A Candidate Region on 11p13 for Systemic Lupus Erythematosus: A Linkage Identified in African-American Families

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Systemic Lupus Erythematosus (SLE), a chronic, complex disease, is the prototype for systemic human autoimmune diseases. Although environmental factors are crucial in triggering the condition, twin and family studies, as well as genetic linkage and association studies, have established its strong genetic predisposition. During the past few years, there has been considerable interest in identifying genomic segments linked to SLE through either a whole genome scan or a candidate gene approach. The discoid lupus erythematosus (DLE) skin lesion is one of the major, and discriminating, manifestations in SLE, especially in African American patients. In this study we have identified 58 multiplex families—27 African American, 26 European American, and 5 others—where at least one SLE patient is also reported to be afflicted with DLE. These families were chosen from the collection of families that are part of our ongoing linkage study for SLE. A genome-wide parametric and nonparametric linkage analysis was conducted with 320 markers. Significant evidence of linkage was identified in only one chromosomal location, 11p13, in the African American families. The maximum 2-point linkage was 5.6 in these pedigrees, obtained at a marker located 47 cM away from the p-terminal end of chromosome 11. The peak multipoint LOD score of 4.6 was obtained very nearby. The segregation of this gene suggests dominant inheritance. These results reveal an important genetic effect related to discoid rash at 11p13 in African Americans with SLE, and demonstrate, through increasing genetic homogeneity, the power of pedigree stratification to detect linkage in complex diseases. ***Keywords: discoid lesion/genome scan/linkage/SLE. J Investig Dermatol Symp Proc 9: 64–67, 2004***

Systemic lupus erythematosus (SLE) is a disease of diverse clinical, serological, and pathologic features in which immune responses are directed against a multitude of self antigens. This astonishing variation might be explained by the inheritance of different combinations of susceptibility genes, possibly in combination with different environmental factors. Evidence for a genetic basis is established through significant familial aggregation with 7%–12% increased risk among the first- or second-degree relatives of a proband (Vyse and Todd, 1996), an increased concordance rate in identical twins (15–69%) as opposed to dizygotic twins (2%–5%) (Deapen *et al*, 1992), and genome scans showing moderate support for genetic linkage in a number of genomic locations (Gaffney *et al*, 1998; Moser *et al*, 1998; Shai *et al*, 1999; Gaffney *et al*, 2000; Gray-McGuire *et al*, 2000; Magnusson *et al*, 2000; Kelly *et al*, 2002; Lindqvist *et al*, 2002; Harley 2002). The relative risk ratio for the siblings of an affected proband (λ_s) varies from 20 to 40 (Wandstrat, 2001). Significant gender differences are observed in prevalence, age at onset, premorbid conditions, clinical expression, course of illness, response to treatment, and morbid risk. In addition, there are important racial differences in disease manifestations (Kelly *et al*, 2002; Harley, 2002). Clearly, SLE is a heterogeneous and complex genetic disease.

Complex genetic phenotypes are characterized by multiple loci with multiple common susceptibility alleles that have apparently low penetrances, with no allele being necessary or sufficient for clinical disease (Lander, 1996; Chakravarti, 1999). Their identification is difficult, requiring very large numbers of multiplex families for analysis. Various combinations of contributing alleles at multiple genes in individual patients may give the appearance of disease phenocopies. Clinical heterogeneity of the disease may also arise from different genotype–phenotype interactions. Consequently, analysis of such diseases requires large numbers of carefully genotyped and phenotyped families coupled with strategies to incorporate the locus heterogeneity into the analysis.

