus erythematosus (SLE). Auto-antibodies found in SLE form immune complexes which can stimulate IFN α production by activating endosomal Toll-like receptors and interferon regulatory factors (IRFs), including IRF5. Genetic variation in IRF5 is associated with SLE susceptibility; however, it is unclear how IRF5 functional genetic elements contribute to human disease.

Methods—1034 patients with SLE and 989 controls of European ancestry, 555 patients with SLE and 679 controls of African-American ancestry, and 73 patients with SLE of South African ancestry were genotyped at IRF5 polymorphisms, which define major haplotypes. Serum IFN α activity was measured using a functional assay.

Results—In European ancestry subjects, anti-double-stranded DNA (dsDNA) and anti-Ro antibodies were each associated with different haplotypes characterised by a different combination of functional genetic elements (OR > 2.56, $p > 0.003$; 1.9×10^{-14} for both). These IRF5 haplotype-auto-antibody associations strongly predicted higher serum IFN α in patients with SLE and explained > 70% of the genetic risk of SLE due to IRF5. In African-American patients with SLE a similar relationship between serology and IFN α was observed, although the previously described European ancestry-risk haplotype was present at admixture proportions in African-American subjects and absent in African patients with SLE.

Conclusions—The authors define a novel risk haplotype of IRF5 that is associated with anti-dsDNA antibodies and show that risk of SLE due to IRF5 genotype is largely dependent upon particular auto-antibodies. This suggests that auto-antibodies are directly pathogenic in human SLE, resulting in increased IFN α in cooperation with particular combinations of IRF5 functional genetic elements.

SLE is a systemic autoimmune disorder affecting multiple organ systems including the skin, musculoskeletal, renal and haematopoietic systems. Humoral autoimmunity is a hallmark of SLE, and patients frequently have circulating auto-antibodies directed against dsDNA, as well as RNA binding proteins (RBP). Anti-RBP autoantibodies include antibodies which recognize Ro, La, Smith (anti-Sm), and ribonucleoprotein (anti-nRNP), collectively referred to as anti-retinol-binding protein. Anti-retinol-binding protein and anti-dsDNA auto-antibodies are rare in the healthy population.¹ These auto-antibodies can be present in sera for years preceding the onset of clinical SLE illness² and are likely pathogenic in SLE.³⁴

Interferon regulatory factor (IRF)5 is a transcription factor that induces transcription of IFN α and IFN α -induced genes.⁵ Genetic association studies of IRF5 have defined haplotypes which confer either susceptibility to or protection from SLE in European ancestry individuals.⁶ These haplotypes are characterised by multiple functional genomic variants⁶ (figure 1A), which presumably alter IRF5-mediated transcription and subsequent risk of SLE.

IFN α is a pleiotropic type I interferon which can break self-tolerance by activating antigen presenting cells after uptake of self material.⁷ Serum IFN α activity is elevated in many patients with SLE,^{8–10} and high IFN α is associated with presence of anti-double-stranded DNA (dsDNA) and anti-RBP antibodies.¹⁰¹¹ Some patients treated with recombinant human IFN α for malignancy and viral hepatitis have developed de novo SLE, which typically resolves after the IFN α is discontinued.¹²¹³ These data suggest a role for IFN α in SLE susceptibility.¹⁴

In our previous work, we have demonstrated that abnormally high serum IFN α is common in unaffected SLE family members, suggesting that IFN α is a heritable risk factor for SLE.⁸ The idea is supported by studies demonstrating that SLE-risk genetic variants in the IFN α pathway have been associated with high serum IFN α levels or increased sensitivity to IFN α .^{15–18} Further support for the genetic contribution to this trait in patients with SLE is provided by a recent genome-wide study that detected novel genetic variants associated with serum IFN levels in patients with SLE.¹⁹

We have previously shown that an SLE-risk haplotype of IRF5 was associated with high serum IFN α in patients with SLE.¹⁶ The differential effect of IRF5 genotype on serum IFN α was most prominent in patients with either anti-dsDNA or anti-RBP antibodies.¹⁶ We have recently demonstrated a similar model of association between genetic variants in the IRF7 locus and serum IFN α and particular auto-antibodies.^{15,20} Given that the genetic effect of IRF5 variants upon serum IFN α was dependent upon the presence of SLE-associated auto-antibodies, we hypothesised that these auto-antibodies may be required for a biological effect related to IRF5 variants and, if so, then SLE susceptibility related to IRF5 may depend upon these auto-antibodies. To test this hypothesis, we detected and modelled the associations between IRF5 haplotypes, auto-antibodies and serum IFN α in a large cohort of patients with SLE.

