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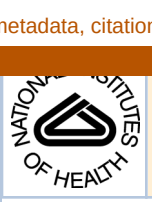

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Analysis of autosomal genes reveals gene–sex interactions and higher total genetic risk in men with systemic lupus erythematosus

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Abstract

Objectives—Systemic lupus erythematosus (SLE) is a sexually dimorphic autoimmune disease which is more common in women, but affected men often experience a more severe disease. The genetic basis of sexual dimorphism in SLE is not clearly defined. A study was undertaken to examine sex-specific genetic effects among SLE susceptibility loci.

Methods—A total of 18 autosomal genetic susceptibility loci for SLE were genotyped in a large set of patients with SLE and controls of European descent, consisting of 5932 female and 1495 male samples. Sex-specific genetic association analyses were performed. The sex–gene interaction was further validated using parametric and nonparametric methods. Aggregate differences in sex-specific genetic risk were examined by calculating a cumulative genetic risk score for SLE in each individual and comparing the average genetic risk between male and female patients.

Results—A significantly higher cumulative genetic risk for SLE was observed in men than in women. ($P = 4.52\times10^{-8}$) A significant sex–gene interaction was seen primarily in the human leucocyte antigen (HLA) region but also in *IRF5*, whereby men with SLE possess a significantly higher frequency of risk alleles than women. The genetic effect observed in *KIAA1542* is specific to women with SLE and does not seem to have a role in men.

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Conclusions—The data indicate that men require a higher cumulative genetic load than women to develop SLE. These observations suggest that sex bias in autoimmunity could be influenced by autosomal genetic susceptibility loci.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a prototypic autoimmune disease characterised by the production of autoantibodies directed against nuclear self-antigens. The aetiology of SLE is incompletely understood, but genetic factors play an important role in the susceptibility to the disease. There is a high degree of concordance between monozygotic twins in SLE.[1] Previous studies have identified a number of genetic loci which are associated with disease susceptibility in SLE.[2]

SLE is a sexually dimorphic autoimmune disease which occurs more than nine times more frequently in women than in men.[3] While it is more prevalent in women, men who develop SLE often experience a more severe disease.[4] The sex chromosome complement and hormonal differences might play a role in the female sex bias of SLE. However, the degree to which sex-specific genetic differences contribute to SLE susceptibility has not been fully studied.

Sex-specific genetic variation has been observed in a number of quantitative traits including systolic blood pressure, forced expiratory volume and serotonin levels.[56] For example, a polymorphism in the angiotensin-converting enzyme is associated with hypertension among men but not women.[7] Sex-specific genetic effects have also been reported at various loci in autoimmune disorders.[8] Numerous instances of sex differences in genetic associations have been reported in multiple sclerosis, an autoimmune disease with a modest sex bias towards women.[3] Interestingly, genetic associations with multiple sclerosis observed in *CCL17* and *CCL22* were only present in men.[9] Furthermore, polymorphisms in the *IFNG* locus were shown to be associated with multiple sclerosis in men but not in women.[10] Sex differences in associations in the *IFNG* locus have also been observed in rheumatoid arthritis, but in women only.[11]

Sex-specific genetic associations appear to play a role in a number of autoimmune diseases. We sought to understand the extent to which sex-specific genetic effects are involved in the aetiology of SLE by studying known SLE susceptibility loci in a large sample set of men and women with SLE and controls of European descent.

METHODS

Study design

We investigated sex-specific differences in allelic frequencies between men and women with SLE among 18 independent autosomal genetic susceptibility loci for SLE. Eighteen single nucleotide polymorphisms (SNPs) tagging these independent previously published and established genetic susceptibility loci were used.[12] Each genetic locus was represented by one tag SNP, except for the human leucocyte antigen (HLA) region and interferon regulatory factor 5 (*IRF5*) which were assessed using two and three tag SNPs, respectively, representing two independent previously established genetic effects for SLE in the HLA region and three independent genetic effects in *IRF5*.[213] We genotyped a total of 4248 patients with SLE and 3818 normal healthy controls. All the patients included in this study fulfilled at least four of the 11 American College of Rheumatology classification criteria for SLE.[1415] All participants included in the study signed an informed consent approved by the Institutional Review Boards of our institutions.

