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Report

Evidence for a Susceptibility Gene, *SLEV1*, on Chromosome 17p13 in Families with Vitiligo-Related Systemic Lupus Erythematosus

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Both systemic lupus erythematosus (SLE) and vitiligo are autoimmune disorders that have strong evidence of complex genetic contributions to their etiology, but, to date, efforts using genetic linkage to find the susceptibility genes for either phenotype have met with limited success. Since autoimmune diseases are thought to share at least some of their genetic origins, and since only a small minority (16 of 92) of the European-American pedigrees multiplex for SLE in our collection have one or more affected members with vitiligo, we hypothesized that these pedigrees might be more genetically homogeneous at loci important to both SLE and vitiligo and, hence, have increased power for detection of linkage. We therefore evaluated genomewide microsatellite-marker–scan data for markers at an average marker density of ~11 cM in these 16 European-American pedigrees and identified a significant linkage at 17p13, where the maximum multipoint parametric LOD score was 3.64 ($P < 4.3 \times 10^{-5}$) and the nonparametric linkage score was 4.02 ($P < 2.8 \times 10^{-5}$), respectively. The segregation behavior of this linkage suggests a recessive mode of inheritance with a virtually homogeneous genetic effect in these 16 pedigrees. These results support the hypotheses that SLE and vitiligo may share important genetic effects and that sampling on the basis of clinical covariates dramatically improves power to identify genetic effects.

Multiple genes characterize the etiology of complex genetic traits, with alleles at any single susceptibility gene making only small-to-moderate contributions to the total risk (Lander 1996; Chakravarti 1999). In this model, susceptibility alleles have low penetrances and are common in the general population, with no single allele being necessary or sufficient for clinical disease. This makes their identification difficult, requiring analysis of large numbers of multiplex families. More specifically, the vast majority of families ascertained through an affected proband will have no other affected member, when the disease is actually caused by the epistatic interaction of two or more recessive loci (Majumder and Nath 1992; Majumder 1993). Moreover, various combinations of contributing alleles at multiple genes in individual patients may result in disease phenocopies. Consequently, finding genetically homogeneous multiplex families for such a disease phenotype is very difficult.

However, there have been a few notable successes in the identification of complex disease genes by grouping of families according to clinical covariates. An association with a lower age at onset, which segregates in a Mendelian fashion, can lead to gene identification by classic positional cloning methods, such as that achieved for the breast-ovarian cancer gene *BRCA1* (Miki 1994; Wooster 1995). Stratification based on the presence of at least one case of the follicular variant of papillary thyroid carcinoma has provided very convincing evidence of linkage to 2q22 (McKay et al. 2001). The principal aim behind this method of grouping of families is to achieve a sample of families with a high degree of genetic and clinical homogeneity. Sample heterogeneity may also be reduced either by use of narrower inclusion criteria or by selection on the basis of special traits that are shared among the families. Identification of traits that tend to be shared by family members—particularly by affected individuals—is a means of identification of useful subgroups of families and may identify traits that share common genetic com-

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ponents. With this in mind, we considered families segregating for systemic lupus erythematosus (SLE) (MIM 152700) and vitiligo (MIM 193200), both apparently autoimmune disorders.

SLE is a complex disease in which humoral immune responses are directed against a multitude of self-antigens. Both environmental and genetic factors are involved in the pathogenesis. Evidence for a genetic basis is established through significant familial aggregation, with a 7%–12% increased risk in 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 (Gaffney et al. 1998, 2000; Moser et al. 1998; Gray-McGuire et al. 2000; Lindqvist et al. 2000; Magnusson et al. 2000). Recently, Meng et al. (1996) estimated the heritability of SLE at 57% in a Chinese data set. The relative risk ratio for the sibs of an affected proband is ~15. Significant gender differences are observed in prevalence, age at onset, premorbid conditions, clinical expression, course of illness, response to treatment, and morbidity risk (Hochberg 1997; Lawrence et al. 1998). In addition, there are also important racial differences in disease manifestations (Petri 1998). In all likelihood, SLE is a genetically heterogeneous disease.

Vitiligo is an autoimmune dermatological disorder characterized by hypopigmentary patches that tend to become progressive over time (Larner 1959; Nordland 1987). The prevalence of this disorder is ~1% in the United States (Larner 1959; El-Mofty 1968) and ~0.38% in Denmark (Howitz et al. 1977). Although the prevalence of vitiligo varies significantly with age, the age-specific prevalence rates are not significantly different between genders (Das et al. 1985; Majumder et al. 1993). Familial (Majumder et al. 1988; Nath et al. 1994) and twin (Siemens 1953) studies have shown significant evidence for familial aggregation and have suggested a genetic basis. Although vitiligo aggregates in families, it does not appear to segregate in a simple Mendelian pattern and, like SLE, has a complex genetic etiology. An oligogenic model for vitiligo was proposed by Majumder et al. (1988) and was cross-validated by Nath et al. (1994, 1995). No genome-scan studies on vitiligo have been reported.

