Genomics **70,** 307–314 (2000)
doi:10.1006/geno.2000.6374, available online at http://www.idealibrary.com on IDEAL®

Fine Mapping of the *SLEB2* Locus Involved in Susceptibility to Systemic Lupus Erythematosus

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Received June 13, 2000; accepted September 1, 2000

We have previously reported linkage of systemic lupus erythematosus to chromosome 2q37 in multicase families from Iceland and Sweden. This locus (*SLEB2*) was identified by linkage to the markers D2S125 and D2S140. In the present study we have analyzed additional microsatellite markers and SNPs covering a region of 30 cM around D2S125 in an extended set of Nordic families (Icelandic, Swedish, and Norwegian). Two-point linkage analysis in these families gave a maximum lod score at the position of markers D2S2585 and D2S2985 ($Z = 4.51$, PIC = 0.65), by applying a "model-free" pseudo-marker linkage analysis. Based on multipoint linkage analysis in the Nordic families, the most likely location of the *SLEB2* locus is estimated to be in the interval between D2S125 and the position of markers D2S2585 and D2S2985, with a peak multipoint lod score of $Z = 6.03$, assuming a dominant pseudo-marker model. Linkage disequilibrium (LD) analysis was performed using the data from the multicase families and 89 single-case families of Swedish origin, using the same set of markers. The LD analysis showed evidence for association in the single-case and multicase families with locus GAAT3C11 ($P < 0.0003$), and weak evidence for association was obtained for several markers located telomeric to D2S125 in the multicase families. Thirteen Mexican families were analyzed separately and found not to have linkage to this region. Our results support the presence of the *SLEB2* locus at 2q37. © 2000 Academic Press

The URLs for data in this article are as follows: International RH Mapping Consortium at NCBI, http://www.ncbi.nlm.nih.gov/genemap/; The Human Genome Mapping Resource Center, http://www.hgmp.ac.uk; the Genetic Location Database (LDB), http://cedar.genetics.soton.ac.uk; and The Cooperative Human Linkage Center (CHLC), http://lpg.nci.nih.gov/CHLC/.

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INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune inflammatory disease potentially involving multiple organ systems such as skin, joints, kidney, and brain. Characteristic of the disease is the expression of a variety of autoantibodies, in particular those against double-stranded DNA. The prevalence of SLE is approximately 65/100,000 in Sweden and Iceland (Ståhl-Hallengren *et al.,* 2000; Gudmundsson and Steinsson, 1990; Jonsson *et al.,* 1990) but is potentially higher in African-Americans (Hochberg, 1985). SLE is most prevalent among women, with a female-to-male ratio of 8–9:1. The age of onset appears to be higher in patients of Scandinavian origin (Ståhl-Hallengren *et al.,* 2000) with a median age at onset of 40 years compared to patients from other populations (Hochberg, 1985), with a peak incidence between 35 and 74 years of age. The etiology of the disease is unknown (Alarcón-Segovia and Alarcón-Riquelme, 1998), but epidemiological studies clearly suggest genetic influence in the susceptibility to SLE. The concordance rate in monozygotic twins is estimated to be 25–69%, which is at least 10-fold higher than in dizygotic twins (Block, 1993; Deapen *et al.,* 1992). However, interaction of genetic and environmental factors has been suggested (Alar-

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TABLE 1

Composition of the Family Material

Multicase families		
Population	No. of families	No. of SLE patients
Iceland	9	27
Sweden	15	36
Norway	6	13
Total Nordic	30	76
Mexico	13	32
Single-case families		
Population	No. of families	Parents available
Sweden	89	133

cón-Segovia and Alarcón-Riquelme, 1998; James *et al.,* 1997).

Efforts to understand the genetics of SLE have been attempted through association studies of various candidate genes, among them the MHC class II and class III genes, as well as various genes encoding molecules with possibly relevant immunological functions, such as the Fcγ receptors IIA and IIIA, IL-10, IL-6, T-cell receptors α and β, and mannose-binding protein (Lindqvist and Alarcón-Riquelme, 1999; Tan and Arnett, 1998). The HLA DR and/or DQ genes seem to more strongly influence the production of specific autoantibodies than the overall SLE phenotype itself (Lindqvist and Alarcón-Riquelme, 1999; Tan and Arnett, 1998; Arnett and Reveille, 1992). Deficiency of the MHC class III complement genes C2 and C4 and non-MHC C1q has been associated with the disease in several populations. In particular, partial deficiency of C4A is frequently found in SLE patients and seems to be a genetic risk factor for the disease (Arnett and Reveille, 1992).

Searches for putative susceptibility loci by genome-wide linkage analysis have been performed in several mouse models (Drake *et al.,* 1994; Hirose *et al.,* 1994; Morel *et al.,* 1994; Kono *et al.,* 1994; Watson *et al.,* 1992) as well as in families with SLE (Gaffney *et al.,* 1998; Moser *et al.,* 1998; Shai *et al.,* 1999; Lindqvist *et al.,* 2000). The results obtained for both the murine and the human diseases suggest the presence of multiple susceptibility loci and extensive genetic heterogeneity (Lindqvist and Alarcón-Riquelme, 1999). The genome scans have not yet resulted in the identification of susceptibility genes, but studies of congenic strains with mouse lupus have provided insight into the putative effects of certain loci (Morel *et al.,* 1996; Wakeland *et al.,* 1997).