MATERIALS AND METHODS

Patients and Genotyping

IRF5 genotype and auto-antibody data were obtained from 1034 patients with SLE and 989 controls of European ancestry, and 555 patients with SLE and 679 healthy controls of African American ancestry from the Oklahoma Medical Research Foundation (OMRF) Lupus Family Registry and Repository and OMRF Lupus Genetics Studies in collaboration with University of Uppsala, the PROFILE consortium of investigators²¹ and Imperial College London, as well as from the Hospital for Special Surgery (HSS) Lupus Family Registry and the University of Chicago Translational Research Initiative in the Department of Medicine (TRIDOM). 138 patients with SLE and 125 controls of African–American Gullah ancestry from the Medical University of South Carolina, and 73 patients with SLE of African ancestry from the University of the Witwatersrand, Johannesburg, South Africa were also studied. All patients with SLE met the American College of Rheumatology criteria for the diagnosis of SLE.²² In families multiplex for SLE, only one affected member was chosen randomly for inclusion to prevent bias. We studied four single nucleotide polymorphisms (SNPs) previously used to designate risk and protective haplotypes for SLE in European ancestry individuals (rs2004640, rs3807306, rs10488631 and rs2280714).^{6,23} Genotyping was performed at OMRF using the Illumina GoldenGate and Infinium systems (Illumina, Inc., San Diego, CA) and individuals in the TRIDOM, PROFILE and HSS registries were genotyped using TaqMan Assays-by-Design probes (Applied Biosystems, Carlsbad, CA). SNP genotyping was performed with >99% completeness at the rs3807306, rs10488631 and rs2280714 SNPs in both registries. The rs2004640 SNP was genotyped with 80.2% completeness at OMRF and >99% completeness in the TRIDOM and HSS registries. All tested SNPs had the expected Hardy–Weinberg proportions ($p \geq 0.25$ for all SNPs). The study was approved by institutional review boards in all institutions, and informed consent was obtained from all subjects.

Measurement of auto-antibodies

Antibodies to Ro, La, Smith (anti-Sm) and ribonucleoproteins (anti-nRNP) in the OMRF samples at the OMRF Clinical Immunology Laboratory were measured by precipitin method, and those in the Hospital for Special Surgery and the University of Chicago

samples, by ELISA in their respective clinical laboratories. Standard clinical laboratory cut-off points were used to categorise samples as either positive or negative. Anti-dsDNA antibodies were measured using *Crithidia luciliae* immunofluorescence at all sites, and detectable fluorescence was considered positive. Most subjects were derived from the OMRF registry, and there were no statistically significant differences in antibody prevalence between study sites ($p \geq 0.10$ for each, table 1).

Measurement of serum IFN α activity

Serum IFN α activity was measured in the same sample in which auto-antibodies were measured. We have developed a sensitive and reproducible bioassay to detect serum IFN α activity,⁸²⁴ as ELISA methods for detection of IFN α in human serum have been complicated by low sensitivity and low specificity.²⁵ In this bioassay, reporter cells (WISH cells; American Type Culture Collection (ATCC), Manassas, VA, #CCL125) are used to measure the ability of sera to cause IFN-induced gene transcription. The reporter cells are cultured with patient sera for 6 h and then lysed, and three canonical IFN α -induced transcripts (interferon-induced protein with tetratricopeptide repeats 1 (IFIT-1), myxovirus (influenza virus) resistance 1 (MX-1) and eukaryotic translation initiation factor 2-alpha kinase 2 (PKR)) are measured using reverse transcriptase PCR. Relative expression data from the three transcripts are then normalised using the mean and SD of healthy donor sera ($n=141$) run in the same assay, and data are presented as an IFN α activity score. The IFN-induced transcriptional activity in the reporter cells can be blocked with anti-IFN α monoclonal antibodies, and no significant functional inhibitors have been detected to date.⁸ This assay has been highly informative when applied to SLE, as well as other human autoimmune disease populations.⁸²⁶²⁷

Statistical analysis

The χ^2 statistic was used to analyze the categorical data, and ORs were calculated using standard parameters. The Haploview software V.4.2 was used for haplotype analysis (solid spine of linkage disequilibrium (LD) method) and calculation of r^2 values. Logistic regression was performed using a backward logistic model including the five major haplotypes as predictors and each auto-antibody as an outcome variable serially, discarding non-significant predictors to achieve the best model fit. Attributable risk was calculated as the number of genotypes skewed toward SLE risk in a given patient category divided by the total number of risk-skewed genotypes in the SLE cohort as compared to controls. IFN α data were analyzed as a quantitative trait, with p-values calculated using the Mann–Whitney U test, as the data were not normally distributed.

