CONCISE REPORT

interleukin 1 gene cluster: a population based study in the southeastern United States

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Background: Interleukin (IL)1α and IL1β, and their endogenous receptor antagonist (IL1Ra), have been related to the pathology of systemic lupus erythematosus (SLE), but the role of IL1 polymorphisms in the aetiology of SLE is unknown.
Objective: To examine polymorphisms at IL1α −889(C→T), IL1α +4845(C→T), IL1β −511(C→T), IL1β +3953(G→T), and IL1Ra (86 bp VNTR) in a population based study of SLE in North Carolina and South Carolina.
Methods: Genotypes from 230 cases who met ACR classification criteria, and from 275 controls matched for age, sex, and state, were analysed separately for African Americans and whites. Odds ratios (ORs) were estimated by logistic regression models for each locus alone and also after adjusting for polymorphisms at adjacent loci.
Results: An increased risk of SLE for the IL1α −889C/C genotype compared with carriage of the −889T allele was found in both African Americans (OR = 3.1, p = 0.001) and whites (OR = 2.9, p = 0.005). In African Americans, carriage of the IL1β −511T allele was associated with a higher risk of SLE than carriage of the −511C/C genotype (OR = 2.4, p = 0.017), independent of variation at IL1α −889.
Conclusions: The observed associations support the hypothesis that genetic variation in IL1 is involved in the aetiology of SLE and merit further investigation.

Interleukin (IL)1α and IL1β are proinflammatory cytokines with widespread biological activities, regulated in part by the IL1 receptor antagonist (IL1Ra). Differences in IL1 and IL1Ra production have been seen in systemic lupus erythematosus (SLE).[1 2] The IL1 gene cluster (IL1α, IL1β, IL1Ra) is located on a 430 kb region of chromosome 2 (2q13-21). Polymorphisms include biallelic single nucleotide polymorphisms in IL1α at position −889(C→T)[3] and in exon 5 at +4845(G→T),[4] and in IL1β at position −511(C→T)[5] and in exon 5 at +3953(C→T).[6] Data on allelic variation at these sites in patients with SLE are limited. One study reported that polymorphisms in IL1β (−511 and +3953) were not associated with SLE.[7] Variation in the penta-allelic 86 base pair tandem repeat (VNTR) in intron 2 of IL1Ra,[8] specifically the uncommon allele (IL1Ra allele 2), has been inconsistently linked to SLE susceptibility.[9 10]

The Carolina Lupus Study is a population based case-control study conducted in the southeastern United States. We examined five polymorphic loci in the IL1 gene cluster (IL1α −889, IL1α +4845, IL1β −511, IL1β +3953, and the IL1RA 86 base pair VNTR region) with respect to SLE, and estimated the effects of these polymorphisms on SLE individually and after adjusting for variation at all five loci.

PATIENTS AND METHODS

Study sample

Patients with SLE (diagnosed between January 1995 and July 1999, meeting the 1997 revised American College of Rheumatology (ACR) classification criteria) in 60 counties of North Carolina and South Carolina were referred through 30 community based rheumatologists and four university rheumatology practices. Controls matched for sex and state, identified through state driver's licence records for the 60 study counties, were randomly selected and frequency matched to cases in five-year age groups. Study protocols were approved by the institutional review boards of the National Institute of Environmental Health Sciences and other participating institutions. Details on sample enrolment have been presented previously.[11] The final sample consisted of 265 cases and 355 controls. Ninety per cent of cases were female, 60% were African American, and the mean age at diagnosis was 39 years (range 15–81). Thirty per cent of controls were African American, reflecting the racial distribution of the study area.

Genotyping

Blood specimens were used to obtain DNA from 243 (92%) cases and 298 (84%) controls. DNA specimens were genotyped for IL1α −889(C→T), IL1α +4845(C→T), IL1β −511(C→T), and IL1β +3953(G→T) biallelic restriction fragment length polymorphisms.[3–6] Specimens were amplified using polymerase chain reaction and digested with restriction enzymes Fun4H1, Nco1, AvaI, and α Taq, respectively. IL1Ra 86 bp VNTR genotypes were determined by polymerase chain reaction based methods.[8] Alleles were differentiated by visual determination of size relative to known markers (allele 1 = 4 repeats, allele 2 = 2 repeats, allele 3 = 5 repeats, and allele 4 = 3 repeats).