We have previously reported a genome-wide screen in which we identified a chromosomal location with a putative susceptibility locus for SLE in Swedish and Icelandic families (Lindqvist *et al.,* 2000) located in the 2q37 region. We obtained maximum two-point lod scores of $Z = 4.24$ and $Z = 3.53$ for markers D2S125 and D2S140, respectively. This locus has been named *SLEB2.* We have now analyzed additional markers covering a distance of approximately 30 cM surrounding D2S125, to refine the estimate of the location of *SLEB2* through the use of two-point and multipoint linkage and linkage disequilibrium analysis. We have extended our family material of Nordic origin to a total of 30 multicase families and 89 single-case families. To test the effects of this locus in a very different genetic population, we also analyzed 13 Mexican multicase families. Our results support the presence of a susceptibility locus residing in the 2q37 region in families of Nordic origin and underscore the importance of using family cohorts from genetically related populations to localize genes for complex diseases.

MATERIALS AND METHODS

Family material. The complete family material used in the present study is described in Table 1. Multicase families were selected as those families having at least two or more individuals fulfilling four or more of the 1982 ACR criteria for SLE (Tan *et al.,* 1982). The majority of families from Iceland and Sweden has been described previously (Lindqvist *et al.,* 2000). Pedigree information from the Icelandic genealogy database made it possible to connect

TABLE 2

General Description of the SNPs

Locus	Accession	Gene	Nucleotide pos.	Nucleotide change	Allele frequencies (%/%)
X52022	X52022	RNA for type VI collagen α chain	9458	T=C	64/36
N21131	AA043340	Anonymous EST	213	T=C	47/53
WI-19287	D63780	*Homo sapiens* mRNA for YSK1	1870	T=C	73/27
N31909	AA417959	Anonymous EST	375	C=G	64/36
R86333	A1015191	Chromaffin granule	69	C=T	95/5
A002D44	AA199623	Anonymous EST	107	A=G	58/42
Cda0fd11	AA15760	Anonymous EST	40	A=G	82/18
stSAA009670	NM_002081	Glypican 1	3638	C=T	91/9
stSG314	M64098	HDLBP	4001	G=A	19/81
T79464	D63878a	KIAA0158 gene	1735	A=G	88/12
T79464	D63878b	KIAA0158 gene	3025	A=G	82/18
WI-17974	AB023160	mRNA for KIAA0943 protein	5454	A=G	38/62
W52438	L16991	Human thymidylate kinase	387	C=T	37/67
W52438	L16991	Human thymidilate kinase	761	G=A	43/57

TABLE 3

Sequences of the Amplification Primers and DASH Match and Mismatch Probes

Locus	Primer 1	Primer 2	Probe 1	Probe 2
X52022	ACCaGAGGTCTCAGGTCAGA	TCTTTGTACTGAAACTTCCGTG	AGAGCCAtCTACCAC	AGAGCCAcCTACCAC
N21131	GAACACTAGTGCCCAGCACCA	GTGCGGGCTCTtGGGGCAGA	CACCATTtCTGCCAC	CACCATTcCTGCCAC
WI-19287	TGATGGCCATAGATTTGCCTTGT	TGAGCAGACACAGTACCTGAT	CTTGTGGtGTTGGAT	CTTGTGGcGTTGGAT
N31909	GCATCTTCCCAACGTCGCTGT	GGAAGGAAGGATTGGAAAAGTC	CTGTGCTcCGGGGGAC	CTGTGCTgCGGGGAC
R86333	GTCTCCAACGGGAAAATATACA	CATAAGAATAATCATCAAAGGCA	ATACAACcCTCTTGC	ATACAACtCTCTTGC
A002D44	TAACACTCGGGTGTAAGACATC	TGCCGTCCCGACGTCACGA	ATCGGGACaGGCGTCGT	ATCGGGACgGGCGTCGT
Cda0fd11	GGTTTATTTTATGCATATTACTGT	AAGGTCATGTGATTTGTAATA	ACTGTACCaCAATAAAT	ACTGTACCgCAATAAAT
stsAA009670	TCCCCCCAtCGCTCAGTGTCA	GACTCAAAAGAACACACGTCACC	GTGTCAGcGGGTGAC	GTGTCAGtGGGTGAC
stSG314	GAACCCTCTCCAGCCTGCTGA	TTGAGACAAAgCATTGTGTGGTT	CTGACCCgAACCCAA	CTGACCCaAACCCAA
T79464	CACTCCTGATTTTTATCTAGAAC	ACCTCTACgACTAAGGAACA	TCAGATTTaCCATAATG	TCAGATTTgCCATAATG
T79464	GTTGCTTAATTGTGAACAGCCA	CCTTCACACAATAAGCCATAAC	CAAAAGCTaTATGTTAT	CAAAAGCTgTATGTTAT
WI-17974	GCTtGTGCGATCTCCATTCCT	CGGCGCACaATGAACTCGGAC	GGGCTCCaCGTCCGA	GGGCTCCgCGTCCGA
W52438	TGaCAAGGAGAATTTTTCCCTA	GCCCACGTCTaGCTGTTTAC	CCCTAGAtTGGTGTA	CCCTAGAtTGGTGTA
W52438	TCtCGGAAtGCCCCGTCCCCA	CTGAAGTTGTaGGGTCTGGAC	CCCCAGCgGAGTCCA	CCCCAGCaGAGTCCA

three additional Icelandic individuals affected with SLE in 2 of the families that we have already studied (Lindqvist *et al.,* 2000). In addition, 3 new multicase families have been identified and added to the present study, which represents nearly all expected SLE multicase families in Iceland (between 5 and 15% of all SLE patients have a first- or second-degree relative with SLE; Arnett and Reveille, 1992). Four more families were also added to the cohort of Swedish families described before (Lindqvist *et al.,* 2000). All Swedish families, including the 89 single-case families, originate from Southern or Central Sweden. Furthermore, the grandparents of all patients in the single-case families were also born in Sweden (to eliminate heterogeneity due to possible inclusion of recent immigrants). Six Norwegian families were also included, originating in various parts of the country, but with all grandparents born in Norway. Thirteen multicase families from the Mexico City area were analyzed separately. All such families have grandparents born in Mexico and are mostly Mexicans of Spanish-Amerindian admixture. All individuals, patients and their relatives, have given informed consent for this study.