RESULTS

Different IRF5 haplotypes are associated with distinct serological profiles in SLE

European haplotype structures and frequencies were similar to those previously reported⁶²³ (figure 1B), and antibody prevalence data are summarised in table 1. In European ancestry patients with SLE, we compared haplotype frequencies between patients who either had or lacked particular auto-antibody specificities using logistic regression models. We found strong and strikingly distinct associations between anti-dsDNA and anti-Ro antibodies, and different IRF5 haplotypes (table 2). Anti-dsDNA antibodies were associated with two haplotypes, including the previously reported SLE-risk haplotype (TACA, #1), as well as a second haplotype which has been previously categorised as neutral with respect to SLE risk (TATA, #2, table 2).⁶ The two haplotypes associated with anti-dsDNA are characterised by a promoter insertion,²³ splice site variation and a polyadenylation variant⁶ (table 2), while the exon 6 insertion is present on one of the associated haplotypes and absent on the other.

Anti-Ro antibodies were strongly associated with the previously reported SLE-risk⁶ TACA haplotype (table 2). This haplotype is characterised by presence of the promoter and exon 6 insertions, as well as the splice site variation and the polyadenylation variant. Notably, the main difference between the anti-Ro and anti-dsDNA associations is that anti-Ro is not associated with the TATA haplotype (#2), which lacks the exon 6 insertion. While this insertion may be required for the anti-Ro association, it is also found on other haplotypes that are not associated with anti-Ro such as haplotype #5, suggesting that this element alone is not sufficient. Anti-La antibodies were present in 7% of European ancestry patients with SLE and were almost exclusively found in combination with anti-Ro antibodies. Surprisingly, there was a third pattern of association between anti-La antibodies and IRF5 variants (table 2). Anti-La antibodies were associated with the rare haplotype #3. This haplotype lacks the promoter insertion, contains the splice site variation and exon 6 insertion and lacks the alternate polyadenylation site (table 2). Anti-nRNP and anti-Sm antibodies were not associated with IRF5 haplotypes, although the prevalence of these antibodies was low in European ancestry patients with SLE, and this could limit the power to detect associations with these antibody specificities (table 1).

SLE-risk due to IRF5 is dependent upon serological phenotypes

Examining case-control data, we found out that IRF5 associations within subsets of patients defined by auto-antibodies were strong (table 3). The TACA and TATA haplotypes (#1 and #2) were strongly associated with anti-dsDNA positive patients versus controls ($OR=2.79$, $p=2.9 \times 10^{-20}$), and the TACA haplotype (#1) was strongly associated with anti-Ro positive patients versus controls ($OR=2.57$, $p=1.8 \times 10^{-14}$). In considering the overall risk of SLE due to IRF5 in our SLE cohort, 70.4% of SLE risk attributable to IRF5 was carried by subjects with either anti-Ro or anti-dsDNA auto-antibodies (37.4% and 33% due to anti-dsDNA and anti-Ro, respectively). Anti-La antibodies are found in a subset of patients with anti-Ro so stratifying subjects by anti-Ro captures the genetic effect of anti-La. Thus, a majority of the risk of SLE due to IRF5 was carried by a minority of the patients (393 of 1034 European ancestry patients with SLE in our cohort had anti-dsDNA, anti-Ro or both).

European-derived SLE-risk haplotype demonstrates similar serological association in African-Americans

In African-American subjects, the four SNP haplotype structures were similar to those observed in European ancestry subjects (figure 1C), and many of the tested auto-antibodies were more prevalent than in European ancestry subjects (suppl. table 1). In case-control analysis, we found that the TACA haplotype (#1) was associated with anti-Ro and anti-dsDNA ($OR=2.10$, $p=3.8 \times 10^{-3}$), and no significant associations were observed with other haplotypes (suppl. table 2). This TACA haplotype reported as an SLE-risk haplotype in European ancestry⁶ is not found in the African ancestry HapMap Yoruba population. In our African-American controls, this haplotype is present at 2.3% frequency (suppl. table 2), which is consistent with 19.2% European admixture when compared to European controls. A similar admixture proportion is present in the African-American cases versus European ancestry cases (18.1%). Thus, the frequency of haplotype #1 is consistent with the expected 20% European admixture rate in African-American cases and controls, allowing for a similar disease association.

