Research article

Genetic linkage and transmission disequilibrium of marker haplotypes at chromosome 1q41 in human systemic lupus erythematosus

Robert R Graham, Carl D Langefeld*, Patrick M Gaffney, Ward A Ortmann, Scott A Selby, Emily C Baechler, Katherine B Shark, Theresa C Ockenden, Kristine E Rohlf, Kathleen L Moser, William M Brown*, Sherine E Gabriel†, Ronald P Messner, Richard A King, Pavel Horak‡, James T Elder§, Philip E Stuart§, Steven S Rich* and Timothy W Behrens

Department of Medicine, Center for Immunology, University of Minnesota Medical School, Minneapolis, MN, USA
*Department of Public Health Sciences, Wake Forest University School of Medicine, Winston-Salem, NC, USA
†Department of Health Sciences Research and Division of Rheumatology, Department of Internal Medicine, Mayo Clinic, Rochester, MN, USA
‡Interní Klinika, Olomouc, Czech Republic
§Department of Dermatology, University of Michigan Medical School, Ann Arbor, MI, USA

Correspondence: Dr Timothy W Behrens, University of Minnesota Medical School, 6-126 BSBE Bldg, 312 Church Street SE, Minneapolis, MN 55455, USA. Tel: +1 612 625 4485; fax +1 612 625 2199; e-mail: behre001@umn.edu

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Abstract

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by the production of autoantibodies to a wide range of self-antigens. Recent genome screens have implicated numerous chromosomal regions as potential SLE susceptibility loci. Among these, the 1q41 locus is of particular interest, because evidence for linkage has been found in several independent SLE family collections. Additionally, the 1q41 locus appears to be syntenic with a susceptibility interval identified in the NZM2410 mouse model for SLE. Here, we report the results of genotyping of 11 microsatellite markers within the 1q41 region in 210 SLE sibpair and 122 SLE trio families. These data confirm the modest evidence for linkage at 1q41 in our family collection (LOD = 1.21 at marker D1S2616). Evidence for significant linkage disequilibrium in this interval was also found. Multiple markers in the region exhibit transmission disequilibrium, with the peak single marker multiallelic linkage disequilibrium noted at D1S490 (pedigree disequilibrium test [PDT] global P value = 0.0091). Two- and three-marker haplotypes from the 1q41 region similarly showed strong transmission distortion in the collection of 332 SLE families. The finding of linkage together with significant transmission disequilibrium provides strong evidence for a susceptibility locus at 1q41 in human SLE.

Keywords: 1q41, autoimmunity, linkage, systemic lupus erythematosus, transmission disequilibrium

Introduction

Systemic lupus erythematosus (SLE [MIM 152700] [1]) is an autoimmune disease characterized by the production of autoantibodies with specificity for a wide range of self-antigens. These antibodies cause disease directly by binding to target tissues (e.g. platelets and phospho-
lipids), and indirectly by depositing immune complexes in vascular tissues, leading to organ damage [2]. The current evidence suggests that SLE is a complex genetic disease, with contributions from both environmental (e.g. ultraviolet light, viral infections) and genetic factors.

A number of recent genome-wide screens support the genetic hypothesis in human SLE, and have reported, in aggregate, 48 potential susceptibility loci [3–7] (reviewed [8]). Of these intervals, significant attention has been directed at the 1q41–42 chromosomal region. After the identification of the syntenic region in mouse (Sle1) as a susceptibility interval for murine SLE [9], a targeted study by Tsao et al [10] was the first to implicate this locus in human SLE (marker D1S229; \( P < 0.005 \)). Subsequently, two independent SLE genome scans also found suggestive evidence for linkage at 1q41–42 [3–5]. Poly ADP ribosyl polymerase (PARP; also referred to as ADPRT [adenosine diphosphate ribosyltransferase]) was initially suggested as the relevant candidate gene in the region [11,12], but that finding was not reproduced in other family collections [13].

The work of Moser et al [14] provided the initial evidence that the susceptibility locus in this region might lie centromeric of PARP. In 127 multiplex SLE pedigrees, the best evidence for linkage was at D1S229, with the greatest extent of allele sharing in the white families at the D1S2616 marker. The data reported here confirm the evidence for genetic linkage at 1q41 in 210 SLE sibpair families (families with a pair of affected sibs) from the Minnesota collection [3,4]. Furthermore, we report new evidence for transmission disequilibrium of both single marker alleles and short marker haplotypes from the 1q41–42 interval in 122 trio families (families with a single affected offspring and both parents) and in the total dataset of 332 SLE families. These data suggest that a human SLE susceptibility locus is located centromeric to PARP near the D1S490 marker.