Genotyping of microsatellites. Markers D2S1363, D2S427, and D2S125 are included in the Weber set 6 and were previously used in the genome scan (Lindqvist *et al.,* 1996, 2000). GATA178G09 and GAAT3C11 are from the Cooperative Human Linkage Center (Sheffield *et al.,* 1995). D2S345, D2S2285, D2S2253, D2S1397, D2S140, and D2S2338 are from Généthon (Dib *et al.,* 1996; Gyapay *et al.,* 1996). COL6A3 was recently described (Pan *et al.,* 1998), as were D2S2585, D2S2985, and D2S2986, which are located at the telomeric region of 2q (Rosenberg *et al.,* 1997). Primers were synthesized by Interactiva Biotechnology (Ulm, Germany) with a conjugated amidite fluorophore at the 5′ end of the downstream primer. Each PCR was optimized for use on 877 ABI instruments (Applied Biosystems, Inc., Foster City, CA). Fragment lengths were defined using an ABI 377 sequencer with GeneScan program, version 2.0 (Applied Biosystems, Inc.). Allele-calling was performed using the Genotyper program, version 2.0 (Applied Biosystems, Inc.), and Mendelian segregation of marker alleles was verified using the GAS program (Alan Young, Oxford University).

Search for single-nucleotide polymorphisms within 2q37. Human GenMap98 from the International RH Mapping Consortium at NCBI was used to check every expressed sequence tag (EST) or gene found within the 2q37 cytogenetic region, between D2S345 (AFM288vb1) and stsSG29476. This region covers 19 cM (sex-averaged). EST sequences were retrieved and aligned using ESTBlast software (Gill *et al.,* 1997), which is available through registration at the UK Human Genome Mapping Project Resource Center (Cambridge, UK). The alignments obtained were visually inspected for the presence of potential polymorphisms. Mismatched bases were visualized when possible in the sequence chromatogram using the ESTace viewer from the Genome Sequencing Center, Washington University School of Medicine (St. Louis, MO). Biallelic markers were chosen based on two criteria: (a) that more than seven large (>200 bp) EST entries could be aligned with the original gene/EST sequence with more than 85% homology and (b) that at least two entries contained the less frequent allele.

Based on above criteria, we identified 36 potential SNP markers. A dynamic allele-specific hybridization (DASH) assay (see below) was designed for each of these SNPs and tested in a panel of 10 Nordic unrelated individuals. For 6 of the putative SNPs, differences could not be unambiguously ascertained between the match and the mismatch probes with the DASH assay, and in 16 of them, no variation was detected. Fourteen SNPs were further analyzed in 50 unrelated individuals of Swedish origin and their allelic frequencies are shown

TABLE 4

Microsatellite Marker Order and Intermarker Distances According to the Summary Map from LDB

	cM	Heterozygosity
D2S1363		0.79
	10.0	
D2S427		0.76
	6.0	
COL6A3		nd
	0.5	
D2S1397		0.76
	2.0	
D2S345		0.70
	4.0	
gaat3cll (D2S2949)		0.57
	0.03	
gata178g09 (D2S2968)		nd
	0.9	
D2S2338		0.56
	0.40	
D2S2285		0.64
	0.02	
D2S2253		0.71
	1.0	
D2S125		0.83
	3.0	
D2S140		0.78
	1.0	
D2S2585		0.55
	0.0	
D2S2985		0.23
	1.0	
D2S2986		0.69

Note. nd, no data available.

in Table 2. The primers and probes used for the SNP genotyping assays are shown in Table 3.

Genotyping of the SNPs. The SNPs were genotyped using the DASH method with minor modifications (Howell *et al.,* 1999). The PCRs were performed in a 20-μl reaction including 35 ng of genomic DNA, 9.6 pmol of unlabeled upstream primer, and 2.4 pmol of biotin-labeled downstream primer. The PCR cycling conditions were as follows: 95°C for 8 min and then 2 cycles of 95°C for 20 s, 60°C for 35 s followed by 2 cycles of 95°C for 20 s, 59°C for 35 s and finally 40 cycles of 95°C for 20 s, 58°C for 35 s with a final extension of 10 min at 68°C. For the SNP at AA15760 the annealing temperature was reduced 3°C, ending at 55°C. After amplification, a volume of 10 μl of the PCR was bound overnight to streptavidin-coated microtiter plates (Hybaid Limited, Middlesex, UK). After the nonbiotinylated strand was washed off with alkali, hybridization was performed separately for each of the allele-specific probes (match and mismatch probes), at low temperature. The sample was then steadily heated while fluorescence was monitored using a Perkin–Elmer 7700 TaqMan instrument (Applied Biosystems, Inc.). The amount of fluorescence is proportional to the amount of hybridized double-stranded DNA, and the difference in hybridization between the matched and the mismatched probes can be analyzed.

Linkage analysis. Two-point linkage analysis was performed using the MLINK (Morton, 1955; Ott, 1991) program (FASTLINK 4.0; Cottingham *et al.,* 1993; Scaffer *et al.,* 1994) and the ANALYZE program package (Terwilliger, 1995, 2000; Göring and Terwilliger, 2000a). The presence of linkage heterogeneity was tested for using the admixture test (Smith, 1961). Multipoint analysis was performed using LINKMAP (FASTLINK 4.0).