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Genotyping and data analysis

Genotyping was performed using the Illumina Custom Bead system on the iSCAN instrument. All individuals included in the study were of European descent. Rigorous quality control measures were applied as reported previously.[16] Samples with genotype success rates <90% were excluded, followed by the exclusion of one individual from each pair if the proportion of alleles shared identical by descent >0.4. Samples were evaluated for mismatches between their reported sex and their genetic data and were removed from the analysis if they did not meet the following criteria: men were required to have chromosomal X heterozygosity ≤10% and be heterozygous at rs2557524 and assigned women were required to have chromosomal X heterozygosity >10% and be homozygous at rs2557524. rs2557524 is mapped to a region on chromosomes X and Y that is identical except for this one base, so male samples would generate a heterozygous genotype and female samples would generate a homozygous genotype. Samples with increased heterozygosity (>5SD around the mean) were then removed. Finally, genetic outliers were identified and excluded as determined by principal components analysis and admixture proportions calculated using ADMIXMAP, as previously described.[16] Samples included in the analysis consisted of 344 male patients with SLE and 1151 controls, and 3592 female patients with SLE and 2340 controls.

All markers included in this study passed inclusion thresholds for Hardy-Weinberg equilibrium (P>0.001) and minor allele frequency (>0.01). Analysis was performed using PLINK.[17] Allele frequencies, ORs with 95% CIs and χ^2 with corresponding p values were determined. Sex-specific associations were performed by comparing allele frequencies between cases and controls from each sex separately, followed by case–case analysis. Sex–gene interactions were validated using a parametric analysis for epistasis as implemented in PLINK.[17] Next, a pairwise non-parametric epistasis test was applied using multifactor dimensionality reduction (MDR) analysis.[18,19]

Cumulative genetic risk scores

We examined the aggregate genetic risk in a sex-specific manner by calculating cumulative genetic risk scores for individuals with 100% genotype success rate among the markers included in the study. Only those individuals with 100% genotype success for all markers were included to prevent the underestimation of genetic risk in any individual due to failed genotyping. Three SNPs were not included when calculating the cumulative genetic scores because they were not associated with SLE in our study (*CTLA4*, *PDCD1* and *MBL*). ORs used to calculate the risk score in each sex were those obtained in the sex-specific association analyses in male and female populations, respectively. We assigned a risk score to each individual based on the sum of the products of the natural logarithm of the OR for each sex-specific association and the number of risk alleles present in each individual at each locus. Cumulative risk scores were calculated for a total of 287 men and 2982 women with SLE.

RESULTS

We first performed case–control association tests in men and women separately. The majority of associations previously reported in women-only or combined populations were recapitulated in our male patients (table 1). Moreover, three of these associations attained genome-wide significance ($p<5.0\times10^{-8}$) in men. We then compared risk allele frequencies between men and women with SLE (table 2).

Interestingly, the frequency of the risk alleles in the HLA locus was significantly higher in men than in women with SLE (rs3131379: $OR_{male\text{-}female}$ 1.37 (95% CI 1.14 to 1.66),

