Fine-Mapping Chromosome 20 in 230 Systemic Lupus Erythematosus Sib Pair and Multiplex Families: Evidence for Genetic Epistasis with Chromosome 16q12

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The presence of systemic lupus erythematosus (SLE) susceptibility genes on chromosome 20 is suggested by the observation of genetic linkage in several independent SLE family collections. To further localize the genetic effects, we typed 59 microsatellites in the two best regions, as defined by genome screens. Genotypes were analyzed for statistical linkage and/or association with SLE, by use of a combination of nonparametric linkage methods, family-based tests of association (transmission/disequilibrium and pedigree disequilibrium tests), and haplotype-sharing statistics (haplotype runs test), in a set of 230 SLE pedigrees. Maximal evidence for linkage to SLE was to 20p12 (LOD = 2.84) and 20q13.1 (LOD = 1.64) in the white pedigrees. Subsetting families on the basis of evidence for linkage to 16q12 significantly improved the LOD scores at both chromosome 20 locations (20p12 LOD = 5.06 and 20q13 LOD = 3.65), consistent with epistasis. We then typed 162 single-nucleotide polymorphism markers across a 1.3-Mb candidate region on 20q13.1 and identified several SNPs that demonstrated significant evidence for association. These data provide additional support for linkage and association to 20p12 and 20q13.1 in SLE and further refine the intervals of interest. These data further suggest the possibility of epistatic relationships among loci within the 20q12, 20q13, and 16q12 regions in SLE families.

Systemic lupus erythematosus (SLE [MIM 152700]) is a heterogeneous, systemic autoimmune disease characterized by the production of autoantibodies to a multitude of self-antigens. SLE predominantly affects females, and prevalence rates and disease severity are increased in several nonwhite groups.[1–5] Clinical manifestations vary between individual patients, and there is evidence for complex contributions of genetic and environmental factors to SLE from population and family-based studies.[6–11] Linkage studies in human SLE pedigrees have identified multiple regions that meet genomewide criteria for significant or suggestive linkage,[12] which is consistent with the hypothesis that SLE is a complex polygenic disease.[13–26]

Our group was one of the first to provide evidence for linkage on chromosome 20 (*D20S186,* LOD = 2.62) in an initial study of 105 SLE sib pair pedigrees.[15] The subsequent addition of 82 independent pedigrees further refined the linkage signal with the emergence of two peaks, one at 20p12 (*D20S186,* LOD = 1.77) and one at 20q13.1 (*D20S119, LOD* = 1.64).[18] Evidence supporting linkage to either 20p12 or 20q13 has been identified in three additional, ethnically diverse family collections.[14,16,22] This concordance of evidence supports the hypothesis that SLE susceptibility genes lie in these regions.

In this report, we present the results of a dense fine-mapping effort on chromosome 20 that used 59 microsatellite markers distributed across 20p12 and 20q13 and 162 SNP markers localized to a 1.3-Mb region of maximal linkage on 20q13.1. These analyses were performed in an expanded collection of 230 SLE multiplex pedigrees, including 44 families not previously reported. The expanded fine-map data set refines the linkage intervals previously identified in our genome screens and provides evidence for transmission distortion with several SNP marker alleles. In addition, we apply an ordered subset analysis (OSA), conditioned on linkage to chromosome 16, that establishes evidence consistent with genetic epistasis between loci on chromosome 16 and 20 and identifies a subset of families with enhanced linkage and association to 20p12 and 20q13.1.

Material and Methods

SLE Families

Methods used for the recruitment of families have been described elsewhere.[27] The University of Minnesota Institutional

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Review Board for research on human subjects approved this study, and informed consent was obtained from all subjects.

Microsatellite and SNP Genotyping

The original genome screen marker set comprised 13 microsatellite markers from the chromosome 20 ABI Linkage Mapping Panel (v. 2.0). An additional 59 fine-map microsatellite markers demonstrating high heterozygosity (≥70%) and distributed uniformly across the regions of maximal linkage were selected from the Marshfield Clinic database. Microsatellite genotyping was performed as described elsewhere.[15]

A 1.34-Mb interval of 20q13.1, defined by the markers *rs2425688* and *rs2425817* (44237049–45580708; hg16 [Human Genome Assembly, July 2003]), was targeted for SNP genotyping. The markers were selected from a publicly available database of 711 polymorphic markers in the 1.34-Mb region of interest (1 marker/1.9 kb).[28] Assays for the Illumina Bead Lab System[29] were initially designed for 247 SNPs (1 marker/5.4 kb). To test common protein coding variation, we typed 30 "double-hit" missense SNPs (observed by at least two independent submitters to dbSNP). In addition, we selected an efficient set of 217 tagging SNPs (tSNPs) by filtering out most of the perfect and near-perfect proxies, using a pairwise r^2 method, such that each of the 711 known SNPs of the reference panel were captured by a tSNP with a minimal r^2 of 0.7 (P. de Bakker, personal communication). Genotyping was performed using an Illumina Bead Lab System[29] at the Broad Institute of M.I.T. and Harvard. One hundred sixty-two SNPs were polymorphic and passed strict quality-control filters (Hardy-Weinberg equilibrium [HWE] $P > .01$, fewer than two Mendelian errors, and >95% genotyping efficiency). Seventeen SNPs were excluded from the analysis for the following reasons: 14 were monomorphic in this sample, 2 were out of HWE, and 1 failed to genotype in 13% of the subjects. No SNPs were excluded on the basis of non-Mendelian inheritance. The two SNPs that failed HWE in both the entire and white samples did not demonstrate any significant results for the transmission/disequilibrium test (TDT), pedigree disequilibrium test (PDT), or haplotype runs test (HRT) among the white pedigrees and showed only suggestive evidence by HRT in the complete data set.