Materials and methods

SLE family collection

The collection of 187 affected sibpair families in Cohorts 1 and 2 of the Minnesota collection have been described in detail elsewhere [3,4]. An additional group of 24 sibpair families was collected and members were genotyped for the 11 markers in the 1q41–42 region. One hundred and twenty-two trio families were also collected. This study was approved by the Human Subjects Review Board at the Mayo Clinic and at the University of Minnesota. The clinical features of the sibpair and trio families are provided in the Supplementary material.

Samples and genotyping

Genomic DNA isolation and genotyping of families was performed as described [3,4]. The 12 markers from the 1q41–42 region originally typed by Moser et al [14] were typed in the Minnesota collection. Marker order (Fig. 1) was determined using the public databases described in the Supplementary material.

Data analysis

See Supplementary material for details of linkage analysis and transmission disequilibrium testing.

Results

Genetic linkage in the 1q41–42 region

In the combined data from the original Cohorts 1 and 2 genome screens, the highest LOD score in the 1q41–42 region was 1.33 at marker D1S229 [4]. With the addition of five new fine-map markers and 24 new families, the best evidence for linkage was slightly reduced (LOD = 1.23) and shifted telomeric to marker D1S2616 (Fig. 2). This level of linkage support corresponds roughly to a nominal \( P \) value of 0.05. When only white families were considered, the peak multipoint LOD score remained at marker D1S2616 (LOD = 0.99; Fig. 2).

Transmission disequilibrium in the 1q41–42 region

The standard transmission disequilibrium test (TDT) [15], applied to the data for a single marker allele, failed to identify significant transmission disequilibrium (Fig. 3). Analyses with the C-TDT (the TDT combined with the discordant sibship [S-TDT]), which allows additional families containing discordant sibs to be evaluated for disequilibrium [16], were then performed. Among the 60 families with discordant siblings but missing parental information, several microsatellite alleles in the 1q41 region demonstrated significant evidence of transmission disequilibrium (Fig. 3). Markers D1S490–allele 6 (\( P = 0.0044 \)), D1S229–allele 7 (\( P = 0.0054 \)), and D1S227–allele 5 (\( P = 0.0084 \)) showed the strongest evidence for disequilibrium using the C-TDT.

The TDT and C-TDT are limited by the fact that only one triad or discordant sibling is analyzed from a given family; thus these two tests examine only a subset of the data from general pedigrees. The pedigree disequilibrium test (PDT) improves upon these two tests by generating a measure of linkage disequilibrium for every discordant sibship and triad in a pedigree [17]. Because it examines the entire data set, the PDT can result in much higher power than the other two tests [17]. The version of the PDT used in this analysis has been modified to accept dyads (a single typed parent and an affected offspring). A dyad is informative only when the offspring has a heterozygous genotype different from that of the parent, thus avoiding the bias of using inferred phase assignments [18,19]. Using the PDT, three single marker alleles of D1S490 exhibited evidence of transmission disequilibrium (Fig. 3). Consistent with the C-TDT results, D1S490–allele 6 yielded the strongest PDT result (\( P = 0.0071 \)).
Transmission disequilibrium of marker haplotypes in the 1q41–42 region

Two- and three-marker haplotypes from the 1q41–42 region were next tested for evidence of transmission disequilibrium (Fig. 3). Haplotype clusters were generated for 'exact match' haplotypes with more than 10 founders. Analysis of two- and three-marker haplotypes revealed that haplotypes from D1S490 to D1S549 had the greatest number of transmissions and yielded the most significant P values, with the majority of the significant haplotypes centered on D1S227. The two-marker haplotypes D1S425–allele 10/D1S2827–allele 11, D1S490–allele 7/D1S227–allele 5, and D1S2616–allele 6/D1S549–allele 12 were significant in the TDT, C-TDT, and PDT. The D1S490–allele 7/D1S227–allele 5 and D1S2616–allele 6/D1S549–allele 12 haplotypes had 63% (42:25) and 61% (52:33) transmitted:nontransmitted ratios (T:NT), respectively.