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p=0.0010; rs1270942: $OR_{male\text{-}female}$ 1.40 (95% CI 1.16 to 1.69), p=0.00046). This was also the case for an SNP in *IRF5* (rs2070197: $OR_{male\text{-}female}$ 1.23 (95% CI 1.01 to 1.49), p=0.039). It is important to note that there was no difference in the risk allele frequencies in the control group between men and women (p=0.39, 0.52 and 0.64, for rs3131379, rs1270942 and rs2070197, respectively). Therefore, it was not surprising to see a trend for a higher association OR in men than in women in sex-specific case–control analysis in these loci (figure 1). This trend was further examined by calculating the heterogeneity I^2 index (range 0–100) and *Q* statistic p values to assess heterogeneity between male and female case–control ORs (rs3131379: OR_{male} 2.61 (95% CI 2.08 to 3.27), OR_{female} 2.05 (95% CI 1.82 to 2.30), I^2 index=71.69 and *Q* statistic p=0.060; rs1270942: OR_{male} 2.71 (95% CI 2.16 to 3.40), OR_{female} 2.05 (95% CI 1.82 to 2.30), I^2 index=78.68, *Q* statistic p=0.030; rs2070197: OR_{male} 2.15 (95% CI 1.71 to 2.69), OR_{female} 1.82 (95% CI 1.62 to 2.03), I^2 index=39.73, *Q* statistic p=0.20). A post hoc analysis showed that our study had 100% power to detect genetic associations in the HLA region and *IRF5* in men (α= 0.05), suggesting that a smaller sample size of men than women in our study did not result in inflation of the ORs in our male set.[2021]

Significant allelic differences between men and women with SLE were also observed for rs4963128, a polymorphism located in *KIAA1542* ($OR_{male\text{-}female}$ 1.25 (95% CI 1.06 to 1.48), p=0.0095) (table 2). rs4963128 was associated with SLE in women but not in men in our study (OR_{male} 0.96 (95% CI 0.80 to 1.15), p=0.68; OR_{female} 1.25 (95% CI 1.15 to 1.35), $p=4.7\times10^{-8}$; I^2 index=84.79, *Q* statistic p=0.010).

We next used a case-only pairwise epistasis analysis implemented in PLINK and confirmed the sex–gene interactions found (table 3). We further validated our results using a non-parametric methodology for non-linear epistasis by applying the MDR test (table 3).

Genetic differences associated with anti-dsDNA antibody positivity among patients with SLE were recently reported by Chung *et al*.[22] We investigated sex differences in the prevalence of anti-dsDNA antibodies among our test population to account for possible confounding. No significant difference in the presence of anti-dsDNA antibodies between men and women with SLE was observed (p=0.15). As men with SLE have previously been reported to experience more severe disease than women, it is important to examine if the difference in the frequencies of the HLA region risk alleles and the risk alleles in *IRF5* and *KIAA1542* that we observed between men and women is not influenced by differences in disease severity. We determined the frequencies of severe SLE manifestations in men and women included in the study (renal involvement, neurological involvement, serositis and thrombocytopenia) and found no differences in the frequencies of neurological involvement or serositis between men and women. However, consistent with previous reports, men with SLE in our study were almost twice as likely to have renal involvement as women (OR 1.70 (95% CI 1.34 to 2.17), $p=1.2\times10^{-5}$). Likewise, men with SLE were more likely to have thrombocytopenia (OR 2.26 (95% CI 1.62 to 3.15), $p=5.7\times10^{-7}$), which is an indicator of a more severe disease in SLE.[23] We have previously shown the association between renal involvement and the SLE risk alleles in *ITGAM* and *TNFSF4*.[24] However, the risk alleles in the HLA region and in *KIAA1542* were not associated with renal involvement or thrombocytopenia in that same set of patients also used in this study (p=0.89, 0.72 and 0.82 for renal involvement and p=0.95, 0.84 and 0.96 for thrombocytopenia for rs3131379, rs1270942 and rs4963128, respectively). Similarly, we found no association between the risk allele in rs2070197 (*IRF5*) and renal involvement in our SLE patients or between rs2070197 and thrombocytopenia (p=0.15 and p=1.00, respectively). These data indicate that the difference in allele frequency between men women with SLE in the HLA region, *IRF5* and *KIAA1542* is not explained by a higher frequency of renal involvement or thrombocytopenia in men than in women.