A clinical feature or phenotypic covariate has allowed very complicated genetic situations to be simplified in a few notable successes of gene discovery in complex diseases. BRCA1 is the classic example (Miki *et al*, 1994). The more than 10 genes for syndromic hyper- and hypotension (Lifton, 1996) are others. Stratification based on the presence of at least one case of the follicular variant of papillary thyroid carcinoma provided very convincing evidence of linkage to the 2q22 region (McKay *et al*, 2001). A similar approach (Samuelsson *et al*, 1999) found a significant linkage to human leukocyte antigen on chromosome 6p for familial psoriasis, although the relationship of HLA with psoriasis has been known for many years. Recently, we found that pedigree

Manuscript received September 18, 2002; revised December 30, 2002; accepted for publication December 30, 2002

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Abbreviations: AA, African American; DLE, Discoid Lupus Erythematosus; EA, European American; SLE, Systemic Lupus Erythematosus

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stratification by variable clinical features provides strong evidence for a number of linkages (Scofield *et al*, 2002; Nath *et al*, 2001; Kelly *et al*, 2002; Namjou *et al*, 2002a; Nath *et al*, 2002; Sawalha *et al*, 2002; Quintero-Del-Rio *et al*, in press;

The purpose of pedigree stratification is to achieve genetic homogeneity by reducing phenotypic and clinical heterogeneity, by reducing heterogeneity at the loci being evaluated. If successful, linkage analysis based on SLE families also containing SLE individuals with a relevant clinical covariate has the potential to greatly improve the detection of the susceptibility gene(s) for SLE.

Because DLE manifestations were part of the SLE phenotype for some patients, but not all, and because SLE and DLE are associated at the population level, we hypothesized that SLE patients and their families with DLE manifestations might be more genetically homogeneous at loci important to DLE-related SLE and hence would facilitate the detection of genetic linkage. This prediction has been shown to be true, and a linkage has been found at 11p13 in the African American pedigrees multiplex for SLE that contain at least one affected with DLE.

MATERIALS AND METHODS

SLE family ascertainment Families were recruited from all regions of the United States and Canada. All of the SLE patients met the 1997 revised criteria for SLE diagnosis (Tan *et al*, 1982; Hochberg, 1998). A detailed procedure for recruitment of families has been described elsewhere (Moser *et al*, 1998; Gray-McGuire *et al*, 2000). All the linkage analyses were based only on the families multiplex (more than two affected individuals) for SLE. These families came from three major ethnic backgrounds: European American (EA), African American (AA), Hispanic, and others. A total of 194 SLE families were used in the analysis. Of these, 113 were EA, 66 were AA, and 15 were Hispanic or of another self-identified group.

Stratification based on the presence of discoid lupus erythematosus To evaluate evidence of genetic linkage in DLE-related SLE families in the present study, we identified 26 families of EA origin and 27 families of AA origin, ascertained from the collection of 194 pedigrees multiplex for SLE through the presence of at least one SLE case in the pedigree with DLE. SLE and DLE status were determined by medical record review and direct patient interview. Skin biopsies were not usually obtained. Among the EA families, 58 members were affected with SLE (average of 2.2 affecteds per family), of which 32 also were alleged to have DLE. Among the 27 AA families, 66 members were affected with SLE (average of 2.43 affecteds per family), of which 34 were alleged to have DLE. The difference in proportion of SLE affecteds with DLE manifestations between EA and AA family members was not statistically significant ($\chi^2 = 0.051$, 1 d.f. $p < 0.82$). Because many SLE linkages are race specific, however, we analyzed the data separately for the self-identified AA and EA pedigrees. The number of families with SLE and DLE is given by race in **Table I**.

Genotyping and error checking Genomic DNA was isolated from peripheral blood cells, buccal cell swabs, mouth wash specimens, or EBV transformed cell lines using standard methods. Methods for the genotyping of families have been described elsewhere (Moser *et al*, 1998; Gray-McGuire *et al*, 2000). A total of 318 microsatellite markers with an average heterozygosity of 76% (range 56%–94%) were typed. Average marker spacing was 11 cM (range 2–25 cM). For the initial genome scan, we used the genetic map and intermarker distances from the Marshfield database. Prior to linkage analysis, all family relationships were confirmed using RELTEST (Olson, 1999).