Analyses

Genotypes from 230 cases (144 African-American, 86 white) and 275 controls (73 African-American, 202 white) were examined in parallel analyses for African Americans and whites. Linkage disequilibrium was examined using the estimating haplotypes program (http://linkage.rockefeller.edu/ott/eh.html) ($\alpha = 0.05$). We used the χ^2 statistic (Fisher's exact test for cell size <5) to compare genotype frequency in cases and controls at each locus. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by unconditional logistic regression. We did not adjust for multiple comparisons. Models were run for each locus separately, comparing

Abbreviations: CI, confidence interval; IL, interleukin; IL1Ra, interleukin 1 receptor antagonist; OR, odds ratio; SLE, systemic lupus erythematosus; VNTR, variation in tandem repeat

the genotypes containing the variant allele with the homozygous wild-type genotype. Independent associations at each locus also were estimated in a model containing all five loci to adjust for potential confounding by linkage disequilibrium. Only the main effects at each locus were examined, except for the interaction of the two promoter polymorphisms (IL1α −889 and IL1β −511) with respect to SLE and proteinuria in African Americans. Proteinuria was defined as two or more urine samples containing ⩾3 mg/ml albumin reported in medical records up to six months after diagnosis.

RESULTS

Table 1 shows the frequency of IL1α, IL1β, and IL1Ra genotypes in patients with SLE and controls. Genotype frequencies were determined to be in Hardy-Weinberg equilibrium, and were significantly different ($p<0.05$) in African-American and white controls at all loci except IL1α −889. Genotype frequencies at IL1α −889 differed between cases and controls in both African Americans and whites: the IL1α −889C/T genotype was inversely associated with SLE compared with the IL1α −889C/C genotype in single locus models (African Americans OR = 0.4, p = 0.004; whites OR = 0.5, p = 0.006). Carriage of the IL1α −889T allele was inversely associated with SLE in the five locus model (African Americans OR = 0.3, p = 0.001; whites OR = 0.3, p = 0.005). Viewed as a positive risk factor, the IL1α −889C/C genotype was associated with a threefold increased risk of SLE in both African Americans (OR = 3.1; 95% CI 1.5 to 6.1, p = 0.001) and whites (OR = 2.9; 95% CI 1.4 to 6.0, p = 0.005) compared with carriage of the IL1α −889T allele. We observed no significant effect for number of copies of IL1α −889T. The IL1α +4845 polymorphism was not significantly associated with SLE.

In single locus models, the IL1β −511C/T genotype was significantly associated with SLE in African Americans (OR = 2.5, p = 0.016) compared with the C/C genotype, and carriage of the IL1β −511T allele was associated with SLE (OR = 2.4, p = 0.017) in the five locus model. We observed no significant effect for number of copies of the IL1β −511T allele in African Americans, and no association in whites. The IL1β +3953 polymorphism was not significantly associated with SLE.

Allelic variation at IL1Ra was significantly associated with SLE in African Americans (exact p = 0.01), driven in part by

Table 1 Frequencies of IL1α, IL1β, and IL1Ra genotypes and associations with SLE*

