Genome Screening in Human Systemic Lupus Erythematosus: Results from a Second Minnesota Cohort and Combined Analyses of 187 Sib-Pair Families

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Summary

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by a loss of immunologic tolerance to a multitude of self-antigens. Epidemiological data suggest an important role for genes in the etiology of lupus, and previous genetic studies have implicated the HLA locus, complement genes, and low-affinity IgG (Fcγ) receptors in SLE pathogenesis. In an effort to identify new susceptibility loci for SLE, we recently reported the results of a genomewide microsatellite marker screen in 105 SLE sib-pair families. By using nonparametric methods, evidence for linkage was found in four intervals: 6p11-21 (near the HLA), 16q13, 14q21-23, and 20p12.3 (LOD scores ≥2.0), and weaker evidence in another nine regions. We now report the results of a second complete genome screen in a new cohort of 82 SLE sib-pair families. In the cohort 2 screen, the four best intervals were 7p22 (LOD score 2.87), 7q21 (LOD score 2.40), 10p13 (LOD score 2.24), and 7q36 (LOD score 2.15). Eight additional intervals were identified with LOD scores in the range 1.00–1.67. A combined analysis of MN cohorts 1 and 2 (187 sib-pair families) showed that markers in 6p11-p21 (D6S426, LOD score 4.19) and 16q13 (D16S415, LOD score 3.85) met the criteria for significant linkage. Three intervals (2p15, 7q36, and 1q42) had LOD scores in the range 1.92–2.06, and another 13 intervals had LOD scores in the range of 1.00–1.78 in the combined sample. These data, together with other available gene mapping results in SLE, are beginning to allow a prioritization of genomic intervals for gene discovery efforts in human SLE.

Introduction

Systemic lupus erythematosus (SLE [MIM 152700]) is an autoimmune inflammatory disease that can affect diverse organ systems, including the skin, joints, brain, lungs, and kidneys. The disease is characterized by immune dysregulation, leading to high-level autoantibody production, immune complex deposition, and vasculitis (Rothfield 1985). SLE most commonly presents in women (F:M ratio 9:1) in their childbearing years, and the overall estimated prevalence in the United States is ~40 cases/100,000 individuals (Hochberg 1997*a*).

Epidemiologic studies suggest a strong genetic component for susceptibility to SLE. Twin studies show an overall 10-fold greater risk for disease concordance in MZ, compared with DZ, twins (Block et al. 1975; Deapen et al. 1992), and familial aggregation studies indicate a sibling risk ratio (λ_S [Risch 1990]) of at least 10 (Vyse and Todd 1996). The genetic hypothesis in SLE is also supported by the high incidence of SLE in patients with certain complement deficiencies (C1q, C2, and C4) and associations of disease and autoantibody production with HLA class II alleles (Arnett 1997). Polymorphisms in low-affinity IgG (Fcγ) receptors, which are important for the clearance of immune complexes, are also implicated in the pathogenesis of lupus (Salmon et al. 1996; Wu et al. 1997).

As one approach to understanding the genetic basis for susceptibility to SLE, a number of groups have initiated genomewide marker screens in human SLE (Gaffney et al. 1998; Moser et al. 1998; Shai et al. 1999). Our group at Minnesota has focused on collecting SLE sib-pair families (Kearns et al. 1998), and we recently reported the results of a 341-marker screen in 105 families (MN cohort 1 [Gaffney et al. 1998]). By using nonparametric multipoint methods, we identified 25 markers with LOD scores >1.0. Sixteen of these markers clustered into four distinct intervals: 6p11-p21 (near the HLA region), 16q13, 14q21-q23, and 20p12. These in-

tervals had LOD scores >2.00, and nine additional chromosomal regions were identified with LOD scores >1.00.

Because it appears likely that SLE, like many complex genetic diseases, exhibits significant genetic heterogeneity, we have continued to identify additional families suitable for mapping. Herein, we report the results of a genomewide marker screen in 82 new SLE sib-pair families (MN cohort 2). Overall, the data from this screen support the evidence for linkage in several of the previously reported potential susceptibility intervals. In addition, several new intervals that showed only weak evidence for linkage in the cohort 1 screen were identified in the present study. A combined analysis of MN cohorts 1 and 2, together with supportive data from other published screens in SLE, allow us now to focus our attention on discrete genomic intervals that are likely to harbor human SLE susceptibility genes.