Statistical Methods

Multipoint NPL analysis.—In all pedigrees reported, each marker was examined for Mendelian inconsistencies with the use of PedCheck,[30] and sporadic problem genotypes were converted to "missing" status. Allele frequency estimates were computed using maximum-likelihood methods implemented in the software RECODE (D. Weeks, personal communication). Map distances were based on the Marshfield genetic map,[31] for the linkage analysis of the microsatellite data. The physical map based on the May 2004 freeze was used for the association and haplotype analyses of the SNP markers.

NPL regression analysis, by use of the NPL_{pairs} statistic, was computed. The NPL regression approach is a conditional logistic regression analysis in which the family-specific NPL statistics (e.g., NPL_{pairs}) at one or more loci are the predictor variables. Consider a sample of m independent pedigrees and a chromosomal region with one or more markers and a locus of interest. Let τ_i be the pedigree-specific contribution to the NPL statistic at the locus of interest. The likelihood function for a conditional logistic regression with τ_i as a predictor is

$$\mathrm{Lik}(\beta; y_i, \tau) \propto \prod_{i=1}^{m} \left[\frac{\exp\{y_i \tau_i \beta\}}{1 + \exp\{\tau_i \beta\}} \right] .$$

Here, $y_i = 1$ for all i, and β is the conditional logistic regression parameter. NPL regression tests whether the observed inheritance at a marker deviates from proportions expected under independent assortment—that is, whether allele sharing among affected relatives is greater than expected. It can be shown that the tests from this likelihood are asymptotically equivalent to Whittemore and Halpern's[32] class of tests[33–36] implemented in Genehunter.[37] Although unaffected individuals can be used to help estimate the possible inheritance vectors for that pedigree, an NPL regression analysis is an "affecteds-only" analysis. The primary advantage of the NPL regression approach is that it allows us to evaluate simultaneously, either by joint or conditional hypothesis tests, the effects of multiple loci (i.e., heterogeneity) and to test for interactions among sets of loci (e.g., epistasis). In addition, the NPL regression approach allows for tests of whether the magnitude of sharing at a locus varies by environmental or other phenotypic factors (gene-phenotype interactions). To test for an interaction between two loci, we included the two loci and their statistical interaction in the model and computed the 1-df test of the interaction coefficient. In addition, we tested for interactions between the degree of sharing (identity by descent [IBD]) at a locus and the American College of Rheumatology (ACR) criteria. The basic linkage analysis was confirmed through use of the exponential allele-sharing model implemented in Genehunter-Plus.[38] Both single-point and multipoint analyses were computed, although only multipoint results are reported.

OSA.—We computed a series of OSAs, to test whether the evidence for linkage on chromosome 16q12 influenced the evidence for linkage on chromosome 20p12 or 20q13.[39] For this analysis, a locus was selected within the 16q12 linkage interval, and the family-specific NPL_{pairs} statistics (covariate) were ranked from largest to smallest. Families were sequentially added to the linkage analyses by rank of the NPL_{pairs} statistic at the 16q12 position, and the corresponding LOD scores were computed across chromosome 20 for that family. This process was repeated until all families were added to the linkage analysis. The subset of families that yielded the largest LOD score at 20p12 or 20q13.1 was taken as the OSA-defined family subset. The statistical significance of the change in the LOD score from the complete family set (230 families) to the OSA-defined family set was evaluated by a permutation test, under the null hypothesis that the ranking of the NPL_{pairs} statistic on chromosome 16 is independent of the LOD score on chromosome 20. The resulting empirical P values for the change in the LOD scores were adjusted for all comparisons made in the respective regions of 20p12 and 20q13.1 (i.e., regionwide P value). The OSAs were computed, with subsets at 51 cM, 56 cM, 57 cM, and 62 cM on chromosome 16.

Overlap in pedigrees contributing to linkage at 20p12 and 20q13.1.—It is interesting to consider the overlap in the pedigrees contributing to the two linkage signals on chromosome

20. In the entire sample, there were 52 families (22.5%) contributing evidence for linkage to 20p12, 51 families (22.1%) contributing evidence to 20q13.1, 60 families (26%) contributing to both regions, and 67 families (29.4%) contributing to neither of these two regions. From the entire sample, we estimate that 151 of the 492 individuals (31%) had at least one recombination between the two subsetting loci on chromosome 16 (16 cM apart). The total number of recombinations was estimated to be 181 (37%); this number includes double recombinations as two recombinations. This number is slightly higher than expected, but we reexamined the genotypes for error and include data only for those individuals for whom we are confident in the allele calls. The overlap in pedigrees, on the basis of OSA, is 25 pedigrees (10.8%) contributing linkage evidence to 20p12, 20 (8.7%) to 20q13.1, 20 (8.7%) to both 20p12 and 20q13.1, and 166 (71.9%) to neither of these two regions. The 166 pedigrees not contributing evidence for linkage to either region include the pedigrees that do not link to chromosome 16. From these data, we conclude that the number of recombinations clearly generates partially independent and partially overlapping subsets of pedigrees that contribute to the respective linkage signals.

Family-based association analysis.—Each marker was tested for departures from HWE proportions with the use of a χ^2 goodness-of-fit test. To test for evidence of association in the presence of linkage, single-locus and haplotypic TDT and PDT results were computed.[40] The haplotypic PDT uses the expectation-maximization algorithm to estimate the haplotype frequencies and is generally robust to potential population stratification.

When a variant arises in a population, either through mutation or migration, it will tend to be associated with relatively few haplotypes. If that variant is either older than or younger than background haplotypes, it will, on average, tend to be associated with either shorter (older variant) or longer (younger variant) haplotypes. The HRT tests whether haplotypes transmitted to an affected offspring tend to be different in length than haplotypes not transmitted to affected offspring.[41] The form of the HRT used here weights haplotypes by the inverse of the marker-allele frequencies. The HRT results were computed using the probands from each pedigree. The statistical significance of the sum of the natural logarithms (SOL) of the similarity scores and three similarity score thresholds (>100, >10,000, and >1,000,000) are estimated under a permutation test.

