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FULL PAPER

Fine mapping chromosome 16q12 in a collection of 231 systemic lupus erythematosus sibpair and multiplex families

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Systemic lupus erythematosus (SLE) is a chronic, autoimmune disorder influenced by multiple genetic and environmental factors. Linkage of SLE to chromosome 16q12–13 (LOD score = 3.85) was first identified in pedigrees collected at the University of Minnesota, and has been replicated in several independent SLE collections. We performed fine mapping using microsatellites to further refine the susceptibility region(s), and the best evidence for linkage was identified at marker D16S3396 (LOD = 2.28, P = 0.0006). Evidence of association was suggested in the analysis of all families (D16S3094, P = 0.0516) and improved to the level of significance (P = 0.0106) when only the Caucasian families were analyzed. Subsets of pedigrees were then selected on the basis of clinical manifestations, and these subsets showed evidence for association with several markers: GATA143D05 (renal, P = 0.0064), D16S3035 (renal, P = 0.0418), D16S3117 (renal, P = 0.0366), D16S3071 (malar rash, P = 0.03638; neuropsychiatric, P = 0.0349; oral ulcers, P = 0.0459), D16S3094 (hematologic, P = 0.0226), and D16S3089 (arthritis, P = 0.0141). Together, these data provide further evidence that an important susceptibility gene(s) for SLE is located at 16q12.
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Systemic lupus erythematosus (SLE) is a heterogeneous, systemic autoimmune disease characterized by the production of autoantibodies to a multitude of self-antigens. SLE predominantly affects female subjects, and prevalence rates are increased in several non-Caucasian groups.[1–5] Clinical manifestations vary among individual patients and among ethnic groups and are thought to be due, in part, to underlying genetic heterogeneity. Evidence to support complex genetic and environmental contributions to SLE comes from numerous population and family-based studies.[6–11] Linkage studies in human SLE pedigrees have identified multiple regions that meet Lander and Kruglyak's[12] strict criteria for significant linkage: 1q23, 1q31, 1q41–42, 2q37, 4p16, 6p21, 16q12–13, and 17p13.[13–24]

Chromosome 16q12 is among the most consistently replicated regions in SLE linkage studies. We provided the first evidence for linkage at 16q12 (D16S415, LOD = 3.47) in 105 pedigrees[15] and increased evidence for linkage (D16S415, LOD = 3.85) with the addition of data from 82 independent pedigrees.[18] Evidence to support linkage to the 16q12–13 region has been identified in four additional, ethnically diverse family collections, in studies that employed a variety of experimental designs. First, Shai *et al*[16] reported supportive evidence for linkage in Mexican-American ($N = 43$) and Caucasian ($N = 37$) families at 16q12 (D16S3136, $P = 0.017$) approximately 5 cM centromeric to the D16S415 marker, which resides at the peak of the linkage interval observed in the MN pedigrees. Second, Tsao *et al*[22] identified linkage ($P = 0.001$) to this same region (between D16S3136 and D16S415) in non-Caucasian ($N = 68$) as opposed to Caucasian ($N = 77$) affected sibpairs. Third, Nath *et al*[24] found evidence for linkage to chromosome 16q12 (D16S3253, $P = 0.000005$) in a combination of European-American ($N = 82$) and African-American ($N = 38$) families using parametric linkage analysis methods. Finally, evidence for family-based association at 16q12 was reported in a Chinese population ($N = 157$) (D16S409, $P = 0.0278$; D16S517, $P < 0.0001$).[25] This strength and concordance of evidence for linkage in multiple SLE family collections, rarely observed in complex diseases, serves as a catalyst for fine mapping this region of chromosome 16.

Here, we present the results of a dense mapping effort using microsatellites in an expanded collection of 231 SLE multiplex pedigrees, which includes 44 families not

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previously reported. These additional families consist of Caucasians ($N=32$), Hispanics ($N=4$), African-Americans ($N=4$), Asians ($N=2$), Middle Eastern ($N=1$), and mixed heritage ($N=1$). There were 30 families comprising at least two affected sibpairs and 14 multiplex families. Details regarding family ascertainment and microsatellite marker genotyping of the first 187 University of Minnesota families have been previously described.[15] In total, there were 243 affected sibpairs and 492 affected individuals among the 841 genotyped individuals. The ethnic breakdown of the entire SLE family collection studied (total $N=231$) is as follows: Caucasian ($N=182$, 79%), African-American ($N=21$, 9%), Hispanic ($N=16$, 7%), Asian ($N=5$, 2%), Middle Eastern ($N=1$, $<1\%$), and mixed heritage ($N=7$, 3%). This study was approved by the University of Minnesota Institutional Review Board for research on human subjects, and informed consent was obtained from all subjects.