Families and Methods

SLE Families

The collection of affected sib-pair families for this study has been described in detail elsewhere (Kearns et al. 1998). Families were recruited from all regions of the United States and Canada, and all patients met the 1997 revised criteria for the diagnosis of SLE (Tan et al. 1982; Hochberg 1997*b*). After informed consent was obtained from the patients, verification of the SLE diagnosis was performed by a review of the patients' medical records and an interview with the patients' physicians. All available parents were recruited (5 affected) to facilitate the analysis of identity-by-descent (IBD) allele sharing. If parents were not available, an unaffected sibling was recruited to assist in the reconstruction of parental genotypes. Since our recent report (Gaffney et al. 1998), we have obtained additional information for three of the cohort 1 pedigrees. The affected sisters in one family were determined to be half-sibs on the basis of statistics generated by using the RELATIVE computer algorithm (Goring and Ott 1997). In a second family, a probable patient with SLE who had been collected and genotyped was verified as definitely having SLE, whereas a previously verified sib with SLE in the same family was determined not to meet the criteria. Finally, 2 parental samples from cohort 1 were collected and analyzed in the cohort 2 screen. This additional information altered the calculated LOD scores slightly in the current analysis of cohort 1, compared with the previously reported values (Gaffney et al. 1998). All family relationships in both cohorts 1 and 2 were confirmed by use of RELATIVE (Goring and Ott 1997). This study was approved by the human subjects review board of the University of Minnesota.

Samples and Genotyping

Genomic DNA was isolated from peripheral blood mononuclear cells by use of standard methods, and genotyping of families was done essentially as described elsewhere (Gaffney et al. 1998). For cohort 2, an Applied Biosystems, Inc. (ABI) fluorescently labeled human linkage mapping set (v2.0) was used and optimized for multiplexing markers of similar color into one or two reaction cocktails. PCR (32 cycles) was done on an ABI 877 Catalyst robotic workstation (5-μl reactions: 5 ng of genomic DNA, 2.5 mM MgCl$_2$, 0.2 mM dNTPs [Pharmacia], 0.2 U Amplitaq Gold DNA polymerase [PE Biosystems] in 1 × PCR Buffer II [PE Biosystems]). Individual primer-pair concentrations were in the range of 0.31–3.30 pmol/reaction, on the basis of the results of optimization runs.

Pooled amplification products were electrophoresed through 5% polyacrylamide gels (FMC Bioproducts) for 2 h at 3000 V on an ABI 377 DNA Sequencer. Semiautomated fragment sizing was done by use of GENESCAN software (v2.1; ABI), followed by allele calling with GENOTYPER software (v2.0; ABI). Each genotype was reviewed manually by 2 members of the research team to confirm the accuracy of allele calling. Poorly performing markers (a total of 34 markers randomly distributed across the genome) were excluded from the analysis. The overall data dropout rate for the ~102,000 genotypes analyzed was ~1%.

Data Analysis

Data analysis was done with GENEHUNTER PLUS (Kong and Cox 1997), a modified version of GENEHUNTER (Kruglyak et al. 1996). This software performs multipoint nonparametric linkage analysis by extracting IBD allele-sharing information among all affected family members at each location in the genome. It then derives a nonparametric linkage score (NPL for GENEHUNTER, Z_{lr} for GENEHUNTER PLUS) on the basis of the number of affected individuals sharing the same marker allele IBD. We chose the S_{all} scoring function, to allow comparison of observed IBD allele sharing among all affected family members (not just affected sib pairs) with that expected under the null hypothesis of no linkage. LOD scores were generated by the GENEHUNTER PLUS software by use of the equation LOD = $Z_{lr}^2/2 \ln 10$ for each cohort individually and as a combined set. Families of various ethnic groups were similarly extracted from the master linkage file and analyzed separately. Allele frequencies used in the parameter files for each analysis were generated from the founder genotypes for that data set (cohort 1, cohort 2, combined cohorts, individual ethnic groups). Marker-map positions were obtained from the latest available

Table 1

Composition of 187 SLE Sib-Pair Families by Cohort

	Cohort 1	Cohort 2	Total
No. of sib-pair families	105	82[a]	187
No. of affected sib pairs	114	93	207
No. of affected relative pairs	127	111	238
No. of affected SLE individuals	220	179	399
No. of unaffected parents and sibs	155	101	256

[a] Includes one family with affected first cousins only.

sex-averaged maps compiled by J. Weber (Center for Medical Genetics, Marshfield Medical Research Foundation).

Results

Demographic and Clinical Features of MN Cohort 2

Minnesota SLE cohort 2 consists of 82 families (75 families with 2 SLE sibs, 6 families with 3 SLE sibs, and 1 family with 2 affected first cousins; table 1). Within the cohort there were 93 affected-sib pairs and 111 total affected-relative pairs. There were some differences in the composition of the cohort 2 and cohort 1 families. In addition to being a smaller collection, cohort 2 had fewer parental or unaffected sib samples available than did cohort 1. Whereas only 1 of the 105 families in cohort 1 was an isolated sib pair (no parents or unaffected sibs), there were 14 such families in the cohort 2 sample of 82 families. There were also fewer total available unaffected sibs in cohort 2. The ratio of the number of unaffected parents or sibs divided by the number of families was 1.23 for cohort 2 (101/82), compared with 1.47 for cohort 1 (155/105). The number of half-sib families was comparable between the two groups (four in cohort 2, five in cohort 1).