Using three-marker haplotypes, the strongest evidence for disequilibrium was in the region spanning the markers D1S229 to D1S2641. Notably, the D1S229–allele 7/D1S490–allele 7/D1S227–allele 5 (T:NT = 23:8, 79% T) and the D1S2616–allele 6/D1S549–allele 12/D1S2641–allele 10 (T:NT = 18:6, 75% T) haplotypes yielded biallelic PDT P values of < 0.008.

Global multiallelic disequilibrium statistic

The PDT test also calculates a measure of the linkage disequilibrium for all the alleles of a given marker in the form of a global multiallelic P value. Using this statistic, the 11 markers of 1q32–42 fine map were tested for global transmission disequilibrium (Fig. 4). Importantly, two markers in the 1q32–42 region reach the 0.05 level of significance in the data set from the 332 SLE families. D1S425 results in a global P value of 0.034, which
appeared to be largely driven by the contribution of families in the trio collection \( (P = 0.0056) \). D1S490 showed the most significant result using this test \( (P = 0.0091) \), with both the sibpair and trio collections contributing to the linkage disequilibrium observed. Significant results for this global disequilibrium statistic were not observed for any other markers in the 1q41–42 region, including ADPRT (PARP). Two- and three-marker haplotype analysis with the global PDT identified only one significant result. The D1S425–D1S2827 ‘window’ yielded a PDT global \( P \) value of 0.032.

**Discussion**

The localization of genes in complex genetic diseases is a challenging proposition, given that these disorders are likely to show significant locus heterogeneity, genetic epistasis, and incomplete penetrance, as well as environmental effects. In SLE, all the available evidence points to a similarly complex genetic etiology, with six recent genetic linkage studies implicating as many as 48 genetic loci [3–7,20]. While some of the loci identified are likely to be false positives or relatively minor genes enriched in one population or another, the locus at 1q41 is unique in that it has provided a significant, though modest, linkage signal in three independent SLE populations [3-5,10,14]. Fine-mapping in the 1q41 region by Moser et al [14] also showed that the highest overall LOD score in 127 families of the Oklahoma collection was at D1S229, and the greatest degree of allele sharing in 78 white families was at D1S2616. D1S229 also showed the strongest evidence for linkage in the UCLA collection [10], and was the best marker in genome screens performed on the Minnesota family collection [3,4]. Thus, the evidence for linkage at 1q41 is reproducible in independent collections using the identical markers.

To detect association with SLE, we used the transmission disequilibrium test (TDT), as well as two additional tests, the C-TDT and the PDT. The PDT is the strongest test of association because of its ability to maximize the information extracted from complex pedigrees. Importantly, marker D1S490 in the 1q41 region was significant on the multiallelic PDT (Fig. 4). Supporting the global finding, the C-TDT and PDT tests also identified evidence for transmission disequilibrium with several alleles from D1S490 and nearby markers (Fig. 3). Marker D1S425 in the 1q32 region also demonstrated significant transmission disequilibrium, particularly in the trio collection (Fig. 4).

We sought to confirm and expand upon the results of the single-marker tests by examining haplotypes from the 1q32–41 region for the presence of transmission disequilibrium. Haplotype-based association methods may be more powerful than single-marker tests [21–23]. For example, the same microsatellite allele (i.e. same size microsatellite repeat) may be present on a number of haplotypes, some of which may not be associated with disease. Indeed, haplotype-based association methods employing a dense map of markers have been used to localize genetic effects to small segments of chromosomes [24–29]. The knowledge of marker order and intermarker distances in the 1q41 region allowed the generation of haplotypes with unambiguously determined ‘phase’ in our large collection of SLE families. The D1S425–D1S2827 two-marker haplotype window in the 1q32 region showed evidence for transmission disequilibrium via the global PDT, while no other windows showed evidence of association using this multiallelic test. However, several individual two- and three-marker haplotypes from the 1q32 and 1q41 region showed significant transmission disequilibrium (Fig. 3). These haplotype results should be viewed with some caution, since they are uncorrected for the multiple allele combinations tested, and the possibility of Type I errors may be increased.