To explore this issue further, we genotyped 73 African patients with SLE from Johannesburg, South Africa, and found out that haplotype #1 (TACA) was not present, although the allele marking this haplotype in Europeans (rs10488361C) was found at 2.7% frequency on a different haplotype (suppl. table 3). Furthermore, in 138 patients with SLE and 125 controls of African-American Gullah ancestry with an average of 8% European admixture, the TACA haplotype is present in controls at 0.8% frequency (6.7% of European

control frequency) and 3.3% frequency in cases (16.7% of European case frequency). We conclude that the association of these IRF5-tag SNP haplotypes with SLE in African-Americans²⁸ is due to a European-derived haplotype and is the result of contributions from the sub-phenotypes of anti-dsDNA and anti-Ro. Interestingly, we did not observe any significant associations between IRF5 haplotypes and anti-Sm or anti-nRNP antibodies in this ancestral background, despite a much higher prevalence of these antibodies in African-American patients with SLE than in European ancestry patients with SLE (Suppl. table 1). Our African-American patient sample is smaller than the European ancestry cohort, and the tag SNPs used in this study are derived from studies of European ancestry populations. Thus, our more limited findings in African-Americans could relate to reduced statistical power and our more limited knowledge of IRF5 risk factors in this background. Further studies in this background are clearly warranted.

Impact of IRF5 genotype upon serum IFN α depends on particular auto-antibodies

We next examined serum IFN α in the context of the auto-antibody-haplotype associations we observed (figure 2). Across both ancestral backgrounds, the haplotypes associated with particular auto-antibodies result in higher serum IFN α in the presence of that particular auto-antibody. This supports the contention that auto-antibodies are required for dysregulation of serum IFN α due to IRF5 risk variants. As noted above, the risk of SLE due to IRF5 was largely explained by these haplotype-autoantibody associations. Thus, the auto-antibody-positive patient group in which IRF5 exerts a differential effect on serum IFN α is the group that demonstrates significant risk of SLE due to IRF5 genotype. These data support a model of pathogenesis in which specific auto-antibodies cooperate with particular IRF5 variants to dysregulate IFN α production, resulting in an increased risk of SLE (figure 3).

DISCUSSION

The formation of SLE-associated auto-antibodies precedes clinical disease in many individuals.² It is likely that SLE-associated auto-antibody immune complexes can stimulate IFN α production in humans in vivo via activation of the endosomal TLR system as has been demonstrated in some in vitro systems.³ Gain-of-function variations in IRF5 downstream of endosomal TLRs could then exacerbate the chronic endogenous stimulation provided by nucleic acid containing immune complexes. We have previously shown that subjects who have anti-Ro antibodies but do not have clinical autoimmunity also do not have high serum IFN α .²⁶ These data suggest that background factors are important to the well-described association between serum IFN α and SLE-associated auto-antibodies, and we propose that IRF5 is one such important background factor.

While we can clearly show that SLE-associated auto-antibodies interact with IRF5 genetic variations to modulate serum IFN α in SLE, it is also possible that IRF5 genotype could directly predispose to the formation of these same auto-antibodies (figure 3, arrow with '?'). Endosomal TLR signalling is important in B cell maturation, and interestingly, IRF5 knockout mice show a deficit in the formation of SLE-associated auto-antibodies.²⁹ This could suggest a feed-forward loop, in which IRF5 variants predispose to auto-antibodies that then cooperate with the same IRF5 elements to result in greater IFN α production. The fact that particular auto-antibodies are associated with specific IRF5 variants could indicate some preferential usage of IRF5 isoforms downstream of TLR7 versus TLR9, although this hypothesis is speculative at present. Plasmacytoid dendritic cells are one of the main IFN α producing cell types, and this may be this cell in which IRF5 variants exert their influence upon IFN α levels. In murine systems, plasmacytoid dendritic cells can activate B cells in multiple ways, including direct cell-cell contact,³⁰ and IFN α produced by these cells can prime B cells to respond to TLR9 ligands.³¹ Thus, the feed-forward loop we suggest may

manifest as an interaction between plasmacytoid dendritic cells and B cells in the presence of TLR ligands.