The clinical and demographic features of cohort 2 are presented in table 2 and compared with those of cohort 1. Cohort 2 patients with SLE were slightly older at the time of diagnosis (34 ± 11 vs. 31 ± 11 years) and had disease for a modestly longer period of time (15 ± 9 vs. 12 ± 7 years), compared with cohort 1. Both cohorts are composed of ∼80% white pedigrees. Of the nonwhite families, there was a higher percentage of black pedigrees in cohort 2. The only differences in clinical features between the cohorts were a slightly lower incidence of hematologic manifestations of SLE (leukopenia, autoimmune hemolytic anemia, lymphopenia, and thrombocytopenia) and a higher percentage of patients treated with corticosteroids, anti-malarial, and cytotoxic medications in cohort 2, compared with cohort 1 (table 2). None of these differences reached statistical significance ($P < .05$).

Genome Screen of MN Cohort 2

The cohort 2 pedigrees were genotyped with 366 dinucleotide repeat microsatellite markers (ABI linkage panel, version 2.0), at an average intermarker distance of 8.9 cM across the 22 autosomes. The data were compiled and multipoint nonparametric analysis was done by use of GENEHUNTER PLUS (Kong and Cox 1997). A nominal LOD score threshold of 1.0 was chosen. Twenty-nine positive markers were identified that fell into 12 discrete chromosomal regions (table 3 and fig. 1). Markers in three of the intervals met criteria for suggestive linkage (LOD score >2.2), as defined by Lander and Kruglyak (1995): 7p22 (D7S517, LOD score 2.87), 7q21 (D7S669, LOD score 2.40), and 10p13 (D10S548, LOD score 2.24). The remaining nine intervals contained markers with LOD scores in the range

Table 2

Clinical and Demographic Features of 399 Patients with SLE by Cohort

Characteristic	Cohort 1	Cohort 2
Age at diagnosis ± SD (years)	31 ± 11	34 ± 11
Duration of disease ± SD (years)	12 ± 7	15 ± 9
Sex (F:M)	219:1	175:4
Ethnicity (%):		
White	80	78
Hispanic	8	6
Black	5	15
Asian	3	0
Mixed heritage	4	1
Laboratory/clinical features (%):[a]		
ANA positive[b]	98	98
Anti-dsDNA positive[c]	49	42
Arthritis	86	84
Skin involvement	91	87
Pleuritis	54	54
Hematologic	49	40
Renal disease	31	30
CNS lupus	13	15
Pericarditis	18	20
>1 Miscarriage	10	6
Medication history (%):[a]		
Corticosteroids	76	85
Antimalarials	65	72
Cytotoxic drugs	28	36
Intravenous steroids	19	21

NOTE.—There were no significant differences in any of these variables, between cohorts 1 and 2, with use of generalized estimating equations to adjust for the intrafamilial correlation (Zeger and Liang 1986).

[a] Data are percentage of patients with SLE who have the features at any time during the course of their disease.

[b] ANA, antinuclear antibodies. All ANA-negative patients were anti-dsDNA positive and otherwise fulfilled criteria for SLE.

[c] dsDNA, double-stranded DNA; CNS, central nervous system.

1.02–2.15 (table 3). None of the markers in the cohort 2 sample met Lander criteria for significant linkage (LOD score >3.6).

Combined Analyses of Marker Data from MN Cohorts 1 and 2

We next combined the marker data from cohorts 2 and 1 to determine the overall level of support for linkage in the best intervals identified in the present cohort 2 study and in the previous cohort 1 study (Gaffney et al. 1998). The markers for chromosomes 14–22 (102 in total) were identical for the two cohorts (ABI linkage panel v2.0). Because chromosomes 1–13 in cohort 1 were genotyped with an earlier version of the ABI linkage panel (v1.0), only 69% of the chromosome 1–13 markers were the same between the two cohorts (181/264). Allele frequencies for the parameter files were calculated by combining the parental genotype data from all families. The combined data set represents ~230,000 genotypes from 187 SLE families, for a total of 207 affected sib pairs and 238 affected relative pairs (table 1).