Results

A total of 230 multiplex SLE families were studied—187 pedigrees that were reported elsewhere[18] and 43 newly recruited pedigrees. The clinical and demographic features of these pedigrees are summarized in table 1. In brief, the pedigrees were predominately of self-reported white descent, and SLE-affected individuals were almost exclusively female. All affected individuals met the 1997 revised ACR criteria for the diagnosis of SLE.

Table 1

Summary of Clinical and Demographic Features for SLE Family Cohorts

SLE Family Trait	Cohorts I and II	Cohort III	Total
Pedigree structure:			
No. of pedigrees	187	43	230
No. of sib pairs	207	44	251
No. of avuncular pairs	5	7	12
No. of cousin pairs	7	4	11
No. of subjects	399	94	493
Subject demographics:			
Female (%)	98.5	89.4	96.8
Ethnicity (%):			
White	79.4	75.5	78.7
African American	9.8	8.5	9.5
Other	10.8	16	11.8
ACR criteria in subjects (%):			
Malar rash	69.9	50	66.1
Discoid rash	12.3	8.5	11.6
Photosensitivity	82	52.1	76.3
Oral/nasal ulcers	56.9	43.6	54.4
Joint inflammation	79.5	73.4	78.3
Pleurisy or pericarditis	57.9	45.7	55.6
Renal disorder	30.8	38.3	32.3
Neurologic disorder	26.6	14.9	24.3
Hemolytic disorder	50.9	40.4	48.9
Immunologic disorder	56.9	55.3	56.6
Positive ANA	98.5	97.9	98.4

Chromosome 20p12

Multipoint NPL analysis performed using NPL regression for the 13 genome screen microsatellite markers provided evidence of linkage to 20p12 near *D20S186* (LOD = 2.57, P = .0006) (fig. 1). After typing additional fine-mapping microsatellite markers, the maximal evidence for linkage in this region was modestly reduced and was shifted 820 kb telomeric of the original maximum (*D20S894*, LOD = 1.90, P = .0031) (fig. 1 and table 2). In addition, the 1-LOD support interval decreased from 16 cM to 9.5 cM.

Previous analyses suggested the presence of epistasis between the loci on chromosome 20 and chromosome 16q12, a region of significant linkage in our family collection and in others (C. Langefeld, unpublished data). We sought to exploit this potential interaction as a means to identify homogeneous subsets of families with enhanced linkage on chromosome 20. To do so, a series of OSAs[39] were computed by conditioning on LOD scores obtained at 1-cM intervals from 47 cM to 67 cM across the region of maximal linkage at chromosome 16q12. Conditioning on chromosome 16 (positions 56 cM and 57 cM) yielded significant increases in linkage at 20p12 (table 3 and fig. 2*A*). Position 57 cM on chromosome 16 identified 32 pedigrees (14% of total) demonstrating maximal increase in LOD score to 5.06 at 20p12, near marker *D20S604*, which is located 1.9 Mb

centromeric to *D20S894*, the best marker in the full family set (fig. 2*A*). The increase in the LOD score to 5.06 was statistically significant ($P = .01$). These *P* values are adjusted for all possible tests via permutation methods across the entire chromosome 20. We note that, if the analysis is restricted to white females affected with SLE and the subsetting on chromosome 16q12 is at 51 cM, the LOD score around the *D20S604*/*D20S163* region, on the basis of 80% of the pedigrees, is 3.87 ($P = .03$). This result shows that the 20p12 linkage is not restricted to a small number of pedigrees—72 of the 147 pedigrees (49%) had strictly positive NPL_{pairs} statistics, and only 52 (35%) had strictly negative NPL_{pairs} statistics. Thus, these data support the conclusion that the subset of pedigrees that link to 16q12 also strongly link to 20p12.

Chromosome 20q13.1

In the 20q13.1 region, the multipoint NPL regression analysis using the 13 genome screen markers provided modest evidence for linkage near *D20S119* (LOD = 1.28, $P = .01$) for the full 230-pedigree collection (fig. 1 and table 2). Analysis of the white-only pedigrees increased the evidence for linkage to LOD = 1.64 at the same locus. The addition of 59 fine-map markers increased maximum LOD scores at the same locus (20q13, *D20S119*) to 1.49 ($P = .009$) and 2.06 ($P = .002$) in the entire and white samples, respectively. Similar to 20p12, the linkage at 20q13 appears to be predominately concentrated in the white pedigrees.

The OSA on 20q13.1 identified three positions on chromosome 16 for which the change in LOD score on 20q13.1 was significant (positions 42, 43, and 45 cM) (fig. 2*B* and table 3). A 33-pedigree subset conditioned on linkage at position 45 cM on chromosome 16 dem-

Table 2

Summary of NPL Results

Region and Subset	Genome Scan LOD	Nearest Marker	Fine-Map LOD	Nearest Marker
20p12:				
Overall	2.57	*D20S186*	1.90	*D20S894*
White	2.84	*D20S186*	2.12	*D20S894*
20q13.1:				
Overall	1.28	*D20S119*	1.49	*D20S119*
White	1.64	*D20S119*	2.06	*D20S119*