At present, the most interesting candidate gene in the region showing the strongest evidence for disequilibrium is the estrogen-related receptor gamma (ESRGRG). This gene is found on the same contig as D1S490 (NT_004817) and is an orphan receptor within the steroid hormone receptor superfamily. It is expressed in lymphocytes and other
Transmission disequilibrium of individual alleles and short marker haplotypes in the 1q32–42 region. Single-allele and two- or three-marker haplotype data sets were analyzed using the transmission disequilibrium test (TDT), the combined TDT and discordant-sib TDT (C-TDT), and the pedigree disequilibrium test (PDT). For the single-marker data, the entire collection of 332 families with systemic lupus erythematosus was used, while the two- and three-marker analyses were performed only on the 274 families with at least one typed parent. Only those marker alleles or haplotypes with at least one significant result are shown.

**Figure 4**

Global pedigree disequilibrium test at 1q32–42 in 332 families with systemic lupus erythematosus. The global (multiallelic) P-values found using the pedigree disequilibrium test are graphed for each of the 11 markers that comprised the fine map. The ‘All Families’ data are graphed in the left panel.
tissues and is an interesting candidate, given the suspected role of sex hormones in the pathophysiology of both mouse and human lupus [30,31]. Other genes in the region include those for the cathelicidin antimicrobial peptide (near D1S2618), an innate microbial defense peptide expressed by the skin during inflammation; MARK (near D1S2641), a serine/threonine protein kinase; and at least three uncharacterized genes.

The data reported here provide some additional perspective on the initial reports that PARP might be the relevant gene in this locus. The finding of significant transmission distortion of marker alleles centromeric to PARP in the Minnesota collection suggests the possibility that the disequilibrium initially reported for PARP alleles may be due to more extensive disequilibrium – to include the PARP marker – in the families studied by Tsao and her colleagues compared with other populations and groups of families studied. A dense mapping of this interval by all the various groups and a pooling of data would help to resolve this question. It seems likely that, as in the HLA region [32], there will be a limited number of ancestral haplotypes in the 1q41 region, and that these ancestral haplotypes will be identifiable by typing a dense map of microsatellites. This should facilitate the identification of the responsible gene(s) in the region.

Conclusions

The data reported here confirm the evidence for genetic linkage at 1q41 in 210 SLE sibpair families from the Minnesota collection. Furthermore, we report new evidence for transmission disequilibrium of both single marker alleles and short marker haplotypes from the 1q41–42 interval in 122 trio families and in the total dataset of 332 SLE families. These data suggest that a human SLE susceptibility locus is located centromeric to PARP near the D1S490 marker.

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Supplementary material

Supplementary materials and methods

Establishing marker order in the 1q41–42 region

The 12 markers from the 1q41–42 region originally typed by Moser et al [S1] were typed in the Minnesota collection (see Fig 1 in the main text of this article). Genetic maps for the region were obtained from the Marshfield Clinic (Marshfield, WI, USA; http://research.marshfield-clinic.org/genetics) and the Genome Database (http://www.gdb.org). Since genetic maps have limited resolution in the case of closely linked markers, the available human genomic sequence from the region was analyzed to more accurately assess marker order and intermarker distances. The Human Genome Project Working Draft database (based on October 7, 2000 freeze) at the University of California, Santa Cruz (http://genome.ucsc.edu), was the primary source used to establish marker order. Three markers (D1S425, D1S229, and D1S213) could not be located in this database. The UniSTS database (http://www.ncbi.nlm.nih.gov/genome/sts/epcr.cgi) located D1S425 on contig NT_004656. According to Mapviewer tool on the National Center for Biotechnology Information site (http://www.ncbi.nlm.nih.gov/genome/guide/human), D1S425 lies 4 Mb telomeric of D1S245. BLAST (http://www.ncbi.nlm.nih.gov/BLAST/) analysis of the human genome sequence was used to locate D1S229 on the contig NT_004817, 125 kb centromeric of D1S490. D1S2860 was found to map to chromosome 3 and thus was omitted from the present analysis. Only 1 marker, D1S213, was not located on any assembled contigs or draft sequence.

Samples and genotyping

Panels of markers were optimized such that markers bearing the same fluorescent tag could be multiplexed in polymerase chain reaction (PCR). PCR (32 cycles) was performed on an ABI 877 Catalyst robotic workstation (5 µl reactions – 5 ng of genomic DNA, 2.5 mM MgCl₂, 0.2 mM dNTPs [Pharmacia, Piscataway, NJ, USA], 0.2 U AmpliTaq Gold DNA polymerase [Perkin-Elmer, Foster City, CA, USA] in 1 x PCR Buffer II [Perkin-Elmer]). Individual primer-pair concentrations ranged from 0.31 to 3.30 pmol per reaction, based on the results of optimization runs. Pooled amplification products were electrophoresed through 5% polyacrylamide gels (FMC Bioproducts, Rockland, ME, USA) for 2 h at 3000 V on an ABI 377 DNA Sequencer. Semiautomated fragment sizing was performed using GENESCAN software (v. 2.1, ABI, Foster, CA, USA) followed by allele calling with GENOTYPER software (v. 2.5, ABI).