Since the evidence for linkage to the region was previously detected under the assumption of dominant inheritance (Lindqvist *et al.,* 2000), the two-point as well as the multipoint linkage analysis was performed assuming a dominant mode of inheritance as well. Because it is impossible to know the mode-of-inheritance parameters of a complex disease, and because we assume that the phenotype "affected" contributed significantly more predictive value (vis-à-vis the underlying disease locus genotypes) than the phenotype "unaffected," we used an affected-only dominant pseudo-marker analysis, as described elsewhere (Göring and Terwilliger, 2000a). Such analysis is shown to be equivalent to traditional "model-free" approaches on simple pedigree structures and to be more powerful in large pedigrees, such as the ones we have in this study (Lindqvist *et al.,* 2000). Allele frequencies were estimated by allele counting in the pedigree material (Smith, 1957; Göring and Terwilliger, 2000b). The marker order and intermarker distances were obtained from the summary map of the genetic location database (LDB), with the exception of the distance between D2S125 and D2S140, which is assumed to be 3 cM (telomeric) (Nancy J. Cox, University of Chicago, pers. comm., June 1999). The relative positions of markers from D2S2285 to the SNP T79464b were based on a physical map that was kindly provided by Dr. Graeme Bell, University of Chicago (Horikawa *et al.,* 2000).

Linkage disequilibrium analysis. The presence of linkage disequilibrium was tested using a haplotype relative risk study design (Falk and Rubinstein, 1987; Terwilliger and Ott, 1992). The marker alleles transmitted from both heterozygous and homozygous parents to affected offspring were used as the case sample and the nontransmitted alleles were treated as an independent genetically matched control sample. The likelihood was then computed following the method of Terwilliger (1995) as a function of λ, the proportion of association observed in the sample. The haplotype relative risk test was applied as it is somewhat more powerful than the standard TDT method (Terwilliger, 1995).

RESULTS

We have previously reported evidence of linkage to a region on chromosome 2q37 with markers D2S125 and D2S140 in multicase SLE families from Iceland and Sweden. In the present study we have typed additional markers in the region and included new Nordic families (total $n = 30$). The heterozygosity and microsatellite marker order we assumed were taken from the summary map of the genetic LDB as shown in Table 4.

Two-point lod scores for 15 microsatellite markers on chromosome 2q37 are shown in Table 5. The maximum two-point lod score was obtained with marker D2S125 ($Z = 3.59$), followed by marker D2S140 ($Z = 2.72$) using a dominant pseudo-marker analysis (Göring, 2000a). Markers D2S2585 and D2S2985 are located at the same position according to all available genetic maps. For this reason they were tested jointly, assuming a recombination fraction of $\theta = 0$ between them. The two-point lod score obtained for the joint marker D2S2585/D2S2985 was $Z = 4.51$. Multipoint linkage analysis indicated the best estimate of the position of the locus to be approximately 4 cM telomeric to D2S125 with a peak lod score of $Z = 6.03$ close to the combined marker D2S2585/D2S2985 using the same inheritance model (Fig. 1).

To analyze the region further by linkage disequilibrium (LD) mapping, we used a likelihood based HRR

TABLE 5

Two-Point Linkage Analysis Results for the Nordic Multicase Families

Marker	$P_D = 0.002$		
	LodHom	LodHet	PIC value
D2S1363	0.56	0.56	0.73
D2S427	0.18	0.18	0.65
COL6A3	0.45	0.67	0.80
D2S1397	0.47	0.52	0.59
D2S345	0.31	0.31	0.59
gaat3cll	0.74	0.77	0.27
gata178g09	0.42	0.42	0.60
D2S2338	0.26	0.36	0.46
D2S2285	0.25	0.28	0.58
D2S2253	0.45	0.45	0.64
D2S125	<u>3.59</u>	<u>3.59</u>	0.81
D2S140	<u>2.65</u>	<u>2.72</u>	0.68
D2S2585	<u>2.26</u>	<u>2.33</u>	0.49
D2S2985	<u>1.72</u>	<u>1.72</u>	0.27
D2S2585/D2S985[a]	<u>4.51</u>	<u>nd</u>	0.65
D2S2986	<u>1.32</u>	<u>1.32</u>	0.60

Note. Maximum lod scores obtained by two-point linkage analysis using MLINK (FASTLINK 4.0) as implemented by the ANALYZE package applying dominant inheritance, affected-only analysis, and disease gene frequency of $P_D = 0.002$. LodHom, two-point linkage analysis assuming homogeneity; LodHet, two-point linkage analysis allowing for heterogeneity. Allele frequencies for each marker were obtained by allele counting in pedigree material. The PIC value is the information content of each marker for the multicase family material.

[a] D2S2585/D2S2985 were treated as a single marker. The PIC value for these two markers combined was estimated for the haplotypes, which may be a slight underestimate of the real PIC value when they are analyzed jointly in a two-point analysis with $\theta = 0$ between them.