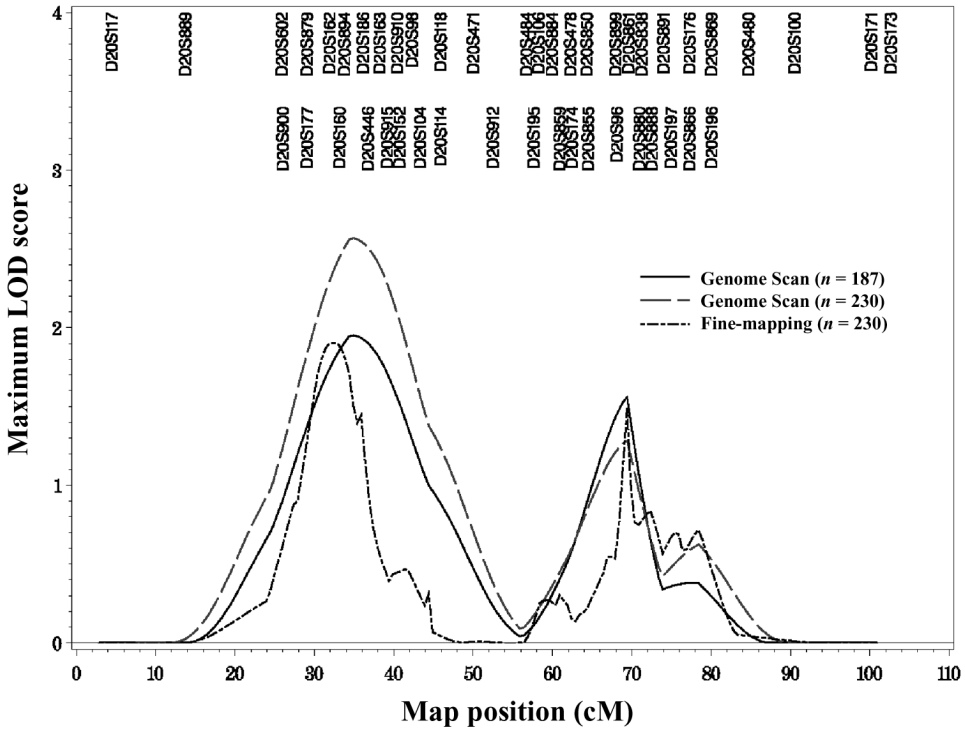

Figure 1 Microsatellite mapping of chromosome 20. LOD scores were calculated using NPL regression statistics and were plotted as a function of genetic distance (cM) along the length of chromosome 20. The LOD score data were derived using 13 genome screen microsatellite markers in 187 SLE sib pair families (*solid line*), the same 13 markers in 230 families (including the same 187 SLE sib pair families) (*coarse hatched line*), and a total of 72 markers, many of which are concentrated in the regions of maximal linkage, in the full 230-family set (*fine hatched line*). For clarity, only selected markers are shown at the top of the figure.

Table 3

Summary of OSA

Chromosomal Region			LOD Score[a]				
Chromosome 16 Position	Chromosome 20 Maximized Position	Nearest Marker	All Pedigrees	Subset Pedigrees	Empirical *P* Value[b]	No. of Pedigrees Used	% of Total Pedigrees
56	33.9 (20p12)	*D20S186*	1.80	4.39	.0158	45	19
57	35.9 (20p12)	*D20S604*	1.44	5.06	.0101	32	14
42	69.4 (20q13.1)	*D20S119*	1.49	3.14	.0122	36	16
43	69.4 (20q13.1)	*D20S119*	1.49	3.40	.0050	40	17
45	69.4 (20q13.1)	*D20S119*	1.49	3.65	.0035	33	14

[a] NPL regression-based LOD score at chromosome 20 position.

[b] Chromosomewide empirical *P* value for the difference between the entire sample LOD and the subset LOD.

onstrated maximal increase in LOD score to 3.65 near marker *D20S861,* ~1 Mb centromeric to *D20S119,* which was the best marker in the full family set.

To more precisely define the linkage effects on 20q13.1, we genotyped 162 SNPs in an ~1.3-Mb region of maximal linkage on 20q13.1 bounded by markers *rs2425688* and *rs2425817.* SNP genotype data was analyzed in the full sample, the white-only sample, and the OSA-defined family sets, by use of the TDT, PDT, and HRT.[41] In the full 230-family set, the HRT demonstrated a broad range of significance ($P \leq .05$) from marker *rs6031869* (44.24 Mb) to marker *rs2251212* (44.73 Mb) (482 kb total distance), an interval that included the following genes: *TOMM34, STK4, KCNS1, WFDC2, WFDC5, WFDC12, PI3, SEMG1, SEMG2, SLPI, MATN4, RBPSUHL, SDC4, C20orf10, C20orf35,* and *PIGT* (fig. 3). Maximal significance by the HRT was centered around two regions—the centromeric *WFDC* gene cluster and the *MATN4* gene (44.44 Mb and 44.62 Mb, respectively) (fig. 3). Within the first region (44.42 Mb) in the full 230-family set, *rs6032006,* located within the *WFDC12* gene, provided the strongest evidence of association with the HRT (SOL $P = .0008$ and >100 $P = .0011$) and the TDT ($P = .0446$), but not with the PDT ($P = .2156$) (table 4). Neighboring SNPs were significant with the HRT, but not with the TDT or the PDT, at the $P = .05$ level. Repeating these analyses in the white pedigrees yielded comparable evidence of association at *rs6032006.* However, neighboring SNPs *rs2664581* within the *PI3* gene (HRT SOL $P = .0013$, HRT >100 $P = .005$, and TDT $P = .0465$), *rs2233896* within the *SEMG2* gene (HRT SOL $P = .0014$, HRT >100 $P = .0042$, and TDT $P = .0465$), and *rs6032064* within the *SEMG2* gene (HRT SOL $P = .0030$, HRT >100 $P = .0058$, and TDT $P = .0329$) also provided supporting evidence of association (table 4).

The next peak of HRT significance was located within the *MATN4* gene region (44.62 Mb) (fig. 3). SNPs *rs2072788* and *rs2072787* provided the strongest evidence of association in the full pedigree set, including the only significant evidence by the PDT analyses (HRT SOL $P = .0058$, HRT >100 $P < .0001$, TDT $P = .0429$, and PDT $P = .0448$) (HRT SOL $P = .0081$, HRT >100 $P = .0001$, TDT $P = .0705$, and PDT $P = .0448$) (table 4). In the entire sample, the evidence of association by the TDT and PDT quickly decayed at neighboring markers. However, within the white pedigrees, the evidence of association for the TDT, but not for the PDT, extended to neighboring SNPs *rs2741500* within the *RBPSUHL* gene (HRT SOL $P = .0086$, HRT >100 $P = .0003$, and TDT $P = .0481$) and *rs2743345* (HRT SOL $P = .0272$, HRT >100 $P = .0006$, and TDT $P = .0165$). Repeating these analyses in the OSA-defined subset of pedigrees did not strengthen the evidence of association within these two regions beyond that reported above.