Data verification

Each genotype was reviewed manually by two members of the team to confirm the accuracy of allele calling. The genotype data from the 11 microsatellites used in the study were analyzed using PEDCHECK and RELATIVE to identify errors in Mendelian inheritance and to confirm familial relationships [S2,S3]. Additionally, the maximum-likelihood haplotypes for each pedigree (generated by GENEHUNTER PLUS [S4], a modified version of GENEHUNTER [S5]) were examined to identify multiple recombinants. Double recombinants were generally found to be genotyping errors and were corrected.

Linkage analysis

Multipoint nonparametric linkage analysis was performed using GENEHUNTER PLUS [S4]. We report the results based on the Sall scoring function, which emphasizes sharing the same allele identical-by-descent (IBD) among all affected family members [S6]. The reported logarithm of odds ratio (LOD) score is calculated as LOD = Zlr/2/ln10. In some instances, families of different ethnic groups were extracted from the master linkage file and analyzed separately. Allele frequencies used in the parameter files for each analysis were generated from the founder genotypes for the analyzed set of data (sibpair families, trio families, all families combined, and individual ethnic groups).
Transmission disequilibrium testing

The alleles from the 11-marker fine map were examined for the presence of transmission disequilibrium using the transmission disequilibrium test (TDT) [S7] and the discordant sibling TDT (S-TDT) test [S8]. Analysis was conducted using the combined TDT/S-TDT program version 1.1, set to accept one missing parent per trio [S8]. This program outputs separate results for the TDT, S-TDT and the combined TDT/S-TDT (referred to herein as C-TDT). In families with multiple affected individuals, only a single affected patient (the index case) was used for the TDT and C-TDT analyses.

Two- and three-marker haplotypes from the 1q32–41 region were tested for the presence of transmission disequilibrium. Maximum-likelihood haplotypes were generated using GENEHUNTER PLUS for the 274 families with at least one typed parent. Markers where phase could not be definitively determined were identified and labeled as missing data for the haplotype analysis. Founder haplotypes were then used to create two- and three-contiguous-marker exact-match clusters using a moving-window approach, as described by Nair et al [S9]. In order to prevent rare haplotypes from dominating the statistical tests and to better approximate the distributional assumptions of these tests, only those haplotypes with at least 10 founders were clustered. Haplotypes represented in fewer than 10 founders were pooled together. Every two- or three-marker ‘window’ was examined by moving the window across the 1q32–42 region one marker at a time from centromere to telomere. The resulting haplotype data were examined using the TDT and C-TDT with each haplotype considered as a single marker.

Additional information from the sibpair and multiplex families was extracted using the pedigree disequilibrium test (PDT) [S10]. The original form of the PDT incorporates information from every possible triad and discordant sibship in a given family, to yield an average measure of disequilibrium for the entire pedigree. In addition, we analyzed dyads (one parent and an affected offspring) when the offspring had a heterozygous genotype different from that of the parent [S11,S12]. The PDT was used to examine the single-marker and the two- and three-marker haplotype data.

Linkage analysis in families carrying ‘risk’ haplotypes

GENEHUNTER PLUS creates maximum-likelihood haplotypes when conducting multipoint linkage analysis. The GENEHUNTER PLUS haplotype output was used to identify families with founders carrying the seven three-marker haplotypes in the 1q41 region (D1S229–D1S2641) that had a TDT transmission:nontransmission ratio of at least 2:1 (66% transmission rate). Multipoint linkage analysis was conducted on the ‘risk’ and ‘nonrisk’ subsets as described above.