Pedigree simulation False positive linkages are more likely when there are a small number of pedigrees contributing to the result. Consequently, we simulated the ascertainment of pedigrees for stratification using the genome scan data by randomly selecting 27 of the 66 AA pedigrees (sampling with replacement) and performing a genome scan analysis on each set of 27 pedigrees. We generated 10,000 resampled data-sets of 27 AA pedigrees (sampling with replacement) and calculated the 2-point LOD score using six models of inheritance. We retained the highest LOD score for each resampled set of 27 and constructed an empirical distribution of LOD scores. From this distribution we also calculated the observed mean LOD score and standard deviation (SD).

Table I. Stratification based on SLE and DLE. The first four entries are about the total collection used to find the pedigrees with DLE patients

Types of Families	Number
AA families	66
EA families	113
Other	15
Total	194
AA families with DLE	27
SLE affecteds	66
SLE affecteds with DLE	32
EA families with DLE	26
SLE affecteds	58
SLE affecteds with DLE	32

Statistical analysis A χ^2 test was used to identify clinical and serologic features associated with SLE with and without DLE (**Table II**).

To screen the genome for linkage, we first calculated 2-point LOD scores using six models of inheritance in the parametric maximum likelihood method (Moser *et al*, 1998; Gray-McGuire *et al*, 2000) with the FASTLINK program (Cottingham *et al*, 1993). Individual pedigree–specific LOD scores were estimated at different recombination values in 0.05 increments from 0.0 (complete linkage) to 0.5 (no linkage). These pedigree-specific LOD scores were then summed together in order to obtain the overall LOD score at each recombination value. Because the disease affects males and females differentially, this difference was also incorporated in two of the models by using two separate penetrance functions for male and female. Once we found a significant linkage to a marker (LOD score > 3.0), the optimum model was chosen from a large set of models generated under fixed population prevalence. The race-specific allele frequencies were obtained from the unrelated individuals from the colleted pedigrees. The multipoint parametric linkage analysis was performed under both homogeneity and heterogeneity. For this we used the GENEHUNTER program (Kruglyak *et al*, 1996).

RESULTS

Race-specific frequencies of all 18 standard clinical and serological criteria between SLE patients with DLE and SLE patients without DLE are shown (**Table II**). None of the features is statistically different between the major groups within the race. Also notable is that thrombocytopenia is more than five times more frequent in the SLE affecteds with DLE in AA, but the sample size is too small for even this difference to be statistically relevant. The difference in proportion of SLE affecteds with DLE manifestations between 26 EA and 27 AA families was not statistically significant ($\chi^2 = 0.051$, 1 d.f. $p < 0.82$). From our entire collection of 194 pedigrees, however, we have 151 AA affecteds and 261 EA affecteds with SLE. Now the proportion of SLE affecteds with DLE manifestations between AA and EA is statistically significant: 34/151 = 23% versus 32/262 = 12% ($\chi^2 = 7.47$, $p = 0.006$). Obviously, not all of the samples here are independent. In this case, then, we may select only one patient from each pedigree with DLE and, interestingly, the proportion is still significant: 27/66 = 40% versus 26/113 = 23% ($\chi^2 = 6.40$, $p = 0.01$).

From the initial genome scan based on 2-point linkage analysis, one marker on chromosome 11, D11S1392, showed significant evidence of linkage (LOD score = 5.2) only in AA pedigrees. The cytogenetic location of this marker is 11p13. When we combined the data between AA and EA, the LOD score at this marker was –2.3, which clearly demonstrated no linkage with this marker. We then maximized the model at this marker for only AA families. This time the LOD score was increased to 5.6 (p-value $= 3.7 \times 10^{-7}$). The estimated recombination fraction where the LOD score was maximized was 0.0. The maximized model was obtained as a dominant allele (disease allele frequency = 0.1) with penetrances of 65% for male and 99% for

Table II. Discoid clinical features (27 AA SLE affecteds with DLE randomly selected and compared with 27 AA SLE affecteds randomly selected from pedigrees without DLE), (26 EA SLE affecteds with DLE randomly selected and compared with 26 EA SLE affecteds from pedigrees without DLE)