	African Americans			Whites		
Genotypes	Cases (n = 144) n (%)	Controls (n = 73) n (%)	OR (95% CI)†	Cases (n = 86) n (%)	Controls (n = 202) n (%)	OR (95% CI)
IL1α −889						
C/C	62 (43)	18 (25)	1.0 (referent)	43 (50)	68 (34)	1.0 (referent)
C/T	57 (40)	43 (59)	0.4 (0.2 to 0.7)	32 (37)	109 (54)	0.5 (0.3 to 0.8)
T/T	25 (17)	12 (16)	0.6 (0.3 to 1.4)	11 (13)	25 (12)	0.7 (0.3 to 1.6)
χ^2 (2df)‡	8.4, p = 0.015			7.6, p = 0.022		
C/T or T/T *v* C/C (single locus model)			0.4 (0.2 to 0.8)			0.5 (0.3 to 0.8)
C/T or T/T *v* C/C (five locus model)			0.3 (0.2 to 0.7)			0.3 (0.2 to 0.7)
IL1α +4845						
G/G	96 (67)	54 (74)	1.0 (referent)	45 (52)	95 (47)	1.0 (referent)
G/T	39 (27)	18 (25)	1.2 (0.6 to 2.4)	27 (31)	83 (41)	0.7 (0.4 to 1.2)
T/T	8 (6)	1 (1)	–	14 (16)	24 (12)	1.2 (0.6 to 2.6)
χ^2 (2df)	2.5, p = 0.28			2.1, p = 0.26		
G/T or T/T *v* G/G (single locus model)			1.4 (0.7 to 2.6)			0.8 (0.5 to 1.3)
G/T or T/T *v* G/G (five locus model)			1.8 (0.9 to 3.5)			1.3 (0.6 to 2.7)
IL1β −511						
C/C	23 (16)	21 (29)	1.0 (referent)	41 (48)	89 (44)	1.0 (referent)
C/T	73 (51)	27 (37)	2.5 (1.2 to 3.8)	36 (42)	87 (43)	0.9 (0.5 to 1.5)
T/T	48 (33)	25 (34)	1.8 (0.8 to 3.8)	9 (10)	26 (13)	0.8 (0.3 to 1.7)
χ^2 (2df)	5.9, p = 0.052			0.48, p = 0.79		
C/T or T/T *v* C/C (single locus model)			2.1 (1.1 to 4.2)			0.9 (0.5 to 1.4)
C/T or T/T *v* C/C (five locus model)			2.4 (1.2 to 4.9)			0.8 (0.4 to 1.3)
IL1β +3953						
C/C	111 (77)	61 (85)	1.0 (referent)	49 (57)	121 (60)	1.0 (referent)
C/T	32 (22)	10 (14)	1.8 (0.8 to 3.8)	31 (36)	67 (33)	1.1 (0.7 to 2.0)
T/T	1 (1)	1 (1)	–	6 (7)	13 (6)	1.1 (0.4 to 3.2)
χ^2 (2df)	2.3, p = 0.31			0.26, p = 0.88		
C/T or T/T *v* C/C (single locus model)			1.6 (0.8 to 3.5)			1.1 (0.7 to 1.9)
C/T or T/T *v* C/C (five locus model)			1.6 (0.7 to 3.5)			1.6 (0.8 to 3.0)
IL1Ra VNTR						
1/1	130 (90)	63 (87)	1.0 (referent)	63 (73)	160 (79)	1.0 (referent)
1/2	6 (4)	3 (4)	1.0 (0.2 to 4.0)	12 (14)	18 (9)	1.7 (0.8 to 3.7)
2/2	1 (1)	0 (0)	–	8 (9)	15 (7)	1.4 (0.6 to 3.4)
1/3	1 (1)	6 (8)	–	3 (3)	7 (3)	1.1 (0.3 to 4.3)
3/3	0 (0)	0 (0)	–	0 (0)	2 (1)	–
1/4	6 (4)	0 (0)	–	0 (0)	0 (0)	–
χ^2 (4df)	12.2, p = 0.016			2.9, p = 0.58		
1/2 or 2/2 *v* other genotypes (single locus model)			1.2 (0.3 to 4.7)			1.6 (0.8 to 3.0)
1/2 or 2/2 *v* other genotypes (five locus model)			0.8 (0.2 to 3.2)			1.7 (0.9 to 3.4)

*Frequencies based on total number shown except as follows. In African Americans: IL1α +4845 based on 143 patients and IL1β +3953 based on 72 controls. In whites: IL1β +3953 based on 201 controls; †OR (odds ratios) and 95% CI (confidence intervals) were derived from logistic regression models comparing the homozygous wild-type genotype with other genotypes. Values are derived from single locus models except where shown for the five locus models. Odds ratios and confidence intervals are not presented for genotypes with fewer than three cases or controls; ‡χ^2 statistic shown. Fisher's exact statistic also calculated for IL1Ra (p = 0.01 in African Americans and p = 0.61 in whites), and for IL1α +4845 (p = 0.33) and IL1β +3953 (p = 0.28) in African Americans.

Table 2 Frequency of estimated haplotypes including five polymorphic loci within the IL1gene cluster in African-American and white CLU study participants*

Estimated haplotypes					Percentage			
IL1α		IL1β		IL1Ra	African Americans		Whites	
−889	+4845	−511	+3953	VNTR	Cases	Controls	Cases	Controls
C	G	T	C	1	32	27	16	19
C	G	C	C	1	20	24	30	28
C	G	C	T	1	0	0	5	1
C	G	T	C	2	1	1	8	4
T	G	T	C	1	12	13	2	2
T	T	C	T	1	3	0	15	16
T	G	C	C	1	8	12	2	5
T	G	T	T	1	2	5	0	0
T	T	C	C	1	5	6	6	6

*Estimated haplotypes shown if carried by at least 5% of participants in either white or African-American cases or controls.

carriage of IL1Ra allele 3, which was significantly less common in African-American cases than in controls (exact $p = 0.006$) and inversely associated with SLE (OR = 0.1, 95% CI 0.0 to 0.7). Variation at IL1Ra was not significantly associated with SLE in whites, though IL1Ra allele 2 was more common in patients with SLE than controls (OR = 1.7, $p = 0.102$).

