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Phenotypic associations of genetic susceptibility loci in systemic lupus erythematosus

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Abstract

Objective—Systemic lupus erythematosus is a clinically heterogeneous autoimmune disease. A number of genetic loci that increase lupus susceptibility have been established. This study examines if these genetic loci also contribute to the clinical heterogeneity in lupus.

Materials and methods—4001 European-derived, 1547 Hispanic, 1590 African-American and 1191 Asian lupus patients were genotyped for 16 confirmed lupus susceptibility loci. Ancestry informative markers were genotyped to calculate and adjust for admixture. The association between the risk allele in each locus was determined and compared in patients with and without the various clinical manifestations included in the ACR criteria.

Results—Renal disorder was significantly correlated with the lupus risk allele in *ITGAM* ($p=5.0\times10^{-6}$, OR 1.25, 95% CI 1.12 to 1.35) and in *TNFSF4* (p=0.0013, OR 1.14, 95% CI 1.07 to 1.25). Other significant findings include the association between risk alleles in *FCGR2A* and malar rash (p=0.0031, OR 1.11, 95% CI 1.17 to 1.33), *ITGAM* and discoid rash (p=0.0020, OR 1.20, 95% CI 1.06 to 1.33), *STAT4* and protection from oral ulcers (p=0.0027, OR 0.89, 95% CI 0.83 to 0.96) and *IL21* and haematological disorder (p=0.0027, OR 1.13, 95% CI 1.04 to 1.22). All these associations are significant with a false discovery rate of <0.05 and pass the significance threshold using Bonferroni correction for multiple testing.

Conclusion—Significant associations were found between lupus clinical manifestations and the *FCGR2A, ITGAM, STAT4, TNSF4* and *IL21* genes. The findings suggest that genetic profiling might be a useful tool to predict disease manifestations in lupus patients in the future.

Systemic lupus erythematosus (SLE) is a chronic relapsing autoimmune disease characterised by deposition of immune complexes in different tissues, such as skin, synovium, lungs, kidneys and other organs. This results in local and systemic inflammation, often progressing to organ dysfunction and failure. SLE occurrence is three to four times higher among Asian and African-American than Caucasian women.[1] In addition, Hispanic, Asian and African-American individuals have an excess morbidity from SLE and a higher prevalence of lupus nephritis than Caucasian individuals.[1–3] The pathogenesis of SLE remains unclear, although there is much evidence demonstrating the involvement of genetic factors in the incidence of this autoimmune disease.[4] The genetic background contributing to the development of SLE includes, in particular, genes encoding disparate proteins that control immune system pathways.[5–7] Whereas most studies have looked for an association between susceptibility loci and SLE, only some have examined the relationship between these markers and select disease manifestations, disease severity and clinical subsets. Many of the clinical manifestations in SLE are correlated, and may indicate different underlying disease mechanisms. Understanding the relationships between SLE risk genes and subtypes of the disease may help to elucidate disease mechanisms and pathways.

The aim of the present study was to investigate the association between 16 confirmed SLE susceptibility loci[8–22] with SLE manifestations in a total of 8329 lupus patients. Our data indicate that genetic factors play a role in the predisposition to various specific disease manifestations in SLE.

Material and Methods

Subjects

A total of 4001 European-derived, 1547 Hispanic, 1590 African-American and 1191 Asian SLE patients was included in the study. The patients' samples were assembled at the Oklahoma Medical Research Foundation after collection through multiple institutions around the world, following ethics committee approval and informed consent. All patients fulfilled the American College of Rheumatology (ACR) criteria for classification of SLE.[23] Clinical data were obtained at each centre either from medical records or pre-established protocols that were reviewed and tabulated at each institution. We chose to examine the clinical ACR criteria including malar rash, discoid rash, photosensitivity, oral ulcers, arthritis, serositis, renal disorder, neurological disorder and haematological disorder.

Genotyping

Genotyping was performed using the Illumina custom bead system on the iSCAN instrument as part of a large lupus candidate gene association study to reduce the cost of genotyping and maximise sample size. The following single-nucleotide polymorphisms (SNP) within 16 confirmed and independent SLE susceptibility loci were studied: rs2476601 (*PTPN22*), rs1801274 (*FCGR2A*), rs2205960 (*TNFSF4*), rs7574865 (*STAT4*), rs231775 (*CTLA4*), rs11568821 (*PDCD1*), rs6445975 (*PXK*), rs10516487 (*BANK1*), rs907715 (*IL21*), rs3131379 (*MSH5* within the human leucocyte antigen (HLA) region), rs1270942 (*CFB*, within the HLA region), rs13277113 (*C8orf13-BLK* region), rs1800450 (*MBL2*), rs4963128 (*KIAA1542*), rs1143679 (*ITGAM*) and rs17435 (*MECP2*/*IRAK1*).[8–22] In addition, 161 admixture informative markers were genotyped and evaluated in our samples. The admixture informative markers were selected to distinguish four continental ancestral populations: Africans, Europeans, American Indians and East Asians.[24–28]