The results of the combined multipoint analysis are shown in figure 1 and summarized in table 4. Evidence for linkage at 6p11-p21 and 16q13, the two intervals with the highest LOD scores in the original cohort 1 study, remained strong in the combined analyses. In the cohort 2 screen, the best marker in the 6p11-p21 interval was D6S276 (LOD score 1.48). This marker lies ~16 cM telomeric of D6S426, the peak marker in the region from the cohort 1 screen (LOD score 4.16). In the combined analysis, the evidence for linkage at D6S426, which lies just centromeric of the HLA region on 6p, showed only a slight increase compared with the results in cohort 1 alone, with an overall LOD score of 4.19. The evidence for linkage at 16q13 strengthened at marker D16S415 when both cohorts were considered, with a combined LOD score of 3.85, compared with a LOD score of 3.47 from cohort 1 families alone. Both the 6p11-p21 and 16q13 intervals exceed the threshold LOD score >3.6 suggested by Lander and Kruglyak (1995) for significant linkage in the combined sample.

Three of the weaker intervals identified in the cohort 1 screen (LOD scores in the range 1.0–2.0) showed an increase in LOD score >0.5 in the combined analysis compared with the cohort 1 alone results: 2p15 (D2S337, LOD score 2.06), 1q42 (D1S235, LOD score 1.92), and 11p15 (D11S922, LOD score 1.60). Of the two chromosome 20 intervals previously identified in the cohort 1 screen, evidence for linkage in the combined analysis of all families dropped at 20p12 (to LOD score 1.77 at marker D20S186), and rose slightly at 20q12 (to LOD score 1.64 at marker D20S119). Cohort 2 showed essentially no evidence for linkage at 14q21-23,

Table 3

Minnesota SLE Cohort 2 Genome Mapping Results

Interval[a] and Cohort 2 LOD Score[b]	Positive Markers	Map Position[c] (cM)	Information Content[d]
7p22:			
2.37	D7S531	.10	.76
2.87	D7S517	2.16	.77
2.07	D7S513	10.40	.75
1.46	D7S507	23.46	.76
7q21:			
1.51	D7S502	75.72	.67
2.40	D7S669	88.35	.72
1.90	D7S630	93.16	.75
1.05	D7S657	99.58	.73
10p13:			
1.74	D10S1653	39.65	.64
2.24	D10S548	46.24	.62
2.04	D10S197	50.04	.69
1.55	D10S208	56.45	.70
7q36:			
1.50	D7S636	157.05	.82
2.15	D7S798	165.95	.77
1.41	D7S2465	174.96	.67
2p23:			
1.14	D2S162	15.20	.74
1.46	D2S168	24.19	.75
1.67	D2S305	34.74	.72
1.47	D2S165	39.83	.75
6p22.3-p21.3:			
1.06	D6S422	28.43	.74
1.48	D6S276	35.47	.67
2q35:			
1.25	D2S325	201.06	.74
1.45	D2S126	212.44	.72
16p13:			
1.21	D16S404	7.15	.70
18p11.23:			
1.03	D18S63	10.59	.71
1.19	D18S452	16.92	.77
1p36:			
1.06	D1S468	.00	.60
8q23:			
1.05	D8S1784	114.81	.59
4q32:			
1.02	D4S413	162.84	.68

[a] Chromosome locations were determined on the basis of data from the Genome Database.

[b] LOD = $Z_{lr}^2/2 \ln 10$ (Kong and Cox 1997).

[c] Map positions were from the Center for Medical Genetics, Marshfield Medical Research Foundation database.

[d] Information content for the marker designated in each interval (Kruglyak et al. 1996). The average information content for all the markers in cohort 2 was .69.

one of the better intervals identified in cohort 1, and in the combined sample, only a single marker (D14S65, LOD score 1.20) on chromosome 14 demonstrated a LOD score >1.0.

