Letter to the Editor

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SLEN2 (2q34–35) and SLEN1 (10q22.3) Replication in Systemic Lupus Erythematosus Stratified by Nephritis

To the Editor:

Systemic lupus erythematosus (SLE) is a chronic, sometimes life threatening, systemic autoimmune disease. African Americans (AA) have a 2–4 times higher incidence of SLE, a higher rate of SLE nephritis, and a less favorable prognosis than European Americans (EA) (Petri 1998). Renal disease occurs in 40%–75% of patients with SLE, within 5 years of disease onset (García et al. 1996), contributing significantly to the disease morbidity and mortality rates of these patients. Patients with focal and diffuse proliferative glomerulonephritis, as described in the World Health Organization's classification of SLE nephritis, have a clearly increased risk of developing severe renal impairment (Clark 1994).

Confirmation of genetic linkage effects has become a major impediment to progress in elucidating complex genetic disorders. Explanations include false-positive reports of linkage and uncertainty that originates from the unknown complexity of the overall genetic heterogeneity in the sampled populations. In addition, when genetic effects are modest in magnitude across the sample and are dependent on variables, such as ethnicity, replication may require very large samples before confident conclusions are possible.

Proposed standards for robust genetic linkages suggest that significance of the original linkage effect be at the $P < .05$ level for any linkage in a genome scan (LOD $\geqslant \sim 3.3$) and that the effect at replication achieve a sufficiently unlikely probability ($P < .05$ or LOD $\geqslant 1.2$) for the original location (Lander and Kruglyak 1995). Consequently, replication studies (either studies of a subsequent sample collected by the same investigator or studies by an independent group of investigators) or a combined data analysis can help distinguish the more robust findings from the false positives, as was done in this study.

We sought to replicate findings, reported elsewhere (Quintero-Del-Rio et al. 2002), of genetic linkage at 10q22.3 (SLEN1 [MIM 607965]) and 2q34–35 (SLEN2 [MIM 607966]), in 70 pedigrees with SLE nephritis (pedigrees multiplex for SLE that contain at least one individual with SLE who is also affected with lupus nephritis). An independently collected sample of 71 pedigrees with SLE nephritis is now available to reapply the nonparametric analysis of sibship allele sharing by the Haseman and Elston algorithm (S.A.G.E. SIBPAL). A total of 307 microsatellite markers were typed from the Weber Screening Set version 8, with an average marker spacing of 11 cM and an average heterozygosity of 76.8% (SD of ±6%; range 60%–94%). Of the total 304 multiplex pedigrees available from the Oklahoma lupus genetics collection (see Lupus Multiplex Registry and Repository Web site), 164 multiplex pedigrees—of which 141 were AA or EA pedigrees—contained at least one member affected with SLE and nephritis.

Sample 1, published elsewhere (Quintero-Del-Rio et al. 2002), contained 205 sib pairs (98 AA and 107 EA) from 71 pedigrees with SLE nephritis (table 1). Sample 2, the replication sample reported here, contains 191 sib pairs (55 AA and 136 EA) from 70 pedigrees with SLE nephritis (table 1). A total of 396 sib pairs (153 AA and 243 EA) from 141 pedigrees with SLE nephritis were available for the combined analysis.

Of the 325 individuals affected with SLE who are from these 141 pedigrees with SLE nephritis, lupus nephritis was ascertained in 238 (103 [32%] with proteinuria, 18 [5.5%] with cellular casts, and 117 [36%] with both), with a similar distribution of proteinuria and cellular casts between the two samples.

The original linkage scan of 71 pedigrees with SLE

Table 1

Group Counts in Samples of Pedigrees Multiplex for SLE and Containing an Affected Member with Nephritis

	No. in					
	Sample 1		Sample 2		Samples 1 and 2 Combined	
Group	AA	EA	AA	EA	AA	EA
Pedigrees	40	31	27	43	67	74
Individuals affected with:						
SLE	93	83	57	92	150	175
SLE and nephritis	61	46	37	54	98	100
Sib pairs	98	107	55	136	153	243
Concordant affected sib pairs	21	42	15	23	36	65

nephritis (Quintero-Del-Rio et al. 2002) produced a linkage effect ($P = .00002$) between D2S1384 and D2S434 at 2q34–35 (SLEN2) in the AA pedigrees (fig. 1*A*). This effect was sufficient to establish linkage (Lander and Kruglyak 1995). The 27 AA pedigrees of sample 2 produced a linkage effect ($P = .002$) at the same position between D2S1384 and D2S434 at 2q34–35, which is more than sufficient to confirm SLEN2 linkage (fig. 1*A*). The combination of samples produces a high probability of linkage at this location (.9999994) and a low probability that the observed relationship is a random finding ($P = .0000006$).

The original linkage scan on pedigrees with SLE nephritis (Quintero-Del-Rio et al. 2002) produced a $P = .00000015$ with D10S2470 at 10q22.3 (SLEN1) in the EA pedigrees (fig. 1*B*). The confirmatory set of 43 EA pedigrees with SLE nephritis resulted in $P = .001$ between D10S1432 and D10S2327 on chromosome 10. This region is 14 cM centromeric to the maximum area of linkage in sample 1 (fig. 1*B*). Both independent samples of pedigrees resulted in a combined $P = .00001$ between D10S2327 and D10S2470.