Statistical analysis

Before data analysis, all SNP that did not meet the following criteria were excluded: minor allele frequency of 0.01 or greater, genotype success rate 0.90 or greater and Hardy–

Weinberg equilibrium p≥0.001. Next, we excluded samples with a genotype success rate less than 0.90. The remaining samples were then evaluated for duplicates or related individuals, and one individual from each pair was removed if the proportion of alleles shared identical by descent was greater than 0.4 (or >0.35 in Gullah samples). Samples with increased heterozygosity (>5 SD around the mean) were then removed from the analysis. Samples were assessed for mismatches between reported gender and genetic data, and 78 individuals with gender discrepancies were removed from the analysis. Finally, genetic outliers were removed from further analysis as determined by principal component analysis and admixture proportions calculated using ADMIXMAP software.[29–32] After applying the quality control measures detailed above, samples included in our analysis consisted of 3675 European-derived, 1566 African-American, 1139 Asian and 1426 Hispanic lupus patients.

Genetic association analysis was performed using PLINK version 1.07[33] in each ancestral background separately, followed by meta-analysis. Association analysis was performed as logistic regressions using SLE subphenotypes as outcome variables and using the ancestry proportions of each individual provided by ADMIXMAP as covariates. The meta-analysis was conducted using standard methods based on the Cochran–Mantel–Haenszel test[34] using PLINK version 1.07 and Comprehensive Meta-Analysis software. The Breslow–Day test was performed for all SNP to assess heterogeneity of the OR in different populations.[35] Multiple testing was corrected by Bonferroni and false discovery rate methods,[36] and only results that are significant using a false discovery rate of 0.05 and survive Bonferroni correction were reported.

Results

Characteristics of the patients with SLE in each cohort are given in table 1. Our clinical data confirmed a higher prevalence of renal disorder in African-American (50.1%), Asian (46.9%) and Hispanic individuals (46%) compared with European-derived lupus patients (34.7%).

Of the studied SNP, rs2476601 (*PTPN22*), rs11568821 (*PDCD1*), rs1143679 (*ITGAM*), rs3131379 (*MSH5*) and rs1270942 (*CFB*) were excluded from further analysis in the Asian samples as a result of a minor allele frequency of less than 0.01. All 16 SNP passed the inclusion criteria in European-derived, African-American and Hispanic samples. To investigate if the SLE susceptibility loci predispose to any particular disease manifestation in SLE, we calculated allele frequencies in each locus in patients with and without the various ACR clinical SLE features and determined OR adjusted for admixture in each ethnic group, then performed a meta-analysis across ethnicities.

We identified no significant differences with the *PTPN22*, *CTLA4*, *PDCD1*, *BANK1*, *PXK*, *MSH5*, *CFB*, *C8orf13-BLK*, *MBL2*, *KIAA1542* and *MECP2* in any of the above clinical ACR criteria (data not shown). However, we found a statistically significant association between some clinical manifestations and the lupus risk alleles in the *FCGR2A*, *ITGAM*, *STAT4*, *TNFSF4* and *IL21* genes. The most significant was the association between renal disorder and the lupus risk allele in *ITGAM* ($p=5.0\times10^{-6}$, OR 1.25, 95% CI 1.12 to 1.35, $p_{Bonferroni}=7.99\times10^{-5}$). This association seems to be driven by the European-derived cohort ($p=4.7\times10^{-7}$, OR 1.39, 95% CI 1.22 to 1.58; table 2). We also detected a significant association between the *TNFSF4* risk allele and renal disorder (p=0.0013, OR 1.14, 95% CI 1.07 to 1.25, $p_{Bonferroni}=0.020$). This association is driven mainly by the European component because the strongest association was found in the European-derived cohort (p=0.0030, OR 1.18, 95% CI 1.06 to 1.33) and only a trend of association was shown in Hispanic individuals (p=0.052, OR 1.17, 95% CI 1.00 to 1.37), but not in African-American (p=0.74, OR 1.05, 95% CI 0.80 to 1.38) or Asian individuals (p=0.81, OR 1.02, 95% CI