Of the four strongest LOD scores obtained in the cohort 2 analysis alone (7p22, 7q21, 10p13, and 7q36),
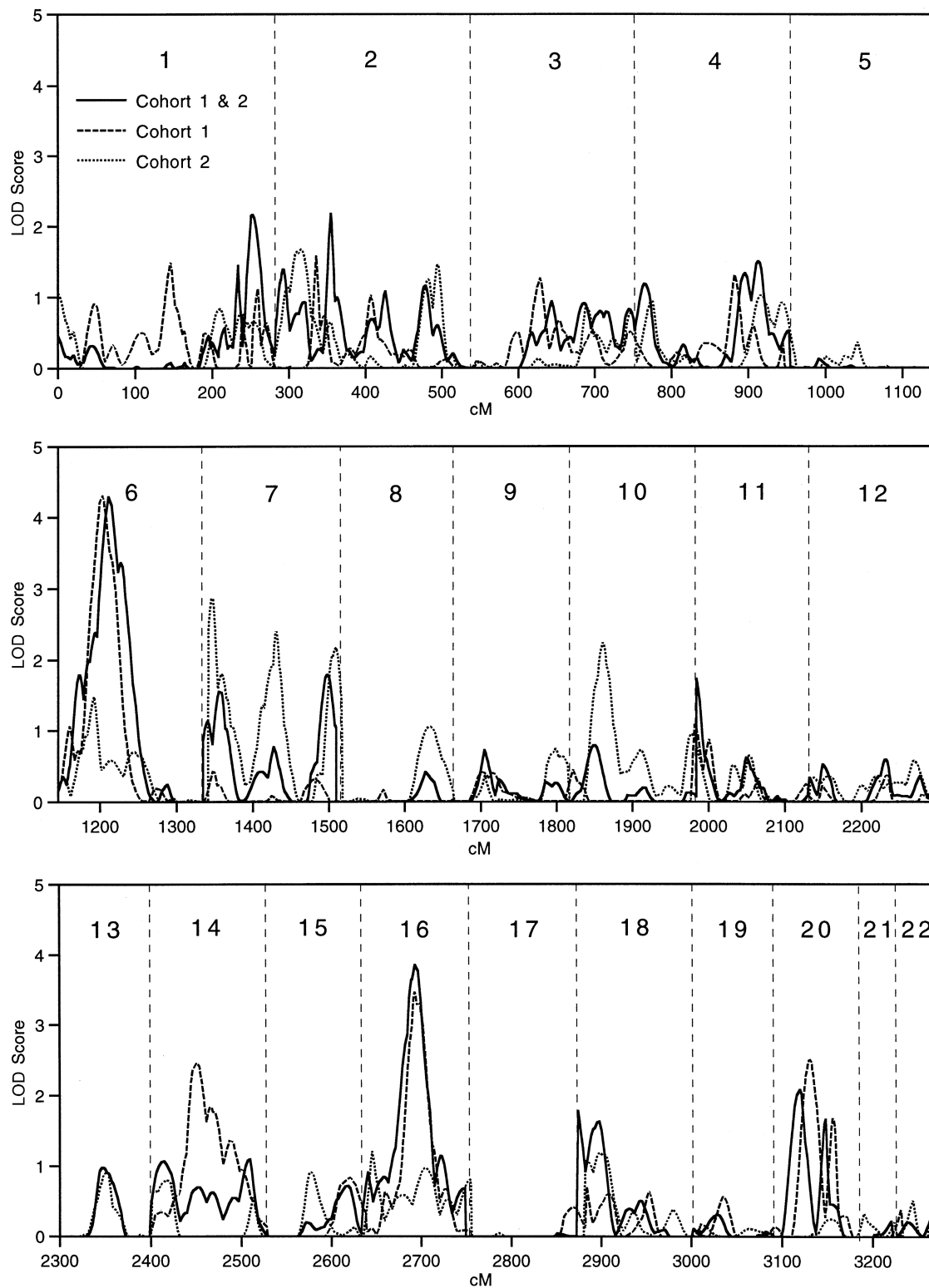
Figure 1   Nonparametric multipoint linkage analysis in 187 sib-pair families. Shown are the LOD-score values for 82 cohort 2 families (*dotted line*), 105 cohort 1 families (*dashed line*), and the combined cohorts (187 families; *solid line*) across the 22 autosomes. The data were generated by use of GENEHUNTER PLUS (Kong and Cox 1997) and represent 366 polymorphic markers at an average intermarker interval of 8.9 cM.

Table 4

Combined Mapping Results for Minnesota SLE Cohorts 1 and 2

	LOD Score[a,b]			Positive Marker	Map Position[c] (cM)	Information Content
Interval	Cohort 1	Cohort 2	Combined			
6p11-p21	.96	.74	1.68	D6S289	28.53	.78
	.50	.93	1.35	D6S422	34.26	.80
	.65	1.48	1.93	D6S276	43.01	.73
	...	.65	2.38	D6S1610	50.53	.60
	4.16	...	4.19	D6S426	59.04	.60
	3.34	.43	3.10	D6S257	78.52	.76
	...	.67	1.94	D6S460	95.77	.54
	.71	.65	1.34	D6S462	101.68	.54
16q13	.74	.57	1.21	D16S3068	36.59	.77
	2.99	.44	2.86	D16S3136	51.75	.71
	3.47	.72	3.85	D16S415	60.45	.69
	1.31	.82	2.14	D16S503	73.19	.74
2p15	1.44	.65	2.06	D2S337	73.09	.82
7q36	.47	1.50	1.69	D7S636	157.05	.82
	...	2.04	2.06	D7S798	162.37	.61
1q42	...	.65	1.56	D1S2800	247.90	.75
	.68	...	1.79	D1S446	249.41	.72
	1.38	...	1.92	D1S235	252.75	.68
	...	.62	1.06	D1S2785	264.93	.46
18p11	.85	1.08	1.78	D18S59	.10	.74
	.47	1.19	1.63	D18S452	23.69	.80
20p12	.93	.25	1.33	D20S115	22.78	.70
	2.03	.21	1.77	D20S186	30.86	.76
	1.97	.02	1.11	D20S112	36.42	.78
20q12	1.59	.33	1.64	D20S119	58.94	.80
11p15	.94	...	1.60	D11S922	.10	.73
	...	.64	1.60	D11S4046	1.00	.72
4q32-q33	1.30	.59	1.33	D4S424	145.19	.72
	.59	1.02	1.50	D4S1597	162.39	.66
2p24	.55	.99	1.39	D2S162	10.66	.79
1q41	.92	...	1.33	D1S229	233.51	.73
4p15	.23	.94	1.31	D4S403	16.38	.67
7p22	.26	1.46	1.30	D7S507	24.65	.80
	.16	1.20	1.07	D7S493	28.22	.84
14q32	.64	.56	1.20	D14S65	110.84	.71
2q34	.33	1.16	1.16	D2S325	195.29	.79
2q21-q33	1.03	...	1.14	D2S151	144.44	.59
13q31	.16	.91	1.02	D13S170	54.65	.82