The HRT also identified a smaller region of significance, from marker *rs6065921* to marker *rs2425754* (fig. 3). The HRT achieved maximal statistical significance at *rs1535045* (HRT SOL $P = .0117$ and HRT >100 $P = .0022$) within the *TNFRSF5* (*CD40*) gene. Two SNPs flanking the *CD40* gene exhibited statistical significance for both the TDT and HRT: *rs6065926* (HRT SOL $P = .0251$, HRT >100 $P = .0040$, TDT $P = .0424$, and PDT $P = .3579$) and *rs2064405* (HRT SOL $P = .0409$, HRT >100 $P = .0461$, TDT $P = .0101$, and PDT $P = .4074$). These results appear to be concentrated in the nonwhite pedigrees, since the significance of this effect was reduced in the white-only analysis.

In the 40 pedigrees identified by OSA at position 43 on chromosome 16, the HRT analysis exhibited significance over a broader region, from marker *rs2425688* to marker *rs6094192* (746 kb total distance). However, the region of peak significance shifted 4.3 kb downstream from the *MATN4* SNP *rs2072788* to a region centering over *SDC4* (fig. 3). The marker displaying the strongest evidence of association within the OSA subset of pedigrees was *rs2072786* (HRT SOL $P < .0001$,

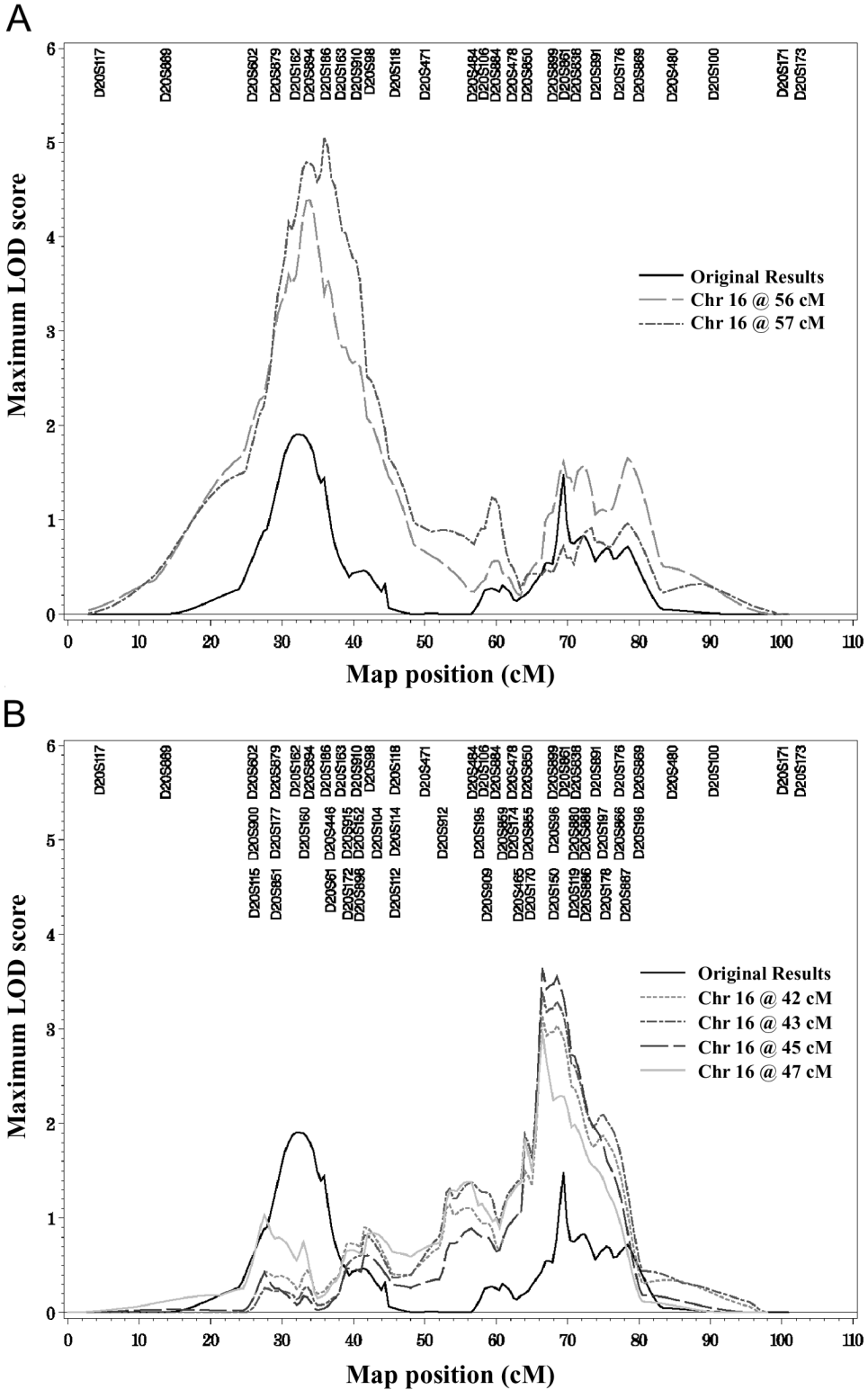

Figure 2 OSA for chromosome 20p12 and 20q13.1. OSA was performed as described in the "Material and Methods" section. *A*, OSA results for chromosome 20p12 conditioned on linkage to positions 56 cM and 57 cM on chromosome 16 (*hatched lines*). LOD score results from the full set of 230 sib pair families and 72 microsatellite markers are shown for comparison (*solid line*). *B*, OSA results for the 20q13.1 region conditioned on linkage at positions 42 cM, 43 cM, 45 cM, and 47 cM on chromosome 16 (*hatched lines*). The positions of selected microsatellite markers are shown in the upper portion of each panel.