0.85 to 1.22). In addition, we also found an association between *FCGR2A* and malar rash (p=0.0031, OR 1.11, 95% CI 1.17 to 1.33, $p_{Bonferroni}$=0.049), *ITGAM* and discoid rash (p=0.0020, OR 1.20, 95% CI 1.06 to 1.33, $p_{Bonferroni}$=0.031), *STAT4* and oral ulcers (p=0.0027, OR 0.89, 95% CI 0.83 to 0.96, $p_{Bonferroni}$=0.042) and *IL21* and haematological disorder (p=0.0027, OR 1.13, 95% CI 1.04 to 1.22, $p_{Bonferroni}$=0.042). We next performed a genetic model analysis testing for a dominant, recessive and additive model for the associations detected. Model analysis was performed in the European-derived set as all the associations detected are primarily driven by the European-derived lupus patients (table 3). Our data suggest an additive model for the association between *FCGR2A* and malar rash, *ITGAM* and discoid rash, *ITGAM* and renal disorder, *TNFSF4* and renal disorder and *IL21* and haematological disorder. An accurate model could not be predicted for the association between *STAT4* and oral ulcers.

In order to investigate further the role of *IL21* in the presence of haematological manifestations in SLE, we examined the specific ACR haematological subphenotypes when available. We failed to find an association between *IL21* and the presence of lymphopenia, haemolytic anaemia or thrombocytopenia. However, a statistically significant association was found between the *IL21* risk allele and leucopenia (p=0.0039, OR 1.14, 95% CI 1.04 to 1.24).

An additional ethnicity-specific association between disease susceptibility loci and clinical manifestations is the association between the risk allele in *KIAA1542* and serositis in Hispanic individuals (p=0.0021, OR 1.35, 95% CI 1.12 to 1.64, $p_{Bonferroni}$=0.033).

Discussion

Genotype–phenotype associations between risk alleles and disease subtypes may provide insight into disease aetiology and mechanisms in SLE. In the present study, a panel of SNP previously implicated to confer a risk of SLE in different populations was analysed for association with clinical manifestations in a total of 8329 SLE patients. To our knowledge, this is the first study to explore a wide panel of SLE susceptibility genes with clinical manifestations in a large multi-ethnic set of lupus patients.

ITGAM (CD11b) encodes integrin-α_M, which regulates several immune system pathways. In our study we have found that the *ITGAM* risk allele was associated with an increased risk of renal disorder and risk of discoid rash in lupus patients (OR 1.25 and 1.20, respectively, table 2). We therefore replicated the recent observation that the functional *ITGAM* rs1143679 polymorphism may influence the risk of renal disorder and discoid rash in SLE.[37–40] It is important to note that part of our European-derived sample overlaps with the study of Kim-Howard *et al*.[37] However, we did not detect the previously reported association between *ITGAM* and the risk of arthritis and neurological disorder in SLE patients.[383941] *ITGAM* has been described as a possible mediator in the inflammatory processes in SLE. In addition, a recent study has shown that in MRL/*lpr* mice, which show glomerular hypercellularity, tubular damage and perivascular cell infiltration, the frequency of CD11b+ GR-1^{low} (myeloid differentiation antigen) cells increased during disease progression in the kidneys and in peripheral blood.[42] The *ITGAM* rs1143679 polymorphism corresponds to a non-synonymous variant that predicts changes in the structure and function of the protein product.[16] CD11b deficiency in mice was shown to enhance the differentiation of naive T cells to interleukin (IL) 17 producing T-helper type17 cells.[43] Serum IL-17 concentrations were higher in patients with discoid lupus and SLE compared with normal controls.[44] These findings suggest a possible role for *ITGAM* in the cutaneous manifestations of lupus.

Our analysis revealed for the first time that the *TNFSF4* risk allele is associated with renal disorder (OR 1.14). TNFSF4 (or OX40L or CD134L) is a member of the tumour necrosis factor superfamily and is expressed on antigen-presenting cells. Its receptor, TNFSFR4 or OX40 or CD134, is expressed on activated T cells and the binding of both is a costimulator of T cells.[45] Serum levels of TNFSF4 were significantly higher among SLE patients with nephritis than among those without nephritis[46] suggesting that TNFSF4 may act as markers of lupus nephritis. In addition, the expression of TNFRSF4 on CD4+ T cells is associated with nephritis and disease activity in patients with SLE.[4647] Observations made in a mouse model of lupus nephritis demonstrate the involvement of TNFRSF4–TNFSF4 interactions in the development of glomerulonephritis.[48] TNFSF4 was also colocalised with immune deposits lining the epithelial side of the glomerular capillary wall in patients with lupus nephritis.[49] A recent study provides evidence that treatment with anti-CD134 monoclonal antibody or the hybridised fusion protein rhCD134:Fc may possess the capacity to alter cytokine production in peripheral blood mononuclear cells from patients with lupus nephritis.[50]