[a] An ellipsis (...) indicates that the marker was not run in that cohort.

[b] Data are as described in table 3.

[c] Position of the highest LOD score for the combined data set, as calculated by GENE-HUNTER PLUS. The nearest marker to each designated map position is shown.

only 7q36 (D7S798, LOD score 2.06) and 7p22 (D7S507, LOD score 1.30) showed LOD scores >1.0 in the combined pedigree collection.

Partitioning of the Sample by Ethnicity

Previous genetic epidemiology studies in SLE (reviewed in Hochberg 1997*a*), as well as the recent mapping studies (Gaffney et al. 1998; Moser et al. 1998; Shai et al. 1999; Tsao et al. 1999), indicate that the genetic susceptibility underlying SLE may differ between racial groups. Thus, we partitioned the data sets by ethnicity. We first examined the group of European-white pedigrees (65 families) within cohort 2, and compared

the results with the entire 82-family sample (table 5, top). Although there were minor changes in LOD scores in all identified intervals, six regions demonstrated a change in LOD score >0.5 LOD units. Notably, the LOD score in the 7q21 region, one of the strongest intervals identified in the cohort 2 screen, dropped to a LOD score of 1.45 from 2.40.

Next, we partitioned the combined data set (cohorts 1 and 2) and analyzed the evidence for linkage in the white pedigrees (149 families) and compared these results with those of the entire sample (table 5, bottom). Once again, the partitioned data showed minor changes in LOD scores across the genome. Six intervals showed

Table 5

Mapping Results in White Pedigrees Compared with Entire Family Collection[a]

| | | LOD Score in Cohort 2 | | |
| | POSITION | All Families[b] | White Families[b] | |
INTERVAL	(cM)	(N = 82)	(N = 65)	Difference
7q21	88.35	2.40	1.45	−.95
2q35	212.44	1.45	.56	−.88
1p36	.10	1.06	.51	−.55
4p15	16.65	.89	1.52	+.62
3q24	152.55	.85	1.39	+.54
12q24	135.54	.58	1.09	+.52
		LOD Score in Cohorts 1 and 2		
		All Families	White Families	
		(N = 187)	(N = 149)	Difference
6p11-p21	66.83	4.19	2.87	−1.32
16q13	60.45	3.85	3.22	−.63
1q42	252.75	1.92	1.30	−.63
14q32	110.84	1.20	.44	−.77
2q21-q33	144.44	1.14	.59	−.55
4p16	11.61	1.10	1.63	+.53
7p22	6.28	1.04	1.84	+.80
13q31	54.65	1.02	.41	−.61
3q24	149.59	.81	1.90	+1.09
16p13	21.71	.71	1.50	+.79
11p15	10.81	.68	1.75	+1.07

[a] Only those regions with a difference >0.5 LOD units (white vs. all pedigrees) are shown.

[b] N = number of families for each analysis.

a LOD score drop >0.5 units when only the white families were considered: 6p11-21 (LOD score 2.87, −1.32 LOD units), 16p13 (LOD score 3.22, −0.63 LOD units), 1q42 (LOD score 1.30, −0.63 LOD units), 14q32 (LOD score 0.44, −0.77 LOD units), 2q21-33 (LOD score 0.59, −0.55 LOD units), and 13q31 (LOD score .41, −0.61 LOD units). Our interpretation of these LOD score drops is that pedigrees from all ethnic backgrounds contribute to the evidence for linkage in these regions. Conversely, LOD score increases were observed at 4p16, 7p22, 3q24, 16p13, and 11p15, suggesting the possibility of genetic effects enriched within the white sample.