Table 2 shows the frequency of estimated haplotypes. There was little evidence of linkage disequilibrium among the five loci in African Americans except between IL1α −889 and +4845 in both cases and controls (controls, $p<0.005$; cases, $p<0.0001$). In whites, there was significant linkage disequilibrium for most pairwise comparisons.

None of the genotypes were independently associated with proteinuria, although IL1Ra allele 2 was non-significantly raised in white cases with proteinuria (25% *v* 9%; OR = 3.3, $p = 0.077$). In African Americans no interaction was seen between IL1α −889 and IL1β −511 genotypes for overall risk of SLE ($p = 0.938$), but a highly significant interaction ($p = 0.002$) was seen for risk of proteinuria. Paradoxically, the combined IL1α −889 C/C, IL1β −511T genotype was positively associated with risk of SLE (OR = 2.4, 95% CI 1.2 to 5.1; $p = 0.019$), but inversely associated with proteinuria (OR = 0.4, 95% CI 0.2 to 0.9; $p = 0.027$).

DISCUSSION

As far as we know this is the first study to simultaneously examine these five loci with respect to SLE and to report an association between allelic variation at IL1α −889 and SLE. In both African-Americans and whites, the IL1α −889C/C genotype was associated with a threefold higher risk of SLE than carriage of the T allele. The IL1α −889C/C genotype has been associated with other inflammatory and autoimmune diseases, including scleroderma.[12] In African Americans, carriage of the IL1β −511T allele was also associated with increased risk of SLE, even after adjusting for variation at IL1α −889. A previous study in China reported no association between this locus and SLE.[7] We did not observe a dose effect for the number of copies of the IL1α −889T or IL1β −511T alleles. However, repeating this study with a larger sample size would provide greater power to detect such a relationship.

These two IL1 promoter polymorphisms may affect IL1 production. The IL1α −889C/C genotype has been associated with significantly lower transcriptional activity of the IL1α gene and lower levels of IL1α in plasma compared with the T/T genotype.[13] Variation in IL1α may also affect the production of IL1β: in healthy Finnish subjects, plasma IL1β was lower in those with IL1α −889C/C than in those with the T/T genotype.[14] The IL1β −511 polymorphism, linked to the IL1β −31 TATA box polymorphism that affects DNA-protein interactions in vitro,[15] may reflect potential for altered levels of gene expression. There has been little indication of an effect of IL1β −511, however, on IL1β levels in vivo.[14]

Our results do not support the hypothesis that IL1Ra allele 2 affects the risk of SLE. However, our ability to detect an association might be limited by the low frequency of this allele in our study group, especially among African Americans. The inconsistent findings for this locus might also be due to differences in linkage disequilibrium with other relevant loci in the IL1 gene cluster.[9 10] IL1Ra allele 2 has been associated with increased production of IL1Ra and IL1β, and decreased production of IL1α,[16] suggesting that these genes should be studied collectively.

We considered these analyses to be useful for generating a hypothesis and did not adjust for multiple comparisons. However, a post hoc analysis yielded a highly significant ($p<0.0001$) overall association between IL1α −889C/C and SLE that remained significant ($p<0.0005$) after Boneferroni adjustment. Our confidence in the association between the IL1α −889C/C genotype and SLE is increased by the similar magnitude of effect in African-Americans and whites despite different racial patterns of linkage disequilibrium across the IL1 gene cluster. Population based sampling of controls can help to minimise the effects of selection bias and population stratification. The proportion of African-American controls in this sample reflected the racial distribution in the study area based on census estimates, resulting in fewer African-American controls than white controls or African-American cases. None the less, the observed associations in African Americans were statistically significant.

In conclusion, polymorphisms in two IL1 gene promoter regions were significantly associated with SLE in this study sample. Variation at both loci may affect IL1 and IL1Ra production, supporting the hypothesis that altered or imbalanced IL1 production may affect the risk of developing SLE.

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