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We further investigated sex-specific differences in overall SLE genetic risk between men and women by calculating a cumulative genetic risk score for SLE in each individual included in the study. Scores were calculated based on the ORs obtained in the sex-specific case–control association analyses using the equation shown in figure 2A. Using a Student t test we observe that, on average, male patients have a significantly higher genetic risk than female patients ($p=4.52\times10^{-8}$; figure 2B). Interestingly, but not unexpectedly, the gap between men and women widens upon removal of rs4963128 (*KIAA1542*), the effect specific to women, while the disparity narrows as one HLA SNP is removed and the difference disappears entirely when both HLA SNPs are taken away (p=0.30).

DISCUSSION

We have reported sex-specific genetic differences in SLE. Specifically, the most significant gene-sex interactions were observed in the HLA gene region and *IRF5* gene. A genetic effect unique to women was also observed in *KIAA1542*. Importantly, we demonstrate that, in patients with SLE, men have a higher cumulative genetic risk than women. These findings suggest that sex disparities in SLE can be at least partly related to factors beyond the X chromosome and hormonal differences. Men need to inherit a larger number of risk alleles to develop SLE than women.

Sexual disparities at the HLA locus and *IRF5* gene appear to account for the largest proportion of genetic variation in overall risk between men and women with SLE. HLA genotypes are among the most common genetic factors implicated in autoimmune disorders including SLE.[25] We tested two independent genetic susceptibility loci within the HLA region and provide robust evidence for gene–sex interaction in these two loci. Indeed, male patients have significantly higher risk allele frequencies than female patients in both HLA susceptibility loci.

IRF5 is essential for viral immunity involving signal transduction via Toll-like receptors and MyD88 pathways.[26] *IRF5* has previously been shown to be highly associated with SLE susceptibility in European populations.[27] The association observed in *IRF5* is among the most robust and reproducible genetic effects in SLE.[28] Our data indicate a gene–sex intercalation between *IRF5* and SLE, with men having a higher prevalence of the risk allele in rs2070197 than women with the disease. Previous studies have shown evidence for three independent genetic variants associated with SLE in the *IRF5* locus.[13] We found no evidence for gene–sex interaction in the other two SLE-associated genetic variants in *IRF5*, which are tagged by rs729302 and rs10954213.

While the genetic basis of sexual dimorphism in SLE has not been fully explored, nor are the results of the present study exhaustive, these data suggest that, in a number of previously established risk loci for SLE, men who develop SLE possess a higher aggregate genetic risk as demonstrated by increased genetic risk scores (figure 2). It stands to reason that the effects of the autosomal genetic component of disease would be more pronounced among men with SLE for whom the added risk factors of sex chromosome complement and sex hormonal factors are not typically present. Interestingly, men with Klinefelter's syndrome (47, XXY) who possess an additional X chromosome have been observed to develop SLE at a rate similar to women.[29] Our data suggest that a SLE genome-wide association study in men would likely result in the discovery of novel genetic risk loci for SLE, and would enable a more exhaustive effort to characterise genetic disparities between men and women with the disease.

The requirement for increased genetic risk in the absence of other sex-specific risk factors among men may account for their reduced overall incidence of SLE, while simultaneously

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reinforcing the molecular processes underlying the disease which may lead to the increased severity of disease manifestations when SLE is present. The frequency of renal disease is significantly higher in female SLE patients with an SLE affected male relative compared to those with no affected male relatives.[30] This points to a stronger genetic component of disease among families with male SLE patients. Our data could not explain the difference in disease severity between men and women as the genetic risk loci which are differentially associated with SLE in men compared with women were not associated with renal involvement or other manifestations of severe SLE. These disease severity loci in male patients are likely to be discovered once a genome-wide association study in male patients and controls with adequate power is conducted.

Based on these data, we suggest that men who develop SLE possess a larger genetic component for disease while women likely develop SLE at a substantially higher rate due to the combined effects of hormonal differences, sex chromosome complement and a lower genetic susceptibility requirement.