While anti-dsDNA antibodies and serum IFN α could potentially vary over time, we find strong associations with fixed genetic polymorphisms, supporting the concept of an inherent tendency toward these molecular traits. We and others have frequently observed genetic associations with anti-dsDNA antibodies and serum IFN α , and this precedent also suggests that there is some stability to both of these traits.¹⁵¹⁹³²³³ It seems likely that there is an underlying propensity for some patients to have anti-dsDNA antibodies and high serum IFN α , upon which some variation over time can act as well. The prevalence of anti-dsDNA antibodies was somewhat low in our study, which may reflect the fact that most patients were recruited from the outpatient setting and which may have greater ability to assess the patients underlying intrinsic tendency toward anti-dsDNA antibodies with less variation related to disease activity. All sites in this study measured anti-dsDNA antibodies using the *Crithidia luciliae* immunofluorescence technique, and thus, our data are applicable to anti-dsDNA antibodies measured using this test.

Genetic associations with auto-antibody subsets within an autoimmune disease have been demonstrated previously, for example a PTPN22 variant is associated with anti-CCP positive rheumatoid arthritis.³⁴ The strikingly detailed relationship we demonstrate between specific auto-antibodies and different IRF5 alleles is unprecedented. Our data support the concept that particular IRF5 functional genetic elements contribute to SLE risk through their relationship with auto-antibodies and IFN α production, and it is possible that IRF5 alleles help dictate the particular auto-antibodies produced. These results demonstrate the capacity of informative functional analyses and phenotypical variation to describe the genetic architecture of complex diseases. In a disease as complex as human SLE, it is likely that subset analyses incorporating detailed molecular phenotypes will be required to understand the various genetic contributions to pathogenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

(A) Diagram of the IRF5 gene indicating the location of previously described functional elements in European ancestry. The first three darker boxes indicate alternately spliced first exons (exons 1a, 1b and 1c), lighter boxes indicate subsequent exons (2–9), and the black box indicates the 3' UTR. Arrows show the location of the SNPs included in this study. Haplotype diagrams are shown for European ancestry (B.) and African-American (C.) patients with SLE, constructed using Haploview 4.2 using the solid spine of linkage disequilibrium (LD) method. Pairwise r^2 values are shown in the boxes, and darker shading indicates increasing r^2 values. IRF, interferon regulatory factors; SLE, systemic lupus erythematosus; SNPs, single nucleotide polymorphisms; UTR, untranslated region.

Figure 2.

Serum IFN α activity in patients with SLE and healthy first-degree relatives. A. Serum IFN α activity in European ancestry patients with SLE (n=312) stratified by the number of TACA and TATA haplotypes and the presence or absence of anti-dsDNA antibodies. B. European ancestry patients with SLE (n=312) stratified by the number of TACA haplotypes and the presence or absence of positive test for anti-Ro antibodies (anti-Ro+ antibodies). C. African-American patients with SLE (n=206) stratified by presence or absence of the European-derived IRF5 SLE-risk haplotype (TACA) and presence of anti-Ro auto-antibodies. Units on the Y-axis represent a summed IFN α -induced gene expression score as measured by our functional assay.⁸ Bars indicate the median, error bars show the

interquartile range, p-values calculated from the Mann–Whitney U test. dsDNA, double-stranded DNA; IFN, interferon; SLE, systemic lupus erythematosus.

Figure 3.

Schematic diagram of the proposed relationships between SLE-specific auto-antibodies, IRF5 genotype and IFN α in SLE pathogenesis. 1. IRF5 haplotypes could influence susceptibility to form particular auto-antibodies (arrow indicated by a?). 2. Immune complexes containing SLE-specific auto-antibodies are taken into cells, and the nucleic acid component triggers endosomal TLR7 and TLR9. 3. Particular IRF5 SLE-risk variants augment downstream IFN α production in the setting of different auto-antibodies, resulting in high serum IFN α (4.) and subsequent risk of SLE (5.). IFN, interferon; IRF, interferon regulatory factors; SLE, systemic lupus erythematosus; TLR, toll-like receptors.