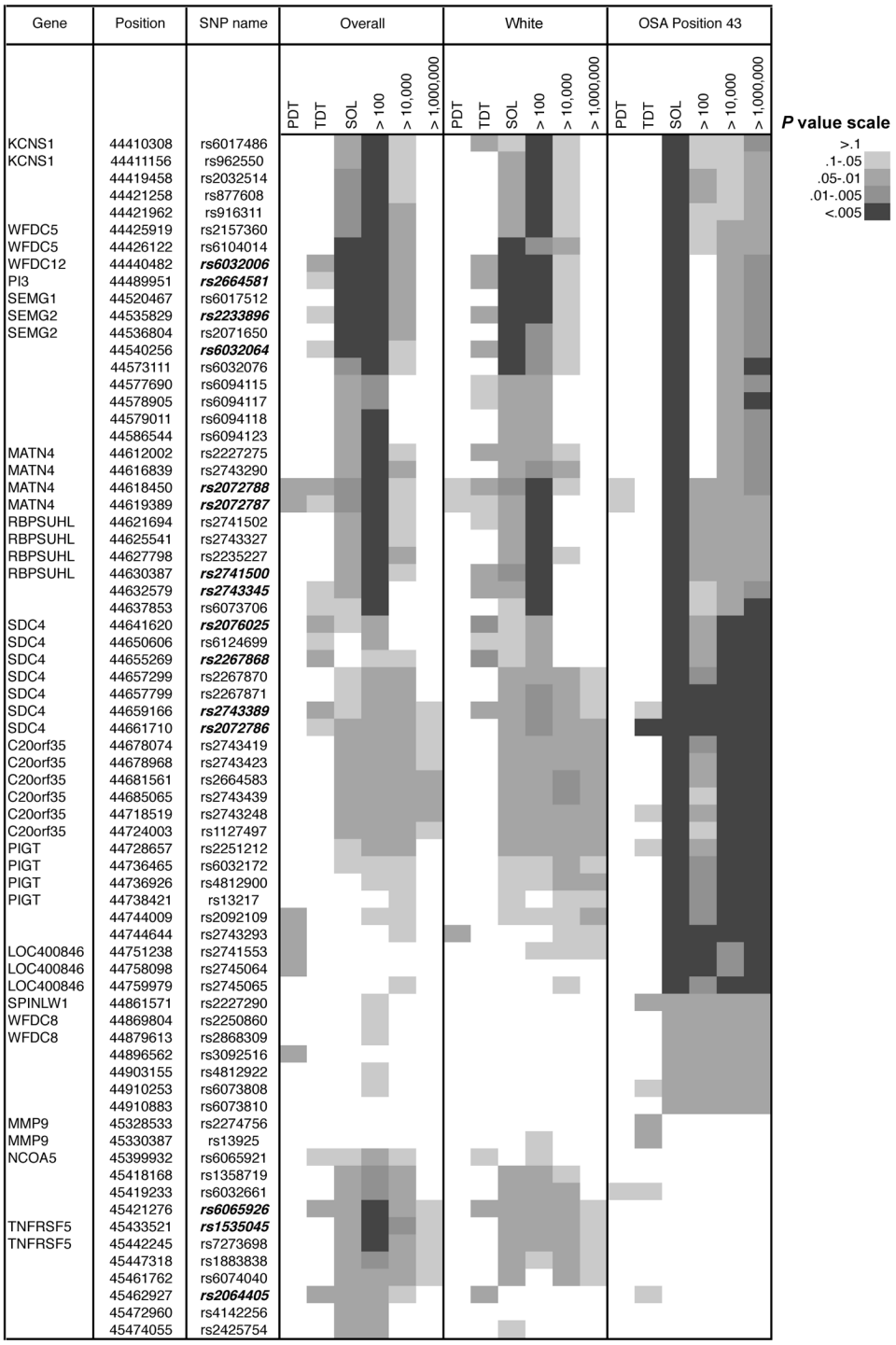

Figure 3 Summary of family-based and haplotype association analysis for selected SNPs from the region of 20q13.1. Seventy SNPs demonstrating the most-significant evidence with family-based PDT, TDT, and haplotypic association HRT are shown. The statistical significance for the HRT is shown as the SOL of the similarity scores and as three similarity score thresholds (>100, >10,000, and >1,000,000) estimated under a permutation test. The evidence for association is represented on a gray scale, as shown in the key to the right. The analyses were performed on the full 230-pedigree family set, the 181-pedigree white family set, and the OSA-defined set of 40 pedigrees conditioned on linkage to position 43 on chromosome 16. The SNPs italicized in bold demonstrate the strongest evidence of association.