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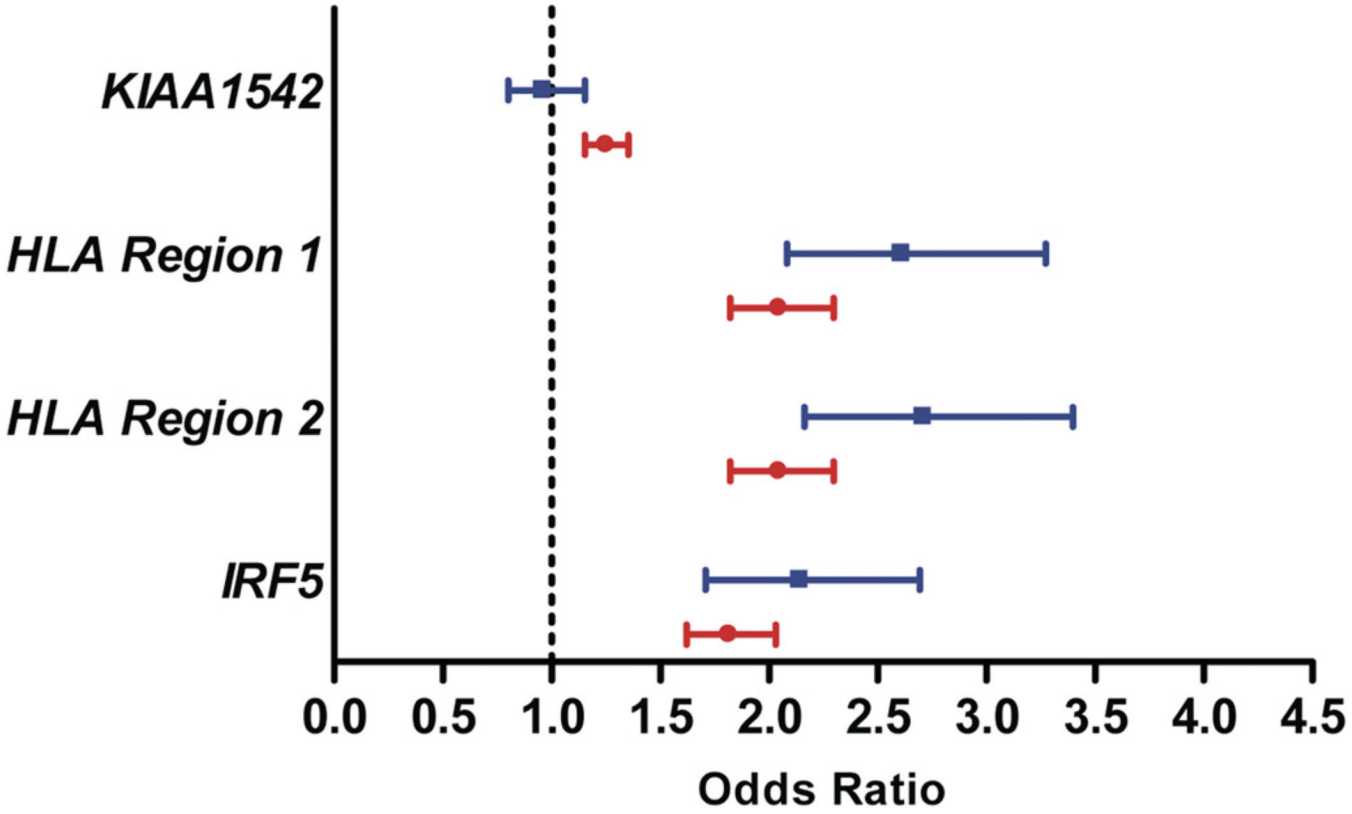

Figure 1.
Sex-specific differences in genetic associations in systemic lupus erythematosus (men in blue and women in red).

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A.

$$Cumulative\ Genetic\ Risk\ Score = \sum_{k=1}^{i} ln(OR_i)n_i$$

$OR_i =$ *Odds ratio of a given effect*

$n_i =$ *Number of risk alleles at a given polymorphic site*

B.