Finally, we also performed a separate genomewide multipoint analysis for the 17 available black families and for the 13 Hispanic families. Despite the small number of families, D2S325 at 2q34 exhibited a LOD score of 1.1 in the black subgroup, whereas the Hispanic collection demonstrated LOD scores of 1.45 at 13q32 (marker D13S159), 1.34 at 1q42 (marker D1S235), and 1.18 at 19q12 (marker D19S414).

Discussion

In this report, we present the results of a genomewide marker screen in Minnesota cohort 2, comprising 82 new SLE sib-pair families. When the data from cohort 2 were analyzed separately, the chromosomal regions with the strongest evidence for linkage were 10p13 and three distinct intervals on chromosome 7. Eight additional regions showed LOD scores >1.00. In the combined data set of both cohorts 1 and 2, the cohort 2 families provided additional evidence for linkage in four of the intervals identified in the previous cohort 1 screen: 16q13, 2p15, 1q42, and 11p15. LOD score increases in these regions were in the range 0.38–0.66 in the combined data set (187 families), compared with cohort 1 alone (105 families). In the combined data set, evidence for linkage at 6p11-p21, near HLA, did not change significantly and remained strong, whereas scores at 14q21-23, one of the better intervals identified in cohort 1, dropped sharply.

Not surprisingly, the mapping results between the two independent MN cohorts were not entirely analogous. Although many factors may contribute to the differences observed, an important issue is the family composition of the second cohort. Cohort 2 contained a smaller number of families than did cohort 1 (82 and 105, respectively), and 14 of the 82 cohort 2 families were isolated sib pairs (neither parents nor unaffected sibs were available), whereas there was only one such family in cohort 1. There were also fewer parents and unaffected siblings available for the second cohort. Thus, cohort 2, overall, has less power to detect linkage than does the cohort 1 collection.

Another consideration is that the markers used for the two screens were not identical. Although the same markers were used for chromosomes 14–22 in both screens, there was only 69% overlap of the markers for chromosomes 1–13 because of differences in the available screening marker sets. Because evidence for linkage can extinguish rapidly as one moves away from a region of interest (Kruglyak 1999), we may have missed evidence for linkage in either cohort on the basis of the markers used for the analysis and the relatively large distances between markers (~9 cM).

Another potential explanation for differences between the two screens is that there is almost certainly significant genetic heterogeneity in human SLE, similar to that suspected in many complex genetic disorders. At least some of the heterogeneity can be attributed to differences between ethnic groups (see table 5 and Moser et al. 1998; Shai et al. 1999). Finally, it is possible that random variation may account for some of the differences we have found. Overall, the data presented underscore the presumed complexity of the genetics in SLE.

Perhaps the strongest supportive evidence for a genuine susceptibility locus in any complex disease, short of actually isolating the gene, is the independent replication of linkage results in independent family collections. This was one of the aims of the present study. However, in addition to the work performed by our group, there are now available two additional published

genome screens in human SLE (Moser et al. 1998; Shai et al. 1999), as well as a targeted marker analysis of distal chromosome 1q (Tsao et al. 1997, 1999), to compare with our results.

Moser et al. at Oklahoma Medical Research Foundation studied 94 multiplex SLE families by using primarily parametric analytical methods and a nominal threshold of LOD >1.5 (Moser et al. 1998). Potential susceptibility loci were identified on 1q23, 1q31, 13q32, and 20q13 in the combined pedigree collection. When the data were stratified by race, additional loci at 1q41 and 11q14-23 were identified in black families, which comprised about a third of the sample. In European-white families, the strongest evidence for linkage was at 14q11, 4p15, 11q25, 2q32, 19q13, 6q26-27, and 12p12-11. Shai et al. at the University of Southern California (USC) performed a genomewide screen in 80 multiplex SLE families (Shai et al. 1999). The two strongest intervals identified in this collection by GENEHUNTER multipoint analyses were 1q44 and 18q21. Significant scores were also obtained at 1p36, 1p21, 1q24, 6p22, 14q23, 16q13, 20p13, and 20q11. Finally, in a targeted study of distal chromosome 1q, Tsao et al. reported evidence for an SLE susceptibility gene in the region of 1q42 (Tsao et al. 1997, 1999).