Supplementary results

Clinical features of systemic lupus erythematosus (SLE) in sibpair and trio families

The composition of the sibpair families studied in this report is summarized in Supplementary Table 1 (see below). Twenty-four additional families were included beyond the families that comprised the original Minnesota Cohorts 1 and 2 [S13,S14]. In addition, a collection of 122 trio families (one SLE patient with both parents) was assembled. Demographics and selected clinical characteristics of the affected individuals within the sibpair and trio collections are presented in Supplementary Table 2. The trio collection was highly enriched for white families (96% compared with 77% in the sibpair families). Compared with patients from the sibpair collection, trio individuals were more likely to be positive for anti-dsDNA antibody, and to have evidence of hematoLogic or renal disease. Lower percentages of trio patients had pleuritis, skin involvement, or CNS lupus. The differences in the clinical manifestations observed may reflect the greater ethnic diversity within the sibpair collection as well as potential differences in familial compared with sporadic SLE.

Linkage analysis in families carrying ‘risk’ haplotypes

The evidence for linkage was examined in the subset of families that carried the ‘risk’ haplotypes, defined as those families carrying three-marker haplotypes from D1S229 to D1S2641 that displayed at least a 2:1 transmission:nontransmission ratio (see Fig. 3 in the main text of this article). Families with the 1q41 risk haplotypes (n = 88) demonstrated a multipoint LOD score of 1.15 at D1S2616, while the remainder of the family collection (n = 23) showed a LOD score at D1S2616 of 0.30. Thus, SLE families carrying the ‘risk’ marker haplotypes with the strongest evidence for transmission disequilibrium were also the families that accounted for most of the evidence for linkage in the 1q41 region.

Localization of the 1q41 effect through haplotype analysis

The two most significant three-marker haplotypes identified in the moving-window analysis were D1S229–allele 7/D1S490–allele 7/D1S227–allele 5 and D1S2616–allele 6/D1S549–allele 12/D1S2641–allele 10. Interestingly, none of the 83 individuals who carried one of these two haplotypes contained both of these on a further extended haplotype. Furthermore, 33 of 55 patients with the 229/490/227 haplotype (60%) also bore allele 6 of D1S2616, while 14 of 28 individuals with the 2616/549/2641 haplotype also bore allele 5 of D1S227. This suggests the possibility that the major effect in this region is between markers D1S227 and D1S2616, an interval of approximately 0.96 megabasepairs. Alternatively, there might be two effects in the region, each carried on one of the haplotypes. The two-marker haplotype data provide some support for the former interpretation, since the
peak number of significant haplotype transmissions is found within the D1S227–D1S2616 interval, with the number of events falling off at surrounding windows. Although the current evidence for linkage disequilibrium at 1q41 extends over quite a broad interval, it is important to note that the marker density in the current study is still rather sparse. Efforts to further localize the gene in this region will require the identification and typing of additional microsatellite markers and single nucleotide polymorphisms (SNPs) in a large cohort of SLE cases and controls.

Supplementary Table 1
Composition of 210 Minnesota SLE sibpair and multiplex families

|                        | Cohort 1 | Cohort 2 | New | Total |
|------------------------|----------|----------|-----|-------|
| SLE families           | 104      | 82       | 24  | 210   |
| Affected sibpairs      | 114      | 93       | 26  | 233   |
| Affected relative pairs| 127      | 111      | 35  | 273   |
| Affected SLE individuals| 220      | 179      | 53  | 452   |
| Unaffected parents and sibs | 155 | 101      | 41  | 297   |

Supplementary Table 2
Demographics and clinical features of 576 SLE patients

|                        | Sibpair | Trio |
|------------------------|---------|------|
| Number of families     | 210     | 122  |
| Number of affected individuals (sex, F:M) | 440:10 | 123:0 |
| Family ethnicity       |         |      |
| White                  | 78      | 96   |
| African-American       | 10      | 1    |
| Hispanic               | 7       | 1    |
| Asian                  | 2       | 1    |
| Mixed heritage         | 3       | 1    |
| Laboratory/clinical features† (%) |         |      |
| ANA positive           | 98      | 97   |
| Anti-dsDNA positive    | 46      | 72***|
| Arthritis              | 85      | 84   |
| Skin involvement       | 92      | 82** |
| Pleuritis              | 53      | 45*  |
| Hematologic            | 48      | 72** |
| Renal disease          | 33      | 53***|
| CNS lupus              | 25      | 15** |
| Pericarditis           | 19      | 20   |

†Data represent the percentage of SLE patients having the indicated laboratory/clinical features at any time during the course of their disease. Differences in the clinical features between the trio and sibpair family collections were determined using generalized estimating equations to adjust for intrafamilial correlation within the sibpair families [S15]. ***P<0.0001, **P<0.01, *P<0.05. ANA = antinuclear antibodies; CNS = central nervous system.

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