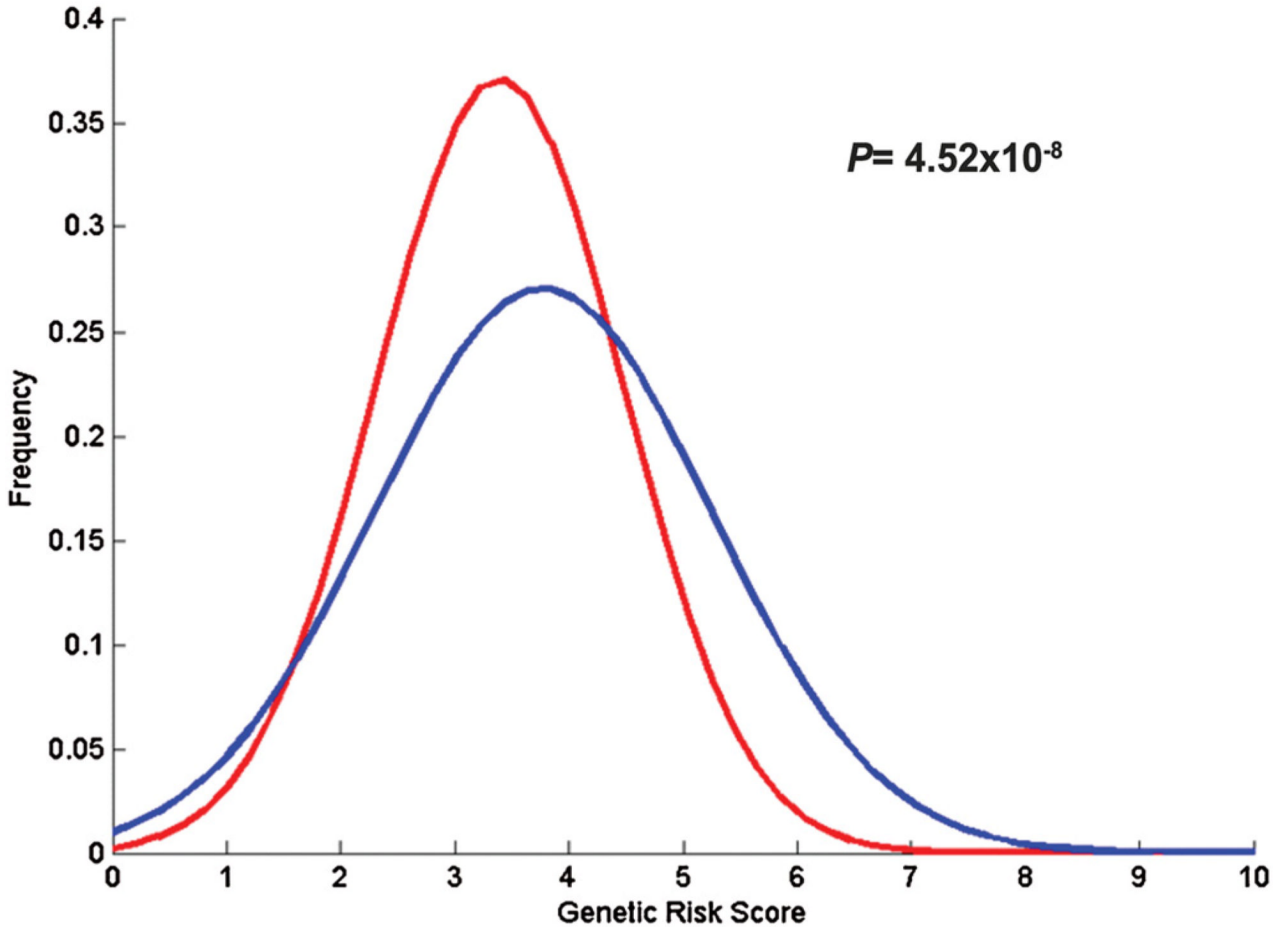

Figure 2.