The *STAT4* rs7574865 polymorphism was originally identified in rheumatoid arthritis and SLE patients in a case–control study[12] and was subsequently confirmed in multiple populations.[91551] Although a previous study reported that *STAT4* is associated with more severe SLE manifestations,[52] we did not find any association between *STAT4* and any of the more severe SLE clinical features in our study. In contrast, we confirmed the previous association between the *STAT4* risk allele and protection against oral ulcers (OR 0.89) in SLE patients.[52] The lack of association between *STAT4* and arthritis in lupus patients, despite the established association between *STAT4* and rheumatoid arthritis, is probably explained by the difference in the pathogenic mechanisms underlying arthritis in these two diseases.[5354]

Another gene associated with SLE is *FCGR2A*, an Fc receptor for immunoglobulin G that mediates in the clearance of immune complexes and is strongly implicated in lupus nephritis in European-derived[55] and African-American individuals.[21] However, we were unable to replicate this association between *FCGR2A* and nephritis in our African-American and European-derived cohorts or in the meta-analysis including the four different populations. Nevertheless, an association between *FCGR2A* and susceptibility to malar rash was detected in our study (OR 1.11). *FCGR2A* was previously associated with susceptibility to subacute cutaneous lupus erythematosus.[56] These data suggest that *FCGR2A* contributes to the phenotypic heterogeneity of SLE, predisposing maybe to a more moderate disease.

Interestingly, we have observed an increase of the *IL21* risk allele in patients with haematological disorder (OR 1.13). This association was due to a higher presence of the *IL21* risk allele in patients with leucopenia (OR 1.14). IL-21 is a cytokine produced primarily by activated CD4+ T cells and is involved in the differentiation and functional activity of T and B cells.[57–59] Plasma levels of IL-21 are significantly higher in SLE patients than in healthy controls, but IL-21 levels do not seem to correlate with SLE activity.[60] IL21R-Fc fusion protein significantly improves disease in MRL/*lpr* lupus-prone mice, suggesting that blocking IL-21 might be a potential therapeutic approach in lupus patients.[61] A decreased expression of IL-21R on peripheral B lymphocytes in SLE has been observed.[62] Furthermore, polymorphisms in *IL21* and *IL21R* have been associated with SLE in different populations.[1863] These findings indicate that the IL-21/IL-21R pathway is important in understanding the pathogenesis of lupus, and abnormalities in this pathway might contribute to the pathological features of SLE, such as leucopenia.

The SNP included in this study were selected as they tag independent lupus susceptibility loci in European-derived lupus patients, although some of these variants have been

replicated in other ethnicities.[2464] It is important to note that the majority of the genotypic–phenotypic associations we reported herein were driven by the European-derived patient set and that these associations tend to get diluted with decreasing European admixture. This perhaps suggests that the tag SNP known to date for the majority of lupus susceptibility genes are non-causal, and more fine-mapping efforts in other ethnicities are needed to localise these genetic effects and to identify causal variants in these disease susceptibility loci.

In conclusion, we found for the first time an association between *TNFSF4* and the risk of renal disorder in lupus patients. We replicated the association between *ITGAM* polymorphism and susceptibility to renal disorder and discoid rash. Furthermore, we reported the association between *FCGR2A* and malar rash, and *IL21* and the presence of leucopenia for the first time, and confirmed the protective effect of the lupus risk allele in *STAT4* on oral ulcers.

Our data indicate that genetic profiling in lupus patients might be a useful tool to predict disease manifestations in the future.

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Table 1
Characteristics and clinical features of SLE patients included in this study