Multipoint nonparametric linkage analysis using MERLIN[26] was first performed on the entire family collection ($N=231$) using the 13 genome-screen microsatellite markers previously reported.[15] These markers span the entire chromosome, with an average intermarker distance of 9.6 cM. A maximum LOD score of 3.17 ($P=0.00007$) was obtained at 71.4 cM (Figure 1).

To assess potential heterogeneity due to ethnicity, we analyzed the Caucasian families ($N=183$) alone. A LOD score of 2.78 was obtained at 67.1 cM in the Caucasian subset, indicating that there was a positive contribution to the overall linkage result (LOD $=0.12$, $P=0.2$) from the smaller group of non-Caucasian families ($N=48$).

We next typed an additional 41 microsatellite markers in the region between 40.6 and 92.1 cM. The average linkage information content across the fine-map region was 0.72. The 47 markers were separated by an average distance of 1.1 cM with the largest gaps occurring near 45.6 cM (between D16S3046 and D16S3113, 4.91 cM), near 62.5 cM (between D16S746 and D16S3044, 3.99 cM), and near 88.2 cM (between D16S3043 and D16S3106, 3.43 cM). Multipoint linkage analysis of the data generated from the dense marker screen yielded a LOD score of 2.38 near D16S3396 (63.8 cM, $P=0.0006$) (Figure 1). Thus, after fine mapping, the best evidence for linkage shifted $\sim$7.6 cM centromeric from the peak observed using the genome-screen markers.

Using data from all microsatellites that mapped between 40.6 and 92.1 cM, we carried out single-marker pedigree disequilibrium test (PDT) analyses to detect excessive allele transmission to affected offspring in the full data set of 231 pedigrees. We found evidence approaching statistical significance for distortion of allele transmission at D16S3094 ($P=0.0516$), which increased to the level of significance ($P=0.0106$) upon examination of the Caucasian pedigree subset ($N=183$). Given that there is effectively no marker–marker linkage disequilibrium among these markers, haplotype analysis on the microsatellites is not a particularly strong inferential tool.

Recent studies from Harley and co-workers[27] have suggested the possibility that subdividing families based on clinical phenotypes may have value in linkage analysis for SLE, in theory by reducing etiologic heterogeneity. In an exploratory analysis, we next evaluated subsets of pedigrees, selected based on clinical criteria for classification of SLE as proposed by the American College of Rheumatology (ACR).[28,29] For any given clinical feature, a pedigree was included if at least one individual with SLE expressed that particular