Table 4

Summary of Association Data for Selected SNPs in the 20q13.1 Region

SNP	Gene	Position	PDT *P* Value	TDT *P* Value	HRT[a] SOL	HRT[a] $>10^2$	HRT[a] $>10^4$	HRT[a] $>10^6$
Full family set ($N = 230$):								
rs6032006	*WFDC12*	44440482	.2156	.0446	.0008	.0011	.0378	.4593
rs2664581	*PI3*	44489951	.4360	.0807	.0013	.0016	.0470	.4960
rs2233896	*SEMG2*	44535829	.5807	.0654	.0013	.0014	.0443	.4969
rs6032064		44540256	.5976	.0654	.0028	.0020	.0568	.5115
rs2072788	*MATN4*	44618450	.0448	.0429	.0058	.0001	.0586	.6031
rs2072787	*MATN4*	44619389	.0448	.0705	.0081	.0001	.0594	.6726
rs2741500	*RBPSUHL*	44630387	.8315	.1255	.0107	.0001	.0657	.5977
rs2743345		44632579	.5526	.0858	.0264	.0002	.1114	.5899
rs2076025	*SDC4*	44641620	.5840	.0269	.0773	.0103	.1848	.5542
rs2267868	*SDC4*	44655269	.8284	.0151	.1036	.0804	.0709	.3683
rs2743389	*SDC4*	44659166	.2742	.0270	.0709	.0250	.0310	.0892
rs2072786	*SDC4*	44661710	.6450	.0984	.0422	.0116	.0162	.0743
rs6065926		45421276	.3579	.0424	.0251	.0040	.0119	.0761
rs1535045	*TNFRSF5*	45433521	.8542	.5736	.0117	.0022	.0075	.0593
rs2064405		45462927	.4074	.0101	.0409	.0461	.0673	.1008
Whites ($N = 181$):								
rs6032006	*WFDC12*	44440482	.2167	.0302	.0008	.0036	.0539	.4502
rs2664581	*PI3*	44489951	.2946	.0465	.0013	.0050	.0614	.4694
rs2233896	*SEMG2*	44535829	.4442	.0465	.0014	.0042	.0591	.4707
rs6032064		44540256	.4608	.0329	.0030	.0058	.0642	.4757
rs2072788	*MATN4*	44618450	.0676	.0482	.0097	.0005	.0801	.5438
rs2072787	*MATN4*	44619389	.0676	.0830	.0172	.0016	.1121	.6176
rs2741500	*RBPSUHL*	44630387	.5267	.0481	.0086	.0003	.1006	.5334
rs2743345		44632579	.9517	.0165	.0272	.0006	.1740	.5339
rs2076025	*SDC4*	44641620	.3582	.0099	.0860	.0123	.2393	.4480
rs2267868	*SDC4*	44655269	.7284	.0098	.0841	.0459	.1053	.1839
rs2743389	*SDC4*	44659166	.1792	.0303	.0430	.0079	.0381	.0514
rs2072786	*SDC4*	44661710	.6778	.1807	.0282	.0060	.0240	.0446
rs6065926		45421276	.2810	.0361	.0270	.0134	.0191	.0655
rs1535045	*TNFRSF5*	45433521	.2130	.2921	.0107	.0124	.0176	.0527
rs2064405		45462927	.4382	.0145	.1008	.2407	.1140	.1294
OSA subset position 43 ($N = 40$):								
rs6032006	*WFDC12*	44440482	.6971	.7452	.0011	.1213	.0281	.0095
rs2664581	*PI3*	44489951	.6971	.5475	.0024	.2585	.0309	.0070
rs2233896	*SEMG2*	44535829	.6971	.7595	.0011	.1365	.0214	.0066
rs6032064		44540256	.6971	.7595	.0027	.2860	.0207	.0072
rs2072788	*MATN4*	44618450	.0548	.1482	.0012	.0151	.0195	.0095
rs2072787	*MATN4*	44619389	.0548	.1505	.0012	.0322	.0295	.0106
rs2741500	*RBPSUHL*	44630387	.8216	.5508	.0022	.0382	.0322	.0115
rs2743345		44632579	.8815	.3199	.0039	.0597	.0352	.0076
rs2076025	*SDC4*	44641620	.1218	.1545	<.0001	.0328	.0011	<.0001
rs2267868	*SDC4*	44655269	.8694	.5312	<.0001	.0166	.0002	<.0001
rs2743389	*SDC4*	44659166	.2467	.0857	<.0001	.0028	<.0001	<.0001
rs2072786	*SDC4*	44661710	.1907	.0026	<.0001	.0006	.0001	<.0001
rs6065926		45421276	.4281	.4383	.4532	.5822	.3048	.5083
rs1535045	*TNFRSF5*	45433521	.2294	.7675	.5280	.5449	.3707	.6647
rs2064405		45462927	.6547	.0916	.5800	.3959	.4102	.6611

[a] HRT $>10^2$, $>10^4$, and $>10^6$ represent three similarity score thresholds (>100, >10,000, and >1,000,000, respectively) estimated under a permutation test that approximates short, intermediate, and long haplotypes, respectively. SOL is the sum of the natural logarithms of the similarity scores.

HRT >100 $P = .0006$, TDT $P = .0026$, and PDT $P = .1907$). Three neighboring SNPs within the *SDC4* gene (*rs2076025, rs2267868,* and *rs2743389*) were also significant by TDT in the full family and white-only analyses, although the HRT evidence for these SNPs was weaker. No statistically significant association was observed between any these SNPs and the presence of various ACR disease criteria.

Discussion

These data provide additional evidence that SLE-predisposing genes reside within the greater chromosome 20p12 and 20q13 linkage regions. The evidence for linkage and association was observed in an expanded set of sib pair and multiplex families, with the use of 72 chromosomewide microsatellite markers and 162 SNPs targeted to a 1.34-Mb region of maximal linkage on 20q13.1. Our results compare favorably with those reported in independent, ethnically diverse SLE family collections that used a variety of experimental designs.[14,16,22] First, Shai et al.[16] reported evidence for linkage with the use of nonparametric methods in Mexican American ($N = 43$) and white ($N = 37$) families at 20p12 (*D20S115,* $P = .012$), ~3.86 Mb telomeric to *D20S186,* the best marker at 20p12 in the Minnesota pedigrees. Second, Tsao et al.[22] identified linkage to the 20p12 region (*D20S162,* $P = .005$; *D20S604,* $P = .03$) in a mixed-ethnicity cohort of 145 SLE-affected sib pairs. Markers *D20S162* and *D20S604* are located 1.48 Mb telomeric and 1.06 Mb centromeric of *D20S186,* respectively. Similar to the Minnesota pedigrees, linkage to 20p12 in the Tsao et al.[22] study was contributed primarily by white ($N = 77$), as opposed to nonwhite ($N = 68$), affected sib pairs. Third, Moser et al.[14] reported evidence for linkage to chromosome 20q13 in a collection of 94 multiplex pedigrees at marker *D20S481* (LOD = 2.49), 120 kb telomeric from the best marker reported in the Minnesota pedigrees (*D20S119*). Again, the white multiplex SLE families contributed predominantly to this score.