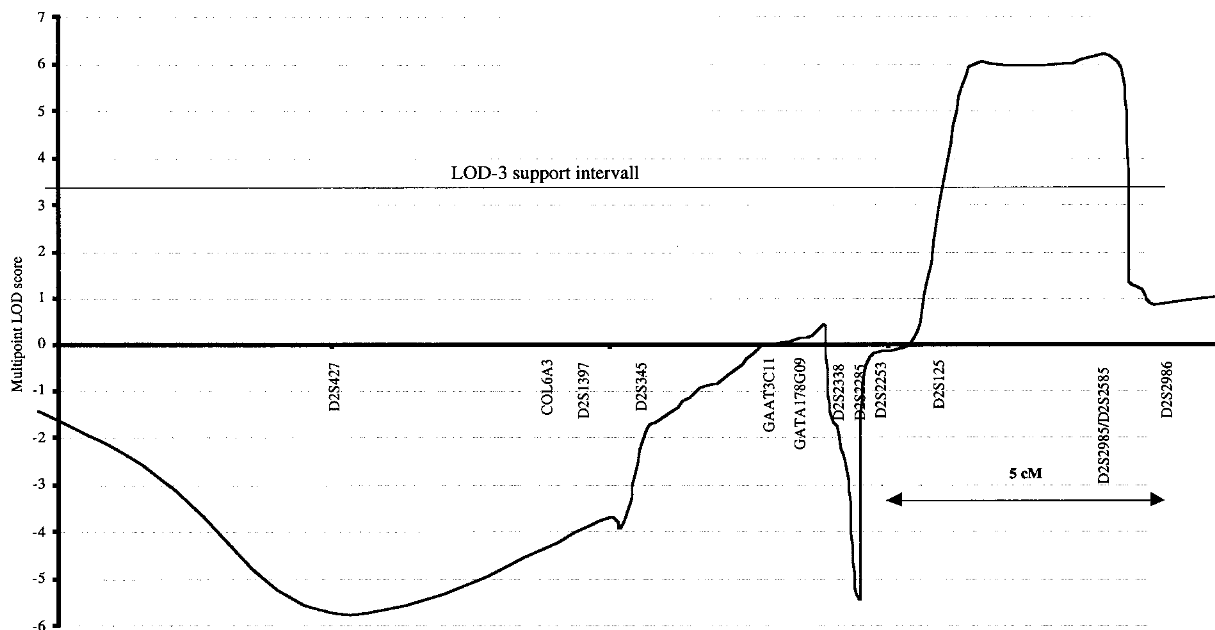

FIG. 1. Multipoint curve. Multipoint analysis assuming dominant inheritance (P_D = 0.002) using LINKMAP (FASTLINK 4.0). The number of alleles for each marker was reduced to facilitate the calculations. Reduced allele frequencies were used in the calculation. The marker order and the intermarker distances were obtained from the LDB summary map. On all genetic maps available the markers D2S2585 and D2S2985 were located at the same position, so the multipoint linkage analysis was performed using the markers jointly. The lod-3 support interval is indicated.

ratio test (Terwilliger, 1995). LD mapping was performed using the microsatellite markers and SNPs. A group of 89 single-case families was also included. Fourteen SNPs located in genes and ESTs surrounding D2S125 were analyzed. A physical map made available to us (G. Bell, Horikawa *et al.,* 2000) allowed us to infer the most likely order of the following markers: D2S2285, D2S2253, D2S125, D2S140, stsAA009670, Cda0fd11, StsG314, and T79464 (a and b). These loci are marked with an asterisk in Table 6. The most likely physical order of the remaining loci was inferred from LDB and GeneMap98 (NCBI).

The multicase and single-case families were first analyzed separately and then jointly. In the multicase families, the strongest evidence for association was found with microsatellite marker D2S2585 ($P = 0.003$, HRR-LTR), but weak evidence was seen also with D2S2986 ($P = 0.01$, HRR-2xn) and the SNP W52438b and Cda0fd11 ($P = 0.02$ and $P = 0.01$, respectively with HRR-2xn). A tendency toward lower P values was observed for the region telomeric from D2S125, although none reached statistical significance after correction for multiple testing using Bonferroni. In the single-case families, the microsatellite GAAT3C11 showed strongest association ($P = 0.002$) that persisted when multicase and single-case families were combined ($P = 0.0003$) (Table 6). None of these findings, however, remained statistically significant after correction for multiple testing.

To test for linkage to the 2q37 locus in families from a different population, we genotyped a set of families from the Mexican population ($n = 13$). The maximum two-point lod score obtained was for D2S2338 ($Z = 0.71$) (Table 7), while no other marker showed any indication of linkage.

DISCUSSION

Herein we have sought to strengthen the linkage of the *SLEB2* locus on chromosome 2q37, described initially in a genome scan performed on Swedish and Icelandic families (Lindqvist *et al.,* 2000). We have now analyzed additional microsatellite markers, 5 of which were included in our genome scan report, and 14 SNPs covering a distance of approximately 30 cM in a total of 30 multicase families of Nordic origin. The two-point and multipoint analyses using the microsatellite markers suggest an approximate location of the *SLEB2* locus near D2S125. The multicase families and a new group of single-case families were analyzed for LD with microsatellite markers and SNPs. The strongest evidence of LD was found in the multicase families with D2S2585 ($P = 0.003$). GAAT3C11 gave the strongest evidence for association in single-case families and when all families were combined. The exact location of GAAT3C11, however, is unknown, but according to the available genetic maps, this marker is centromeric to D2S125. We are at present trying to define its exact location through physical mapping. Although none of the P values remained significant after correction for mul-

TABLE 6

Linkage Disequilibrium Analysis in the Nordic Multicase and Single-Case Families

	Multicase		Single case		Multicase and single case	
Markers	HRR-LRT	HRR-2xn	HRR-LTR	HRR-2xn	HRR-LTR	HRR-2xn
D2S1363	0.50	0.32	0.50	1.00	0.50	0.30
D2S427	0.21	0.20	0.50	1.00	0.21	0.20
D2S1397	0.50	0.25	0.50	0.91	0.50	0.59
D2S345	0.50	0.25	0.50	1.00	0.50	0.25
COL6A3	0.50	0.31	0.50	1.00	0.50	0.31
X52022	0.50	0.47	0.14	0.08	0.50	0.25
gata178g09	0.38	0.11	0.50	0.74	0.25	0.43
D2S2338	0.50	0.45	0.50	1.00	0.50	0.85
N21131	0.50	0.85	0.50	0.33	0.50	0.35
WI-19287	0.50	0.70	0.11	0.13	0.06	0.07
R86333	0.50	1.00	0.13	0.22	0.09	0.14
A00D44	0.50	0.09	0.50	0.82	0.50	0.48
D2S2285*	0.50	0.62	0.50	0.11	0.50	0.38
D2S2253*	0.50	0.44	0.50	0.53	0.50	0.37
gaat3cll*[a]	0.05	**0.04**	**0.002**	**0.002**	**0.0003**	**0.0003**
D2S125*	0.50	0.12	0.50	0.94	0.50	0.87
D2S140*	0.50	0.96	0.50	0.89	0.50	0.90
StsAA009670*	0.50	0.31	0.50	0.84	0.50	0.76
Cda0fd11*	**0.01**	**0.01**	0.50	0.69	0.10	0.15
StsG314*	0.50	0.92	0.06	0.07	0.50	0.62
T79464a*	0.31	0.37	**0.04**	0.05	**0.04**	0.05
T79464b*	0.50	0.99	**0.02**	**0.03**	0.06	0.08
WI-17974	0.50	0.41	0.50	0.27	0.50	0.25
W52438a	0.50	0.53	0.50	0.92	0.50	0.49
W52438b	**0.04**	**0.02**	0.50	0.68	0.50	0.37
D2S2585	**0.003**	**0.006**	0.50	0.57	**0.04**	**0.03**
D2S2985	0.50	0.92	0.50	0.83	0.50	0.96
D2S2986	**0.03**	**0.01**	0.50	0.78	0.43	0.25