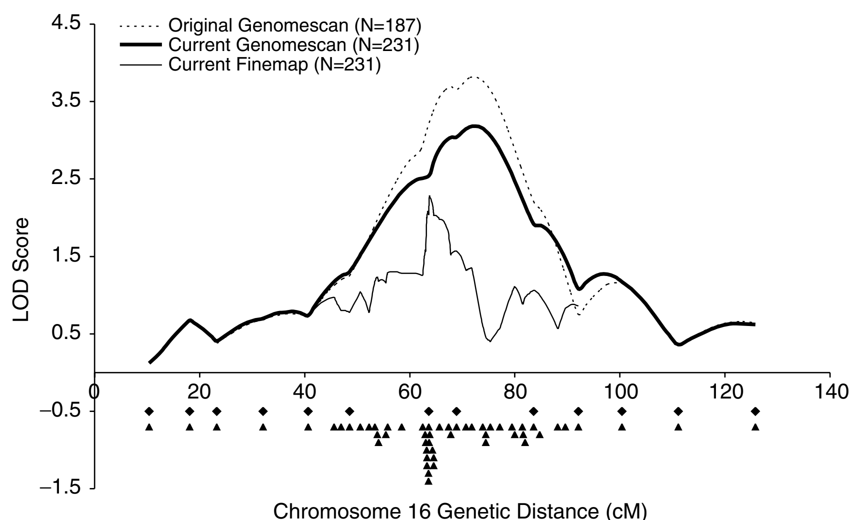

Figure 1 Multipoint linkage analyses on chromosome 16 in 231 SLE families. LOD scores are shown for nonparametric linkage analysis using the NPLpairs statistic computed using the software MERLIN. LOD scores are plotted against the genetic distance in centimorgans (cM). The dashed line shows the results from previous genome scans in the Minnesota collection using 13 microsatellite markers in 187 multiplex pedigrees. The thick solid line shows the genome-scan results using the same 13 microsatellite markers in the entire collection of 231 families. The thin solid line shows the linkage results from the fine map generated using 41 additional microsatellite markers in the region between 40.6 and 92.1 cM in 231 families. The approximate positions of the original genome-screen markers are represented by diamonds, and the fine-map markers together with the genome-screen markers used for the analysis are shown as triangles. Marker order and distances between markers were determined using maps available from the Center for Medical Genetics, Marshfield Medical Research Foundation http://research.marshfieldclinic.org/genetics/ and the University of California Santa Cruz http://genome.cse.ucsc.edu/. Genetic and physical maps were used to extrapolate the genetic distances when intermarker genetic distances were zero.

Table 1 Subsetting of SLE families based on clinical characteristics[a]

ACR criteria	*No. (%) individuals*	*No. (%) pedigrees*
Arthritis	469 (94)	217 (94)
Discoid rash	118 (24)	53 (23)
Hematologic[b]	351 (71)	159 (69)
Immunologic[c]	403 (81)	184 (80)
Malar rash	439 (88)	204 (88)
Neuropsychiatric	219 (44)	99 (43)
Oral ulcers	393 (79)	183 (79)
Photosensitivity	456 (92)	211 (91)
Renal	194 (39)	87 (38)
Serositis	408 (82)	192 (83)

[a]Pedigree subsets were generated based on at least one individual in the family having the clinical feature.
[b]Hematologic criteria include hemolytic anemia, leukopenia, lymphopenia, and thrombocytopenia.
[c]Immunologic criteria include the presence of anti-dsDNA, anti-Sm, or anti-phospholipid antibodies in serum.

phenotype. Frequencies of the ACR clinical features within the individuals and families studied are presented in Table 1. We did not include analysis of the subset defined by presence of antinuclear autoantibodies (ANAs) because all but one pedigree met this criterion. The analysis of pedigree subsets provided evidence for modest levels of association with multiple markers in the region of interest using the PDT: GATA143D05 (renal, $P = 0.00637$), D16S3035 (renal, $P = 0.0418$), D16S3117 (renal, $P = 0.0366$), D16S3071 (malar rash, $P = 0.03638$; neuropsychiatric; $P = 0.0349$; oral ulcers, $P = 0.0459$), D16S3094 (hematologic, $P = 0.0226$), and D16S3089 (arthritis, $P = 0.0141$) (see Figure 2).

Interestingly, the evidence for association in the clinical subset analysis appeared to cluster in more than one region. The first region included the markers identified in the renal subset (GATA143D05, D16S3035, and D16S3117, mapping between 63.6 and 63.7 cM) and clustered within the major linkage peak identified near D16S3396 (at 63.8 cM). Evidence for association was also found with a second group of three markers (D16S3071, D16S3094, and D16S3089, between 75.3 and 80.0 cM), which were located near a second, smaller observed linkage peak (D16S3089, maximum LOD = 1.11, at 80.0 cM).

SLE is clearly a heterogeneous disease at both the genotypic and phenotypic levels.[27] The reduction in LOD score following the addition of 44 pedigrees with both the genome-screen and fine-mapping markers is likely to be explained by increased genetic heterogeneity introduced by the addition of unlinked pedigrees. This is consistent with the evidence for heterogeneity described by Nath *et al*[24] in which maximum evidence for linkage was observed in only 35% of pedigrees (HLOD = 4.85). The reduction in overall evidence for linkage could also result from an increase in genotyping errors, or