(A) The equation by which cumulative genetic risk scores were calculated. Scores were obtained for each patient by multiplying the natural logarithm of the OR for each of the associated loci by the number of risk alleles present at each locus. Cumulative risk was then calculated in each patient by summing the risk scores for 15 out of 18 risk loci included in this study. Three loci were not included when calculating the cumulative genetic risk scores because they were not associated with systemic lupus erythematosus in our study (*CTLA4*, *PDCD1* and *MBL*). (B) Distribution curves for cumulative genetic risk scores for systemic lupus erythematosus in men (blue) and women (red) showing a higher genetic risk in men

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than in women ($p=4.5 \times 10^{-8}$). Sex-specific ORs (table 1) were used to calculate the cumulative genetic risk score in male and female patients.

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Table 1

Genetic associations in men and women with systemic lupus erythematosus compared with normal healthy male and female controls of European descent

		Male				Female			
		Risk allele frequency				Risk allele frequency			
SNP	Gene locus	Case	Control	OR (95% CI)	p Value	Case	Control	OR (95% CI)	p Value
rs2476601	*PTPN22*	0.102	0.081	1.28 (0.96 to 1.71)	0.089	0.110	0.0802	1.41(1.24 to 1.61)	1.5×10^{-7}
rs1801274	*FCGR2A*	0.528	0.499	1.13 (0.94 to 1.34)	0.18	0.542	0.513	1.13 (1.05 to 1.21)	0.0017
rs2205960	*TNFSF4*	0.276	0.209	1.44 (1.19 to 1.75)	2.3×10^{-4}	0.269	0.2167	1.33 (1.22 to 1.45)	1.6×10^{-10}
rs7574865	*STAT4*	0.285	0.213	1.47 (1.21 to 1.79)	1.2×10^{-4}	0.309	0.2302	1.49 (1.37 to 1.63)	4.0×10^{-20}
rs231775	*CTLA4*	0.352	0.346	1.03 (0.86 to 1.23)	0.79	0.362	0.3475	1.07 (0.87 to 1.01)	0.11
rs11568821	*PDCD1*	0.878	0.875	1.03 (0.79 to 1.34)	0.83	0.889	0.8851	1.04 (0.93 to 1.18)	0.47
rs6445975	*PXK*	0.311	0.280	1.16 (0.96 to 1.40)	0.12	0.291	0.2564	1.19 (1.10 to 1.30)	3.6×10^{-5}
rs10516487	*BANK1*	0.746	0.675	1.42 (1.17 to 1.72)	4.3×10^{-4}	0.737	0.701	1.19 (1.10 to 1.30)	2.8×10^{-5}
rs907715	*IL21*	0.680	0.655	1.12 (0.93 to 1.34)	0.23	0.687	0.656	1.15 (1.06 to 1.24)	5.2×10^{-4}
rs3131379	*HLA Region 1*	0.222	0.099	2.61 (2.08 to 3.27)	1.3×10^{-17}	0.172	0.0923	2.05 (1.82 to 2.30)	1.7×10^{-34}
rs1270942	*HLA Region 2*	0.225	0.097	2.71 (2.16 to 3.40)	5.9×10^{-19}	0.172	0.0921	2.05 (1.82 to 2.30)	2.1×10^{-34}
rs729302	*IRF5*	0.737	0.684	1.23 (1.02 to 1.49)	0.032	0.745	0.675	1.41 (1.30 to 1.53)	1.3×10^{-16}
rs2070197	*IRF5*	0.204	0.106	2.15 (1.71 to 2.69)	2.6×10^{-11}	0.172	0.1028	1.82 (1.62 to 2.03)	9.3×10^{-26}
rs10954213	*IRF5*	0.699	0.639	1.31 (1.09 to 1.58)	0.0038	0.678	0.618	1.30 (1.21 to 1.41)	1.9×10^{-11}
rs13277113	*C8orf13-BLK*	0.321	0.229	1.60 (1.32 to 1.93)	1.3×10^{-6}	0.288	0.2421	1.27 (1.16 to 1.38)	6.5×10^{-8}
rs1800450	*MBL*	0.143	0.140	1.03 (0.80 to 1.31)	0.83	0.146	0.1392	1.05 (0.85 to 1.06)	0.34
rs4963128	*KIAA1542*	0.664	0.673	0.96 (0.80 to 1.15)	0.68	0.712	0.664	1.25 (1.15 to 1.35)	4.7×10^{-8}
rs1143679	*ITGAM*	0.201	0.138	1.57 (1.26 to 1.96)	5.4×10^{-5}	0.193	0.1202	1.75 (1.58 to 1.95)	1.3×10^{-25}