	African-American		European-American	
Clinical features	SLE with DLE (N = 27)	SLE without DLE (N = 27)	SLE with DLE (N = 26)	SLE without DLE (N = 26)
Malar Rash	12	9	13	12
Photosensitivity	6	5	16	13
Oral lesions	8	3	9	8
Arthritis	21	19	9	8
Pericarditis	8	6	1	4
Pleuritis	6	6	9	7
Nephritis	11	16	6	4
Neurologic	4	6	6	4
Hemolytic Anemia	2	3	3	2
Leukopenia	16	12	6	8
Lymphopenia	15	15	10	11
Thrombocytopenia	5	1	3	2
Anti-dsDNA	15	17	9	11
Anti-Sm	8	3	2	0
ACL	8	10	8	8
Anti-nRNP	15	11	4	3
Anti-Ro	9	10	7	8
Anti-La	3	3	4	3

NOTE: Clinical features are defined in Tan *et al* (1982) or Hochberg (1997). Anti-Sm, anti-nRNP (nuclear ribonuclear protein), anti-Ro and anti-La are determined by Ouchterlony double immunodiffusion. ACL is anti-cardiolipin

female. On the other hand, the LOD and HLOD scores for all AA families were –0.17 and 1.74, respectively, at this marker. We did not find any statistically significant evidence of linkage in EA families in the genome scan.

We also performed the multipoint linkage analysis considering all the 14 markers on chromosome 11 together. This time the maximum multipoint LOD score under the homogeneity model was 4.3 (p-value $= 8.7 \times 10^{-6}$); under the heterogeneity model, the LOD score was 4.6 (p-value $= 4.2 \times 10^{-6}$) with 81% of the families being linked ($\alpha = 0.81$). Both linkage analyses reached the peak LOD scores, which are very close to D11S1392 (**Fig 1**).

We also performed a simulation experiment to evaluate our results. From the 10,000 simulations, none of the multipoint LOD scores exceeded the value of 5.6, which gives a p-value below 0.0001. In fact, the LOD scores under the null hypothesis ranged from –2.07 to 3.48, the average was –0.44, and the estimated standard deviation was 2.47.

Interestingly, earlier we detected this genomic location for SLE susceptibility by stratifying the pedigree based on thrombocytopenia (Scofield *et al*, 2002). There are eight families in common with the present study. These eight families yielded a LOD score of 4.5, based on the best-fitting model (the model that yielded the maximum LOD score of 5.6 for 27 AA families) for the present study. We also performed a genome scan using the remaining 19 (27–8) nonthrombocytopenic families to search for additional evidence of linkage. No new significant linkage was found in this subgroup (data not shown).

DISCUSSION

One major SLE susceptibility locus was detected on the short arm of chromosome 11 (11p13) by stratifying the pedigrees according to the presence of DLE. The estimated linkage results were significant in both 2-point and multipoint analyses (exceeding the genome-wide significance criteria established by Lander and Kruglyak in 1995 (Lander and Kruglyak, 1995). DLE is more frequent in AA than in EA SLE patients in most studies (Jacyk and Damisah, 1979). This is also evidenced from our study. Interestingly, we earlier detected this genomic location for SLE susceptibility by stratifying the pedigree based on thrombocytopenia (Scofield *et al*, 2002). From both of these studies, it is clearly highly likely that there is a gene that contributes the SLE susceptibility.

One striking feature of our results is that the 11p13 putative gene for SLE shows linkage in the AA families but not in the EA families, although the frequency of DLE is no different between EA and AA. Clearly, the SLE susceptibility gene at this locus is race specific, a pattern similar to that seen in studies of other complex diseases (Lernmark and Ott, 1998).