Note. Likelihood-based haplotype relative risk test for association as performed by the HRRLAMP program applied by the ANALYZE package (J. Terwilliger). The likelihood ratio test, HRR-LRT, and the 2xn table χ^2 test; HRR-2xn results presented as P values. Significant values are in bold ($P < 0.05$). The SNP N31909 is not included due to genotyping problems. The positions of the loci marked with an asterisk have been confirmed by physical mapping (G. I. Bell, University of Chicago, pers. comm., Sept 2000; Horikawa *et al.,* 2000).

[a] Marker GAAT3C11 is located centrometric to D2S2253 according to GeneMap98.

tiple tests, several markers telomeric to D2S125 showed a tendency for association. Although at the present moment we cannot delimit the region with confidence, we have confirmed the presence of this susceptibility locus for SLE in our group of 30 Nordic families. Independent confirmation of suggestive linkage to D2S125 in American multicase families for SLE has been recently obtained (J. B. Harley, Oklahoma Medical Research Foundation, pers. comm., May 2000).

The use of SNPs for fine mapping of susceptibility loci for complex diseases has been widely proposed particularly because SNPs occur at a higher frequency than microsatellites. However, the utility of SNPs in fine mapping depends on several factors such as the degree of linkage disequilibrium between the SNP and the actual disease mutation and the strength of the effect of the actual mutation in the disease. The SNPs we have studied here are located within genes and transcript covering 20 cM surrounding D2S125, but may be too far away from the disease mutation. A recent study analyzing SNPs surrounding the APOE allele involved in Alzheimer disease could detect association with SNPs only if as close as 50 kb from the disease allele with a weak association showing at 400 kb from APOE (Martin *et al.,* 2000). We are at present completing the physical map for 2q37.3 and searching for new SNPs in the region.

The difference between the linkage results for families of Nordic and those of Mexican descent might lead one to believe that this locus confers susceptibility for development of SLE mainly in Nordic populations. Presumably, the common genetic background of the Nordic families made it possible to detect the effect of this locus, which might otherwise not have been possible using a more admixed population with a greater degree of genetic heterogeneity. A much larger number of Mexican families is needed to demonstrate the presence of this locus in this population. As is evident from the mouse lupus models as well as the recent results from genome-wide screens of human SLE, the disease exhibits pronounced genetic heterogeneity. Therefore, it is rather likely that susceptibility loci may differ in nature between ethnic populations.

TABLE 7

Two-Point Linkage Analysis in the Mexican Cohort

Marker	LodHom	LodHet
D2S1363	0.03	0.03
D2S427	0.01	0.01
PLAP	0.00	0.00
COL6A3	0.01	0.01
D2S1397	0.00	0.00
D2S345	0.01	0.01
gaat3cll	0.01	0.01
gata178g09	0.00	0.00
D2S2338	0.71	0.71
D2S2285	0.00	0.00
D2S2253	0.00	0.00
D2S125	0.01	0.17
D2S140	0.00	0.00
D2S2585	0.00	0.00
D2S2985	0.00	0.00
D2S2986	0.00	0.00

Note. Two-point maximum lod scores obtained by MLINK (FASTLINK 4.0) computed using the ANALYSIS package under dominant inheritance ($P = 0.002$) and affected-only analysis. LodHom, assuming homogeneity; LodHet, allowing for heterogeneity. Allele frequencies for each marker were obtained by allele counting in the pedigree material itself.

ACKNOWLEDGMENTS

This work has been supported by European Commission Grant BMH4-98-3489 and NorFa Grant 00.15.062-O, as well as by the following individual grants: the Swedish Medical Research Council to M.E.A.-R. (Grant 12673) and G.S. and L.T. (Grant 13489); the Åke Wibergs Stiftelse to M.E.A.-R.; the King Gustaf V:80th Jubilee Fond to M.E.A.-R.; the Margareta Rheumatology Research Foundation to I.L.; the Swedish National Association against Rheumatism to M.E.A.-R., G.S., L.T., and I.L.; the Landspitalínn and University of Iceland Research Funds to K.S.; the Research Council of Norway to R.J. (Grant 115563/320); and the Hitchings–Elion fellowship from the Burroughs Wellcome Fund to J.D.T. The authors are indebted to Dr. Graeme Bell and Yukio Horikawa from the University of Chicago for kindly providing physical mapping information on 2q37 prior to publication.

REFERENCES

Alarcón-Segovia, D., and Alarcón-Riquelme, M. E. (1998). Ethiopathogenesis of systemic lupus erythematosus: A tale of three troikas. *In* "Systemic Lupus Erythematosus" (R. G. Lahita, Ed.), pp. 55–69, Academic Press, San Diego.

Arnett, F. C., and Reveille, J. D. (1992). Genetics of systemic lupus erythematosus. *Rheum. Dis. Clin. North Am.* **18:** 865–892.