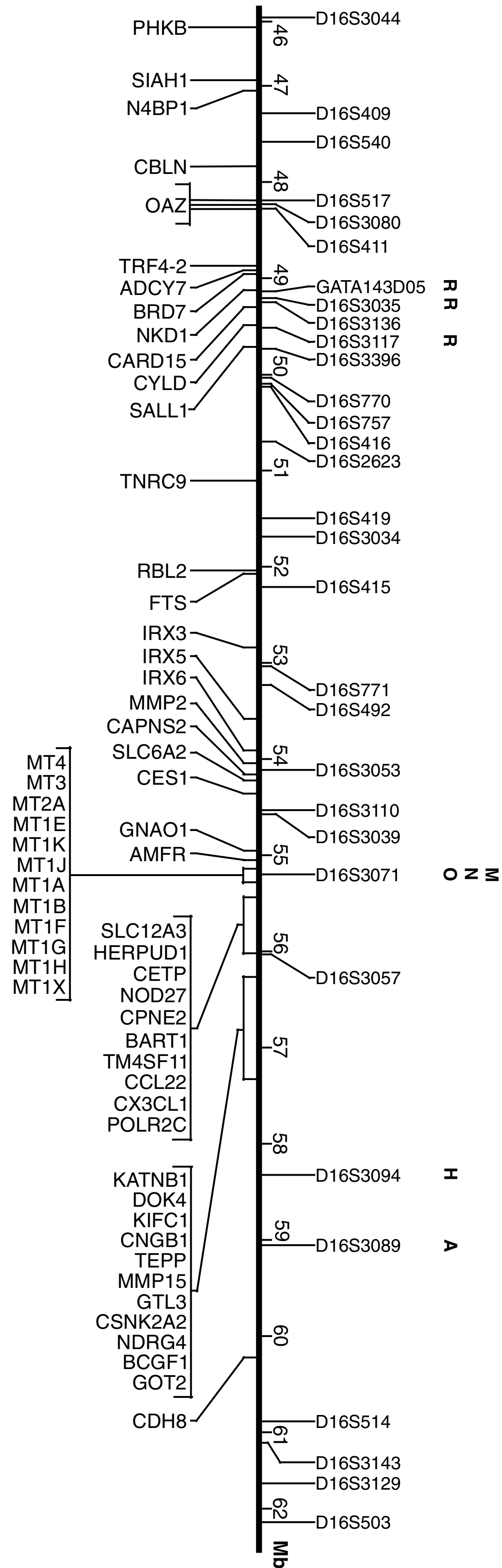

Figure 2 Map of the chromosome 16q12 region and summary of PDT. Shown are the markers used in this study and the major known genes in the region. The locations of markers that showed association with family subsets, subdivided based on clinical features, are also highlighted. R: renal; M: malar rash; N: neuropsychiatric; O: oral ulcer; H: hematologic; A: arthritis.

inaccuracies of marker order. However, we attempted to carefully screen these data for genotyping errors during the genotyping process and subsequently via testing for inconsistencies with simple Mendelian inheritance patterns and unlikely double recombinants.

Evidence for association in renal pedigrees was found for multiple markers within the fine-mapping linkage peak at 63.8 cM, a region that contains a number of interesting candidate genes, most prominently CARD15. Recent data have indicated a role for CARD15 mutations in genetic susceptibility to autoimmune Crohn's disease, an inflammatory disorder of the small bowel, and psoriatic arthritis.[30–32] CARD15 (also known as NOD2) functions in innate immune responses to intracellular lipopeptides leading to activation of NF-kappaB.[33] We have recently examined the known CARD15 polymorphisms implicated in Crohn's disease in our SLE family collection, but these single-nucleotide polymorphisms (SNPs) do not show evidence for association (R Graham *et al*, unpublished data). This could be an issue of statistical power, and may require a larger data set. Alternatively, there may be additional genetic variation within the gene, and a more comprehensive analysis of CARD15 in SLE is needed.

The family subsets defined by presence of malar rash, neuropsychiatric complications, and oral ulcers all showed significant association with D16S3071, a marker that resides within a cluster of metallothionein genes (MT4–MT1L). These genes encode for low molecular weight heavy metal-binding proteins, which have a role in zinc and copper homeostasis, and may protect against damage from reactive oxygen species. This is of interest given previous data indicating that SLE T cells show evidence for oxidative stress,[34] and the known relationship between oxidants and apoptotic cell death. Stress-induced apoptosis may play a role in exposing certain autoantigens commonly targeted by autoantibodies in SLE.[35,36] Furthermore, transcripts for several metallothionein ((MT)-2A, 1H, 1X, 1E, 1F, 1H, 1L) genes were recently shown to be upregulated in the peripheral blood cells of patients with SLE by microarray analysis (E Baechler *et al*, unpublished data). The metallothionein family of genes has not previously been implicated in genetic risk for SLE or other autoimmune diseases.

In summary, our data have identified an area of peak linkage centered around the CARD15 locus on chromosome 16q12 at 63.8 cM. Evidence for microsatellite allele association is also observed in this region within families enriched for renal disease. Other subsets of families show association with markers telomeric to the CARD15 locus. The 16q12 region has now been typed by the HapMap project at a resolution of approximately one SNP every 4–5 kb (www.hapmap.org), and these data provide a high-level view of linkage disequilibrium patterns across this region, as well as a host of validated SNP markers. We are currently typing a dense map of SNPs across this region, with the goal of identifying the precise genetic variation that contributes to SLE susceptibility.

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