The interpretation for SLEN1 is less straightforward than it is for SLEN2. The magnitude of the linkage effect is sufficient to confirm linkage, but not at the original location. Overall, the linkage effect is diminished, but it is sufficiently robust to remain established. The issue of whether this linkage is confirmed may be controversial,

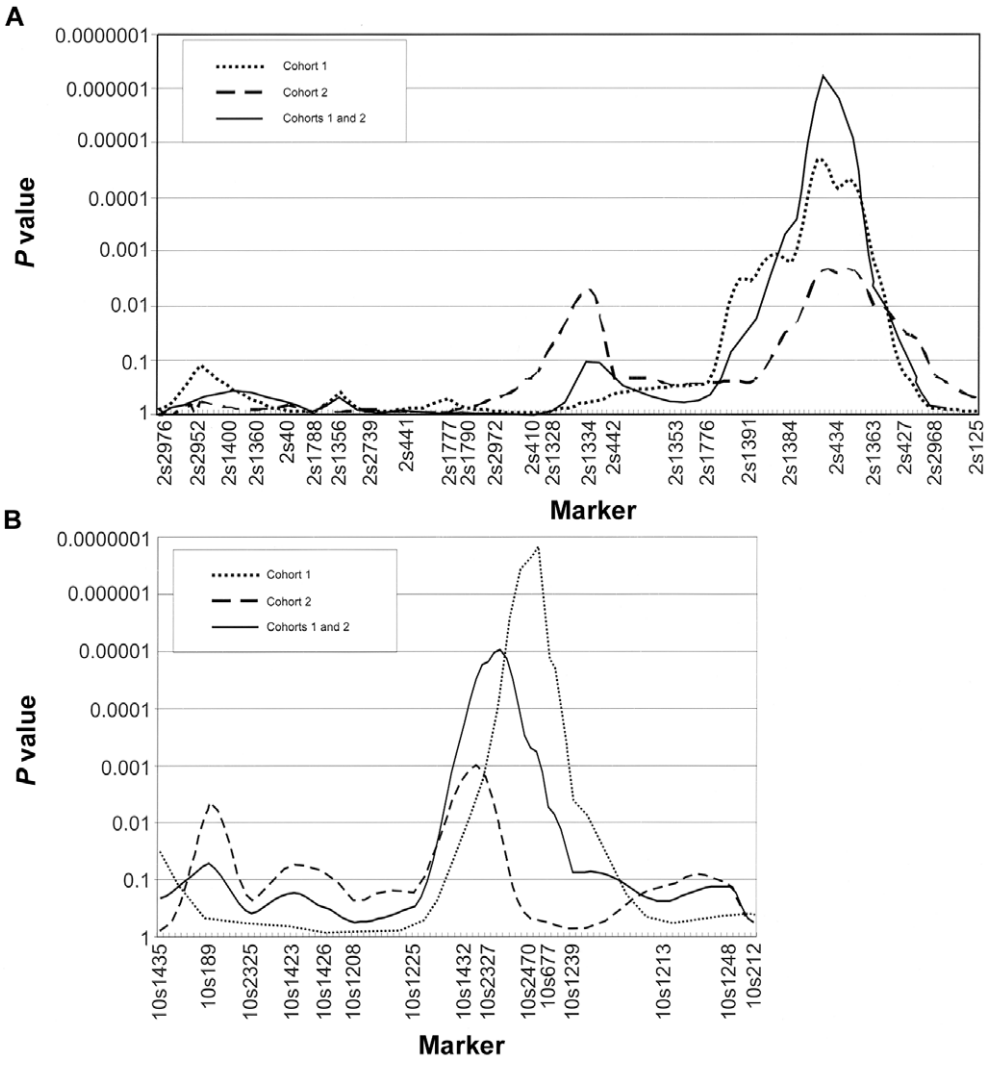

Figure 1 Linkage observed in pedigrees multiplex for SLE and stratified by race and lupus nephritis. *A*, Effect at 2q34–35 in African American pedigrees. *B*, Results at 10q22.3 in European American pedigrees. S.A.G.E. SIBPAL was used for the analysis. Centimorgans are plotted on the *X*-axis, and *P* values are plotted on the *Y*-axis. The dotted lines represent the published results of sample 1 at 2q34–35 and 10q22.3 (Quintero-Del-Rio et al. 2002), whereas the dashed lines represent the confirmation group (sample 2) findings, and the solid line represents the combined effect from both samples.

and, therefore, more data is desirable, as with most complex human genetic effects.

The average contribution to the maximum linkage effect at SLEN2 from sample 2 (55 sibships; $P = .002$) is very similar to that from sample 1 (98 sibships; $P = .00002$): 0.046 and 0.048 log units of P^{-1} per sibship, respectively. This level of consistent independent contribution between samples is unusual among complex genetic diseases.

SLEN2 is syntenic to *bxs1* (*sle7*) in the BXSB murine model of SLE, located at 32.8 cM on mouse chromosome 1. Similar to SLEN2, *bxs1* may make a contribution to lupus nephritis (see Mouse Genome Informatics and NCBI GenBank Web sites).

The sample 2 results, through successful replication, and the combined data analysis of the large study sample provide powerful evidence supporting an SLE susceptibility locus at 2q34–35 and provide additional evidence supporting linkage at 10q22.

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Electronic-Database Information

URLs for data presented herein are as follows:

Lupus Multiplex Registry and Repository, http://www.omrf.ouhsc.edu/lupus (for Oklahoma lupus genetic collection study)
Weber Screening Set version 8, http://research.marshfieldclinic.org/genetics/sets/Set8ScreeningFrames.htm
Mouse Genome Informatics, http://www.informatics.jax.org/javawi/servlet/Marker?key=75947
NCBI GenBank Human to Mouse Homology Region Maps, http://www.ncbi.nlm.nih.gov/Omim/Homology/human2.html
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/
S.A.G.E. SIBPAL, http://darwin.cwru.edu/index.php

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