Phenotypes	European-derived, n (%)	African American, n (%)	Asian, n (%)	Hispanic, n (%)
Total individuals	4001	1590	1191	1547
Women	3655 (91.3)	1465 (92.1)	1095 (92)	1412 (91.3)
Men	346 (8.7)	125 (7.9)	96 (8)	135 (8.7)
Age of onset (mean±SD)	33.6±13.7	34±12.3	26.3±10.8	29.5±11.7
Malar rash	2262/3583 (63.1)	733/1587 (46.2)	572/1191 (48.0)	915/1544 (59.3)
Discoid rash	617/3376 (18.2)	530/1588 (33.4)	90/1189 (7.6)	194/1543 (12.6)
Oral ulcers	1673/3538 (47.3)	538/1586 (33.9)	430/1188 (36.2)	620/1541 (40.2)
Photosensitivity	2512/3793 (66.2)	747/1585 (47.1)	401/1187 (33.8)	915/1540 (59.4)
Arthritis	3211/3911 (82.1)	1316/1586 (83.0)	741/1190 (62.3)	1098/1545 (71.1)
Renal disorder	1226/3533 (34.7)	795/1586 (50.1)	559/1191 (46.9)	700/1522 (46.0)
Serositis	1455/3587 (40.6)	683/1516 (45.1)	229/1018 (22.5)	398/1365 (29.2)
Neurological disorder	606/3330 (18.2)	380/1492 (25.5)	101/1067 (9.5)	219/1543 (14.2)
Haematological disorder	2213/3348 (66.1)	1055/1414 (74.6)	930/1189 (78.2)	870/1373 (63.4)

SLE, systemic lupus erythematosus.

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Table 2

Genotypic–phenotypic associations in SLE

	Meta-analysis					European-derived		Hispanic		African-American		Asian	
Gene (clinical manifestation)	**N**	**p Value***	**OR (95% CI)**	**$p_{Bonferroni}$**	**p_{heter}**	**p_{admix}**	**OR (95%CI)**	**p_{admix}**	**OR (95% CI)**	**p_{admix}**	**OR (95% CI)**	**p_{admix}**	**OR (95% CI)**
FCGR2A (malar rash)	4	0.0031	1.11 (1.17 to 1.33)	0.049	0.54	0.011	1.14 (1.03 to 1.27)	0.15	1.11 (0.96 to 1.30)	0.90	1.01 (0.88 to 1.16)	0.12	1.16 (0.96 to 1.39)
ITGAM (discoid rash)	3	0.0020	1.20 (1.06 to 1.33)	0.031	0.79	0.033	1.18 (1.01 to 1.39)	0.066	1.31 (0.98 to 1.75)	0.14	1.17 (0.95 to 1.43)	NA	NA
STAT4 (oral ulcers)	4	0.0027	0.89 (0.83 to 0.96)	0.042	0.94	0.066	0.9 (0.81 to 1.01)	0.060	0.86 (0.74 to 1.01)	0.21	0.88 (0.72 to 1.07)	0.32	0.9 (0.78 to 1.09)
ITGAM (renal disorder)	3	5.0×10^{-6}	1.25 (1.12 to 1.35)	7.99×10^{-5}	0.050	4.7×10^{-7}	1.39 (1.22 to 1.58)	0.41	1.09 (0.89 to 1.34)	0.37	1.09 (0.90 to 1.33)	NA	NA
TNFSF4 (renal disorder)	4	0.0013	1.14 (1.07 to 1.25)	0.020	0.50	0.0030	1.18 (1.06 to 1.33)	0.052	1.17 (1.00 to 1.37)	0.74	1.05 (0.80 to 1.38)	0.81	1.02 (0.85 to 1.22)
IL21 (haematological disorder)	4	0.0027	1.13 (1.04 to 1.22)	0.042	0.54	0.010	1.16 (1.03 to 1.30)	0.78	1.02 (0.86 to 1.22)	0.36	1.09 (0.91 to 1.30)	0.055	1.22 (1.00 to 1.50)

OR were calculated for risk alleles in each susceptibility genetic locus in systemic lupus erythematosus (SLE) comparing patients with and without the various clinical American College of Rheumatology SLE classification criteria. Only significant associations in the meta-analysis with a false discovery rate of less than 0.05 and that also pass the threshold for multiple testing by Bonferroni correction are depicted.

*False discovery rate <0.05.

N, number of cohorts included in the meta-analysis; NA, not applicable; p_{admix}, p value adjusted for admixture; p_{heter}, heterogeneity p value.

Table 3

Genetic model analysis in the European-derived samples for the genetic phenotype associations reported in this study

	European-derived		
Gene (clinical manifestation)	**p Dominant**	**p Recessive**	**p Additive**
FCGR2A (malar rash)	0.018	0.078	0.011
ITGAM (discoid rash)	0.048	0.23	0.032
STAT4 (oral ulcers)	0.19	0.073	0.065
ITGAM (renal disorder)	1.50×10^{-6}	0.0077	3.56×10^{-7}
TNFSF4 (renal disorder)	0.025	0.0049	0.0027
IL21 (haematological disorder)	0.013	0.12	0.0093