Frequency of auto-antibodies and renal disease in the European ancestry patients with SLE studied, shown as the percentage of patients with a positive test or criterion

Table 1

Ancestral background	ANA	Anti-Ro	Anti-La	Anti-Sm	Anti-nRNP	Anti-dsDNA	ACR renal
All-European ancestry	99	24	7	3	11	21	0.31
OMRF n=956	99	23	7	3	10	21	0.31
HSS n=51	100	25	10	4	6	25	0.28
University of Chicago n=27	100	41	7	8	16	26	0.26
p-Value for a difference by site	0.99	0.10	0.24	0.26	0.34	0.70	0.75

p-Value by χ^2 distribution comparing data from OMRF, HSS and the University of Chicago, calculated with two degrees of freedom. 'ACR Renal' indicates the proportion of subjects who met the American College of Rheumatology Criteria for renal disease.²²

ANA, anti-nuclear antibodies, anti-nRNP, anti-ribonucleoprotein antibodies, anti-Sm, anti-Smith antibodies, dsDNA, double-stranded DNA, HSS, Hospital for Special Surgery; OMRF, Oklahoma Medical Research Foundation.

Haplotypes with associated functional elements and corresponding serological associations in European ancestry case–case analysis

Table 2

Tag SNP haplotype	Promoter indel	Splice variant	Exon 6 indel	Poly-A variant	Serological association
1. TACA	Ins	Present	Ins	Present	Ro: OR=1.50, $p=2.0\times10^{-3}$ DNA: OR=1.51, $p=7.4\times10^{-3}$
2. TATA	Ins	Present	Del	Present	DNA: OR=1.68, $p=4.9\times10^{-5}$
3. TCTA	Del	Present	Ins	Absent	La: OR=3.51, $p=7.5\times10^{-3}$
4. GCTA	Del	Absent	Del	Present	-
5. GCTG	Del	Absent	Ins	Absent	-

IRF5 haplotypes are shown as each of the four alleles in order from 5' to 3' (rs2004640, rs3807306, rs10488631, rs2280714). Serological associations were detected using case–case logistic regression analysis within the European ancestry SLE cohort; best fit model is shown for each auto-antibody. p-Values shown are calculated using a Wald test.

splice variant present=rs2004640 T, splicing of exon 1B possible, Poly-A variant present=rs10954213 A introducing a new polyadenylation site. The lowest p-values in logistic models for association between any haplotype and anti-Smith antibodies (anti-Sm), and between any haplotype and anti-ribonucleoprotein antibodies (anti-nRNP) are $p=0.64$ and $p=0.40$, respectively. Ins, insertion; IRF, interferon regulatory factors; Del, deletion; SLE, systemic lupus erythematosus; SNP, single nucleotide polymorphism.

DNA+ and Ro+ cases=patients with SLE with a positive test for anti-dsDNA or anti-Ro autoantibodies, respectively

Table 3

Haplotype	Controls n=989	All cases n=1034	DNA+ cases n=217	Ro+ cases n=239	DNA+ cases vs controls OR (95% CI)	DNA+ cases vs controls, TATA+TACA vs all other haplotypes	Ro+ cases vs controls OR (95% CI)
1. TACA	0.120	0.198	0.209	0.259	1.95 (1.49 to 2.55) p=1.1×10 ⁻⁶	2.79 (2.23 to 3.49) p=2.9×10 ⁻²⁰	2.57 (2.01 to 3.29) p=1.8×10 ⁻¹⁴
2. TATA	0.336	0.407	0.491	0.414	1.90 (1.54 to 2.35) p=1.9×10 ⁻⁹	-	1.40 (1.14 to 1.71) p=1.3×10 ⁻³
3. TCCTA	0.049	0.049	0.051	0.039	1.03 (0.64 to 1.67) p=0.88	-	0.80 (0.49 to 1.33) p=0.39
4. GCTA	0.118	0.079	0.053	0.057	0.42 (0.27 to 0.65) p=1.9×10 ⁻⁵	-	0.45 (0.30 to 0.68) p=9.2×10 ⁻⁵
5. GCTG	0.343	0.250	0.183	0.220	0.43 (0.33 to 0.55) p=9.3×10 ⁻¹¹	-	0.54 (0.43 to 0.68) p=2.2×10 ⁻⁷

IRF5 haplotypes are shown as each of the four alleles in order from 5' to 3' (rs2004640, rs3807306, rs10488631, rs2280714). DNA+ and Ro+ cases=patients with SLE with a positive test for anti-dsDNA or anti-Ro, respectively

IRF, interferon regulatory factors; dsDNA, double-stranded DNA.