In addition to SLE, linkage to 20q has been reported in families with Grave's disease, a condition characterized by autoimmune hyperthyroidism.[42,43] A major genetic effect in Grave's disease has been mapped to a region ~13 cM centromeric to *D20S119,* near marker *D20S195.* Recent genome scan results in families ascertained for multiple autoimmune diseases through the Multiple Autoimmune Diseases Genetic Consortium also provide evidence for linkage to chromosome 20 (P. Gregersen, personal communication). The strongest linkage evidence in this study was obtained in a subset of families classified as having SLE or in unaffected individuals with high levels of autoantibodies to various antigens, including SSA, SSB, SM, RNP, SCL70, JO1, RIBO, or chromatin. An NPL LOD score of 3.5 was obtained at ~95 cM, a region 25 cM telomeric to the 20q13.1 linkage effect described here (P. Gregersen, personal communication). These observations suggest the presence of multiple autoimmune-predisposing genes on chromosome 20 that individually may have dominant roles in specific autoimmune diseases. At present, it is not possible to determine if these effects act in concert to influence autoimmune susceptibility.

The sum of the genetic evidence to date supports the hypothesis that SLE is a complex genetic disease. Thus, we expect to observe genetic heterogeneity and gene-gene and gene-environment interactions, all of which limit the power of linkage and association methods. Methods that attempt to account for genetic and environmental heterogeneity are attractive means to identify homogeneous subsets of families enriched for specific linkage and/or association effects. In this study, we employed two relatively recent methods (NPL regression and OSA) to explore the potential epistatic relationships among loci within the linkage peak on chromosome 16q12 and the loci on chromosome 20. OSA has the advantage of not requiring a priori specification of covariate trait cutoffs, which, depending on the covariate, may not be intuitively obvious. NPL regression and/or OSA has been successfully used to refine disease-gene location estimates in a variety of complex diseases, including macular degeneration,[44] autism,[45] multiple sclerosis,[46] and diabetic nephropathy.[47] The OSA analysis identified SLE family subsets that share significant linkage to both 16q12 and 20p12 or 20q13.1, consistent with the epistasis hypothesis. Further studies are needed to determine if specific gene families or pathways represented in these chromosomal regions work coordinately in SLE pathophysiology.

The data presented here provide the first evidence for family-based association between genetic markers on chromosome 20 and SLE. A unique aspect of our analysis was the use of a novel haplotype-based analysis method, the HRT.[41] This test evaluates shared consecutive identical-by-state allele matches between haplotypes around a reference marker. Weights are then assigned as an inverse function of allele frequencies at markers within the shared haplotype. HRT is similar in its philosophy to other haplotype-sharing statistics (e.g., maximum identity length statistic[48] and haplotype-sharing statistic[49]) but does not require allele or haplotype removal because of small sample sizes or the use of excess degrees of freedom.[41] Given the differences in the hypothesis being tested, it is not surprising that the HRT and PDT/TDT can differ in statistical significance for a particular SNP. Specifically, the HRT emphasizes differences in length of haplotypes, whereas the PDT/TDT emphasizes pure association with a specific haplotype.[41]

Importantly, the SNPs with the strongest evidence for

association within the 20q13.1 region reside within potentially interesting candidate genes. In the full family set, the strongest evidence ($P < .05$ for PDT, TDT, and HRT) points to *MATN4* as a putative candidate. *MATN4* encodes the protein matrilin 4. Matrilins are a group of noncollagenous extracellular matrix proteins that share von Willebrand factor A domains important for protein-protein interactions.[50] Although widely expressed in human tissues, the molecular function of matrilin 4 is unknown; thus, the mechanism by which matrilin 4 might lead to autoimmunity is equally unclear. Antibodies to a closely related family member, matrilin 1 (MATN1 [MIM 115437]), are hypothesized to play a role in the autoimmune disease relapsing polychondritis.[51]

In the OSA family set, the association evidence suggests a possible role for *SDC4*. *SDC4* encodes the protein syndecan 4, which belongs to a family of transmembrane heparan sulfate proteoglycans that appear to act as receptors or coreceptors involved in intracellular signaling.[52] Syndecan 4 has a number of activities, including modulation of fibroblast growth factor signaling,[53] regulation of cell migration via cross talk with integrins,[54] and control of adhesion via cytoskeletal modifications.[55] Syndecan 4 is expressed from an early pro–B cell step through immature and mature B cell stages.[56] It is almost, but not entirely, restricted to B lymphocyte lineage cells in bone marrow and is absent from B cells that have undergone Ig isotype switching.[56] Syndecan 4 ligation on activated B lymphocytes leads to dramatic morphological changes and extrusion of prominent filopodia, which may facilitate signaling and migration.[56] In coimmunoprecipitation experiments, syndecan 4 has also been shown to be a potential binding partner for the chemokine receptor 4 (CXCR4).[57] This syndecan 4/CXCR4 complex is likely a functional unit involved in stromal cell–derived factor-1 (SDF-1) signaling.[57] Although it is premature to implicate any of the genes identified here as a lupus susceptibility gene without confirmation from independent SLE cohorts, these data suggest that a detailed investigation of these regions might be fruitful.

In summary, the data presented here support the hypothesis that SLE susceptibility genes reside on chromosome 20. In addition, we provide evidence suggesting an epistatic interaction between loci on chromosome 16 and chromosome 20 in an OSA-defined subset of our SLE families. This type of information may be useful in selecting families enriched for particular genetic effects as part of future fine-mapping and resequencing efforts. In addition, large-scale genomewide association studies employing staged case/control designs and high-density SNP arrays—currently under way under the auspices of the SLE Genetics (SLEGEN) consortium—will likely lead to enhanced characterization of the causal variants that influence SLE susceptibility on chromosome 20.

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Web Resources

URLs for data presented herein are as follows:

dbSNP, http://www.ncbi.nlm.nih.gov/SNP/
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for SLE and MATN1)

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