The outcome of this experiment is provocative and has led to a search for possible candidate genes for further study. There are several potentially interesting candidate genes located within this genomic region (11p13), which spans the region between 31 megabases and 39 megabases (from the UCSE genome browser). In fact, from the database search we have found seven potential candidate genes based on their known functions in the immune system (CD44, RAG1), apoptosis (HIPK), blood cell development and regulation (CAT), EBV virus infection (TRAP), previous association with disease (WT1-Wilms tumor), and epithelial cell development (EHF). At this point any of these candidate genes has the potential to contain a mutation or mutations

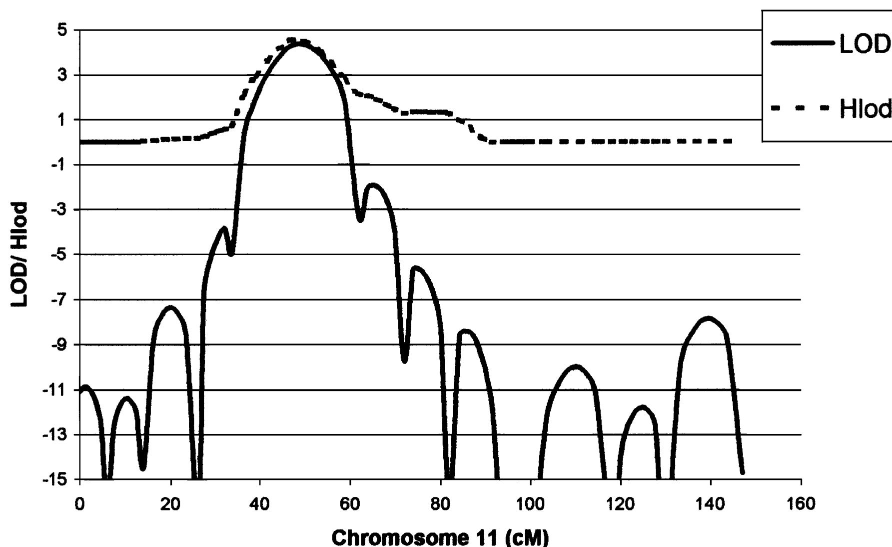

Figure 1. Multipoint model–based linkage assuming homogeneity (LOD score) and heterogeneity (HLOD score) for chromosome 11 in the 27 African American pedigrees containing one or more affected members with discoid lupus erythematosus (DLE). The marker positions and their intermarker distances (in cM) are, from left to right: D11S 1984-6.0-D11S2362-8.0-D11S 1999-12.0-D11S 1981-8.0-A34E8-13.0-D11S1392-15.0-D11S 1985-10.0-D11S2371-10.0-D11S 2002-12.0-D11S 2000-6.0-D11S 1986-18.0-D11S4464-12.0-D11S912-17.0-D11S2359.

that cause the relationship we observed in these data at 11p13. The availability of the genome sequence and accompanying dense SNP map will enable more exhaustive tests to pinpoint the putative susceptibility gene.

Because the presence of DLE manifestations in a family was used as a pedigree stratification criterion, and because DLE is a major criterion for identifying the SLE phenotype, perhaps DLE and SLE have a common autoimmune genetic determinant(s). These results demonstrate that, regardless of the actual number of genes involved in SLE, decreasing sample heterogeneity by subgrouping families on the basis of common associated phenotypes has increased the likelihood of identifying genes for SLE. To the best of our knowledge, the studies presented herein represent the first genome-wide survey for finding DLE-related SLE susceptibility genes.

In summary, we have identified a potentially informative genomic region at 11p13, which may contain the putative SLE susceptibility gene. We have also demonstrated the presence of significant genetic heterogeneity, which, when ignored, leads to underestimation of significant linkage. We have shown significantly increased evidence for linkage with SLE when we separately consider the DLE-related SLE families, a subgroup with potentially homogeneous genetic properties.

ELECTRONIC DATABASE INFORMATION

The URLs for the database information used in this article are as follows:

Candidate gene location for chromosome 11: http://nciarray.nci.nih.gov/cards/index.html

Marshfield database: http://research.marshfieldclinic.org

Lupus Multiplex Registry and Repository: http://omrf.ouhsc.edu/lupus

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

This study was supported by the NIH grants (AR048928, AR-12253, AI24717, AR42460, AR45231, AI131584, AR048940, AR049084, and RR15577). Cohorts A, B, and C were obtained from the Lupus Multiplex Registry and Repository (AR-1–2253).

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