SNP, single nucleotide polymorphism.

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Table 2

Sex–gene disparities between men and women with systemic lupus erythematosus

		Risk allele frequency					
SNP	**Gene**	**Male**	**Female**	**OR**	**95% CI LL**	**95% CI UL**	**p Value**
rs2476601	*PTPN22*	0.102	0.110	1.08	0.84	1.40	0.55
rs1801274	*FCGR2A*	0.528	0.542	1.06	0.90	1.24	0.48
rs2205960	*TNFSF4*	0.276	0.269	1.04	0.87	1.24	0.67
rs7574865	*STAT4*	0.285	0.309	1.12	0.94	1.34	0.21
rs231775	*CTLA4*	0.352	0.362	1.05	0.89	1.23	0.60
rs11568821	*PDCD1*	0.878	0.889	1.11	0.87	1.42	0.38
rs6445975	*PXK*	0.311	0.291	1.10	0.93	1.30	0.27
rs10516487	*BANK1*	0.746	0.737	1.05	0.88	1.26	0.60
rs907715	*IL21*	0.680	0.687	1.03	0.87	1.22	0.71
rs3131379	*HLA Region 1*	0.222	0.172	1.37	1.14	1.66	0.0010
rs1270942	*HLA Region 2*	0.225	0.172	1.40	1.16	1.69	0.00046
rs729302	*IRF5*	0.737	0.745	1.09	0.92	1.31	0.32
rs2070197	*IRF5*	0.204	0.172	1.23	1.01	1.49	0.039
rs10954213	*IRF5*	0.699	0.678	1.10	0.93	1.31	0.25
rs13277113	*C8orf13-BLK*	0.321	0.288	1.17	0.99	1.39	0.069
rs1800450	*MBL*	0.143	0.146	1.02	0.82	1.28	0.85
rs4963128	*KIAA1542*	0.664	0.712	1.25	1.06	1.48	0.0095
rs1143679	*ITGAM*	0.201	0.193	1.05	0.87	1.28	0.62

LL, lower limit; SNP, single nucleotide polymorphism; UL, upper limit.

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Table 3

Evidence for interaction between four known systemic lupus erythematosus (SLE) susceptibility loci and sex in patients with SLE

		Parametric analysis		MDR analysis			
Locus	**SNP**	**χ^2**	**Interaction p value**	**Cross-validation consistency**	**Balanced accuracy**	**χ^2**	**p Value**
HLA	rs1270942	11.60	0.00066	10/10	0.6220	696.05	1.5×10^{-150}
HLA	rs3131379	10.25	0.0014	10/10	0.6218	694.24	3.7×10^{-150}
KIAA1542	rs4963128	6.41	0.011	10/10	0.6222	551.20	3.8×10^{-119}
IRF5	rs2070197	4.07	0.044	10/10	0.6212	675.63	4.0×10^{-146}

A df of 3 was used to calculate p values. It should be noted that the MDR χ^2 and p values reflect a joint effect consisting of the main genetic effect and the interaction effect.

Cross-validation consistency is the number of times MDR found the same model as the data were divided into different segments. Balanced accuracy represents (sensitivity+specificity)/2. Sensitivity = true positives/(true positives +false negatives) and specificity = true negatives/(false positives+true negatives). This gives an accuracy estimate that is not biased by the larger class.[31]

MDR, multifactor dimensionality reduction; SNP, single nucleotide polymorphism.