Block, S. R. (1993). Twin studies: Genetic factors are important. *Arthritis Rheum.* **36:** 135–136.

Cottingham, R. W., Jr., Idury, R. M., and Schaffer, A. A. (1993). Fast sequential genetic linkage computations. *Am. J. Hum. Genet.* **53:** 252–263.

Deapen, D., Escalante, A., Weinrib, L., Horwitz, D., Bachman, B., Roy-Burman, P., *et al.* (1992). A revised estimate of twin concordance in systemic lupus erythematosus. *Arthritis Rheum.* **35:** 311–318.

Dib, C., Faure, S., Fizames, C., Samson, D., Drouot, N., Vignal, A., *et al.* (1996). A comprehensive genetic map of the human genome based on 5,264 microsatellites. *Nature* **380:** 152–154.

Drake, C. G., Babcock, S. K., Palmer, E., and Kotzin, B. L. (1994). Genetic analysis of the NZB contribution to lupus-like autoimmune disease in (NZB X NZW)F1 mice. *Proc. Natl. Acad. Sci. USA* **91:** 4062–4066.

Falk, C. T., and Rubinstein, P. (1987). Haplotype relative risk: An easy reliable way to construct a proper control sample for risk calculations. *Ann. Hum. Genet.* **51:** 227–233.

Gaffney, P. M., Kearns, G. M., Shark, K. B., Ortmann, W. A., Selby, S. A., Malmgren, M. L., *et al.* (1998). A genome-wide search for susceptibility genes in human systemic lupus erythematosus sib-pair families. *Proc. Natl. Acad. USA* **95:** 14875–14879.

Gaffney, P. M., Ortmann, W. A., Selby, S. A., Shark, K. B., Ockenden, T. C., Rohlf, K. E., *et al.* (2000). Genome screening in human systemic lupus erythematosus: Results from a second Minnesota cohort and combined analysis of 187 sib-pair families. *Am. J. Hum. Genet.* **66:** 547–556.

Gill, R. W., Hodgman, T. C., Littler, C. B., Oxer, M. D., Montgomery, D. S., Taylor, S., and Sanseau, P. (1997). A new dynamic tool to perform assembly of expressed sequence tags (ESTs). *Comput. Appl. Biosci.* **13:** 453–457.

Göring, H. H. H., and Terwilliger, J. D. (2000a). Linkage analysis in the presence of errors. IV. Joint pseudomarker analysis of linkage and/or linkage disequilibrium on a mixture of pedigrees and singletons when the mode of inheritance cannot be accurately specified. *Am. J. Hum. Genet.* **66:** 1310–1327.

Göring, H. H. H., and Terwilliger, J. D. (2000b). Linkage analysis in the presence of errors. III. Marker loci and their map as nuisance parameters. *Am. J. Hum. Genet.* **66:** 1298–1309.

Göring, H. H. H., and Terwilliger, J. D. (2000c). Linkage analysis in the presence of errors. II. Marker locus genotyping errors modeled with hypercomplex recombination fractions. *Am. J. Hum. Genet.* **66:** 1107–1118.

Gudmundsson, S., and Steinsson, K. (1990). Systemic lupus erythematosus in Iceland 1975 through 1984. A nationwide epidemiological study in an unselected population. *J. Rheumatol.* **17:** 1162–1167.

Gyapay, G., Schmitt, K., Fizames, C., Jones, H., Vega-Czarny, N., Spillet, D., *et al.* (1996). A radiation hybrid map of the human genome. *Hum. Mol. Genet.* **5:** 339–346.

Hirose, S., Tsurui, H., Nishimura, H., Jiang, Y., and Shirai, T. (1994). Mapping of a gene for hypergammaglobulinemia to the distal region on chromosome 4 in NZB mice and its contribution to systemic lupus erythematosus in (NZBxNZW)F1 mice. *Int. Immunol.* **6:** 1857–1864.

Hochberg, M. (1985). The incidence of systemic lupus erythematosus in Baltimore, Maryland, 1970–1977. *Arthritis Rheum.* **28:** 80–86.

Horikawa, Y., Oda, N., Cox, N. J., Li, X., Orho-Melander, M., Hara, M., *et al.* (2000). Genetic variation in the gene encoding Calpain-10 is associated with type 2 diabetes mellitus. *Nat. Genet.* **26:** 163–175.

Howell, W. M., Jobs, M., Gyllensten, U., and Brookes, A. J. (1999). Dynamic allele-specific hybridization. *Nat. Biotechnol.* **17:** 87–88.

James, J. A., Kaufman, K. M., Farris, A. D., Taylor-Albert, E., Lehman, T. J., and Harley, J. B. (1997). An increased prevalence of Epstein–Barr virus infection in young patients suggests a possible etiology for systemic lupus erythematosus. *J. Clin. Invest.* **100:** 3019–3026.

Jonsson, H., Nived, O., Sturfelt, G., and Silman, A. (1990). Estimating the incidence of systemic lupus erythematosus in a defined population using multiple sources of retrieval. *Br. J. Rheumatol.* **29:** 185–188.

Kainulainen, K., Perola, M., Terwilliger, J. D., Kaprio, J., Koskenvuo, M., Syvänen, A. C., *et al.* (1999). *Hypertension* **33:** 844–849.

Kono, D. H., Burlingame, R. W., Owens, D. G., Kuramochi, A., Balderas, R. S., Balomenos, D., *et al.* (1994). Lupus susceptibility loci in New Zealand mice. *Proc. Natl. Acad. Sci. USA* **21:** 10168–10172.

Lindqvist, A. K., Magnusson, P. K., Balciuniene, J., Wadelius, C., Lindholm, E., Alarcón-Riquelme, M. E., *et al.* (1996). Chromosome-

specific panels of tri- and tetranucleotide microsatellite markers for multiplex fluorescent detection and automated genotyping: Evaluation of their utility in pathology and forensics. *Genome Res.* **6:** 1170–1176.