Although there are significant differences between the reported mapping studies in SLE in terms of family structures, ethnicity, and analytical techniques used, there is an encouraging level of agreement in the mapping results. Table 6 provides a summary of potential SLE susceptibility loci identified in two or more independent screens. These 12 intervals were defined as follows: (1) LOD score >1.0 or NPL score >1.5 and (2) intermarker distance ≤25 cM. Particularly striking were the results from the Minnesota cohort 1 and USC screens. Seven of the 10 regions identified by Shai et al. (1999) were among the 13 top intervals identified in Minnesota cohort 1, and 5 of the best 10 intervals from the combined Minnesota analyses were supported by the USC study. These similarities may reflect the fact that both groups studied predominantly sib-pair families using similar marker panels and that both data sets were analyzed with allele-sharing methods.

Remarkably, the interval at 1q42 (around marker D1S229) shows evidence for linkage in all of the SLE mapping studies reported to date. This interval was originally identified by Tsao et al. (1997), and poly-ADP ribosyl transferase (PARP) was recently suggested as the relevant gene in the region on the basis of strong TDT scores with a polymorphic marker in the 5′ region of the gene (Tsao et al. 1999). Although recent results in our family collection do not support PARP as being the specific gene of interest (unpublished data), this region of the genome will continue to be a focus for future investigations.

Significant evidence for linkage in our combined cohort analysis was also found over a broad interval spanning 6p11-p21, with the best markers (D6S426 and D6S257) mapping just centromeric to the HLA region (located at 6p21.3). Evidence for linkage in this region was also obtained in the USC study, with marker D6S276 (14 cM telomeric to D6S426) giving a LOD score of 1.60. This same marker achieved a LOD score of 1.48 in our cohort 2 screen. The HLA region has long been implicated as a susceptibility locus in SLE (Arnett 1997), as well as in many other autoimmune diseases,

Table 6

Proposed Human SLE Susceptibility Loci Identified in Two or More Mapping Studies

Locus	Gaffney Cohort 1	Gaffney Cohort 2	Moser	Shai	Tsao[a]	Maximum Marker Interval[b] (cM)
1p36	D1S234	D1S468		D1S468		24
1q23			FcγRIIA	D1S484		1
1q41-44	D1S235		D1S3462	D1S2785	D1S229	16
2q32-35		D2S126	D2S1391			17
3q11	D3S1271		D3S2406			10
4p15		D4S403[c]	D4S403			0
4q28-31	D4S424	D4S413	D4S2431			16
6p11-22		D6S426[c]		D6S276		14
14q11-23	D14S276			D14S258		21
16q12-13	D16S415			D16S3136		2
20p12-13	D20S186			D20S115		7
20q11-13	D20S119		D20S481	D20S195		4

[a] Targeted study of chromosome 1q only.

[b] Between the two most widely spaced markers showing evidence for linkage within each locus (25 cM was the maximum allowable intermarker distance). Marker locations were determined on the basis of data from maps available at the Genetic Location Database, University of Southhampton.

[c] On the basis of LOD score from combined Minnesota cohort 1 and 2 analyses.

including type 1 diabetes, psoriasis, multiple sclerosis, and rheumatoid arthritis (Nepom and Erlich 1991). It remains to be determined whether the linkage identified in this interval represents a polymorphic HLA locus or a linked gene(s) within the HLA region.

The 16q13 region identified in the Minnesota and USC cohorts is of interest because of its identification in other autoimmune diseases, including psoriasis (Nair et al. 1997), Crohn's disease (Hugot et al. 1996; Curran et al. 1998; Mirza et al. 1998; Hampe et al. 1999), Blau syndrome (Tromp et al. 1996), type 1 diabetes (Davies et al. 1994), and asthma (Daniels et al. 1996). In addition, the 20p12 region, identified in the Minnesota and USC screens, maps close to a recently identified putative susceptibility locus for psoriasis (Nair et al. 1997; Trembath et al. 1997), whereas the 20q12 locus, identified in all three SLE genome screens, maps to a potential susceptibility region for Grave's disease (Tomer et al. 1998). The clustering of different autoimmune diseases in families (Bias et al. 1986) and the apparent sharing of susceptibility loci between various autoimmune disorders (Becker et al. 1998) suggests that genes within the identified SLE intervals may be important for predisposition to autoimmunity in general.

The first wave of genome screens in human SLE, including the data reported here, support the hypothesis that multiple genes contribute to disease susceptibility. Clearly, there is no single locus operating in all families multiplex for SLE, and the degree of ethnic and genetic heterogeneity appears be quite significant. In this respect, the genetics of SLE resembles that of many other complex genetic diseases. Fine mapping and candidate gene sequencing efforts in these intervals are under way, with a goal of eventually identifying major genes predisposing to human SLE.

Acknowledgments

Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Center for Medical Genetics, Marshfield Medical Research Foundation, http://www.marshmed.org/genetics/

Genetic Location Database, University of Southhampton, http://cedar.genetics.soton.ac.uk/pub/

Genome Database, The, http://gdbwww.gdb.org

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for SLE [MIM 152700])

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