Lindqvist, A. K., and Alarcón-Riquelme, M. E. (1999). The genetics of systemic lupus erythematosus. *Scand. J. Immunol.* **50:** 562–571.

Lindqvist, A. K. B., Steinsson, K., Johanneson, B., Kristjansdottir, H., Gröndal, G., Svenungsson, E., Lundberg, I., *et al.* (2000). A susceptibility locus for systemic lupus erythematosus (*hSLE1*) in chromosome 2q. *J. Autoimmun.* **14:** 169–178.

Martin, E. R., Gilbert, J. R., Lai, E. H., Riley, J., Rogala, A. R., Slotterbeck, B. D., *et al.* (2000). Analysis of association at a single nucleotide polymorphisms in the APOE region. *Genomics* **63:** 7–12.

Morel, L., Rudofsky, U. H., Longmate, J. A., Schiffenbauer, J., and Wakeland, E. K. (1994). Polygenic control of susceptibility to murine systemic lupus erythematosus. *Immunity* **1:** 219–229.

Morel, L., Yu, Y., Blenman, K. R., Caldwell, R. A., and Wakeland, E. K. (1996). Production of congenic mouse strains carrying genomic intervals containing SLE-susceptibility genes derived from the SLE-prone NZM2410 strain. *Mamm. Genome* **7:** 335–339.

Morton, N. E. (1955). Sequential tests for detection of linkage. *Am. J. Hum. Genet.* **7:** 277–318.

Moser, K. L., Neas, B. R., Salmon, J. E., Yu, H., Gray-McGuire, C., Asundi, N., *et al.* (1998). Genome scan of human systemic lupus erythematosus: Evidence for linkage on chromosome 1q in African-American pedigrees. *Proc. Natl. Acad. Sci. USA* **95:** 14869–14874.

Ott, J. (1991). "Analysis of Human Linkage," Johns Hopkins Univ. Press, New York.

Pan, T. C., Zhang, R. Z., Speer, M. C., and Chu, M. L. (1998). CA repeat polymorphism of the COL6A3 gene on chromosome 2q37. *Hum. Hered.* **48:** 235–236.

Risch, N. (1987). Assessing the role of HLA-linked and unlinked determinants of disease. *Am. J. Hum. Genet.* **40:** 1–14.

Rosenberg, M., Hui, L., Ma, J., Nusbaum, H. C., Clark, K., Robinson, L., *et al.* (1997). Characterization of short tandem repeats from thirty-one human telomeres. *Genome Res.* **7:** 917–923.

Scaffer, A. A., Gupta, S. K., Shiram, K., and Cottingham, R. W., Jr. (1994). Avoiding recomputation in genetic linkage analysis. *Hum. Hered.* **44:** 225–237.

Shai, R., Quismorio, F. P., Jr., Li, L., Kwon, O. J., Morrison, J., Wallace, D. J., *et al.* (1999). Genome-wide screen for systemic lupus erythematosus susceptibility genes in multiplex families. *Hum. Mol. Genet.* **8:** 639–644.

Sheffield, D. A., Weber, J. L., Beutow, K. H., Murray, J. C., Even, D. A., Wiles, K., *et al.* (1995). A collection of tri- and tetranucleotide repeat markers used to generate high quality, high resolution human genome wide linkage maps. *Hum. Mol. Genet.* **4:** 1837–1844.

Smith, C. A. B. (1957). Counting methods in genetical statistics. *Ann. Hum. Genet.* **21:** 254–276.

Smith, C. A. B. (1961). Homogeneity test for linkage data. *Proc. Second Int. Congr. Hum. Genet.* **1:** 212–213.

Ståhl-Hallengren, C., Jönsen, A., Nived, O., and Sturfelt, G. (2000). Incidence studies of systemic lupus erythematosus in Southern Sweden: Increasing age, decreasing frequency of renal manifestations and good prognosis. *J. Rheumatol.* **27:** 685–691.

Tan, E. M., Cohen, A. S., Fries, J. F., Masi, A. T., McShane, D. J., Rothfield, N. F., *et al.* (1982). The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum.* **25:** 1271–1277.

Tan, F. K., and Arnett, F. C. (1998). The genetics of lupus. *Curr. Opin. Rheumatol.* **10:** 399–408.

Terwilliger, J. D. (1995). A powerful likelihood method for the analysis of linkage disequilibrium between trait loci and one or more polymorphic marker loci. *Am. J. Hum. Genet.* **56:** 777–787.

Terwilliger, J. D. (2000). A likelihood-based extended admixture model of oligogenic inheritance in "model-based" or "model-free" analysis. *Eur. J. Hum. Genet.* **8:** 399–406.

Terwilliger, J. D., and Ott, J. (1992). A haplotype-based haplotype relative risk statistic. *Hum. Hered.* **42:** 337–346.

Terwilliger, J. D., and Weiss, K. M. (1998). Linkage disequilibrium mapping of complex disease: Fantasy or reality? *Curr. Opin. Biotechnol.* **9:** 578–594.

Terwilliger, J. D., and Göring, H. H. H. (2000). Gene mapping in the 20th and 21st centuries: Statistical methods, data analysis and experimental design. *Hum. Biol.* **72:** 63–132.

Wakeland, E. K., Morel, L., Mohan, C., and Yui, M. (1997). Genetic dissection of lupus nephritis in murine models of SLE. *J. Clin. Immunol.* **17:** 272–281.

Watson, M. L., Rao, J. K., Gilkeson, G. S., Ruiz, P., Eicher, E. M., Pisetsky, D. S., *et al.* (1992). Genetic analysis of MRL-lpr mice: Relationship of the Fas apoptosis gene to disease manifestations and renal disease modifying loci. *J. Exp. Med.* **176:** 1645–1656.