SLE shows familial cosegregation with other autoimmune diseases, such as rheumatoid arthritis, Sjogren syndrome, anti-phospholipid antibody syndrome, scleroderma, and autoimmune thyroid disease. In fact, studies show that 10%–20% of lupus probands have at least one first- or second-degree relative afflicted with an autoimmune disease other than lupus.

Empirical evidence is consistent with the existence of many SLE-susceptibility genes, usually of low penetrance, and there is similar, although separate, evidence with regard to the genetics of vitiligo. The expectation is that, when families are selected as segregating for both phenotypes (by any one of a large number of possible strategies), then there should be increased power to detect what should be a very small number of susceptibility genes.

To evaluate evidence of genetic linkage in families with vitiligo-related SLE, we identified 16 families of European-American origin that were selected on the basis of the presence of at least one case of vitiligo from the collection of 92 multiplex pedigrees with European-American ancestry, ascertained in our ongoing SLE genetic-linkage projects. Most of the time vitiligo was diagnosed on the basis of review of medical records. Fourteen (87%) of the families with SLE and vitiligo were nuclear families. Among these 16 families, there were 41 individuals affected with SLE (the average number of affected individuals per family was 2.5), 20 individuals affected with vitiligo, and 14 individuals affected with both vitiligo and SLE. A total of 95 individuals from these 16 families were used in the analyses. In the linkage analyses, we considered only those individuals with SLE as being affected—that is, those with only vitiligo were considered to be unaffected.

Genomic DNA was isolated from peripheral blood cells, buccal cell swabs, or Epstein-Barr virus–transformed cell lines by standard methods. Methods for 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 2–30 cM). Prior to any linkage analyses, all family relationships were confirmed by RELTEST (Olson 1999).

Since the disease model (which considered mode of inheritance, disease-allele frequency, and penetrances of genotypes at the disease locus) was uncertain, we initially screened the entire genome, using the nonparametric linkage analysis program GENEHUNTER-PLUS, which allows complete multipoint analysis with a large number of highly polymorphic markers. GENEHUNTER-PLUS incorporates both traditional parametric (i.e., LOD scores, under the assumption of assuming genetic homo- or heterogeneity) and nonparametric linkage analysis (Z_{lr} scores, assessed by comparison of the observed setwise IBD sharing among all affected family members versus that expected under the null hypothesis of no linkage) (Kruglyak et al. 1996; Kong and Cox 1997). We chose equal weight for each family and chose the S_{all} scoring function to allow comparison of observed IBD allele sharing among all the affected members versus that expected under the null hypothesis of random segregation. From the Z_{lr} score, the nonparametric LOD score is calculated as $Z_{\mathrm{lr}}^2/2\ln(10)$, which is comparable to the parametric model–based LOD score. We estimated marker-allele frequencies in family founders by

using FASTLINK (Cottingham et al. 1993). Marker map positions were obtained from the latest available sex-averaged maps from the Marshfield database (Center for Medical Genetics, Marshfield Medical Research Foundation). Once we observed, on the basis of the nonparametric method, significant evidence of linkage, we then estimated the disease model at that region by maximizing the model parameters under the constraint that the estimated SLE prevalence be considered to be <0.1%.

The nonparametric analysis revealed significant evidence of linkage ($Z_{lr} = 4.02$, $P = 2.8 \times 10^{-5}$) between the markers D17S974 and D17S1298. On the basis of this Z_{lr} score, the estimated nonparametric LOD score was calculated to be 3.5. We maximized the parameters to obtain the most parsimonious model at this locus, by parametric linkage analysis. The highest LOD score was 3.64 at the position matching that of the peak Z_{lr} score (fig. 1). The most parsimonious model was a moderately common, recessively inherited gene with 16% penetrance in the recessive homozygote and with a disease-allele frequency of .025. When the 1-unit-stepdown method (Conneally et al. 1985) was used, the 95% support interval for the linked region for the location of the putative susceptibility gene was ~23 cM, on the basis of the result of parametric analysis and the most parsimonious model.

We investigated the genetic heterogeneity by calculating Hlod, the LOD score under a heterogeneity model; the estimated proportion of families linked to this locus was essentially 100%, and the Hlod score was almost the same as the .65 under a homogeneity model. Under a dominant model, the highest LOD score obtained was .02, which was significantly different from the LOD score estimated under a recessive model ($\chi^2 = 16.7$, 1 df, $P = 4.5 \times 10^{-5}$). We also performed an affecteds-only parametric analysis, by recoding the unaffected individuals as unknown, so that the LOD score would not be affected by the penetrance function. The results were essentially unchanged (LOD score 3.67).

There are two diagnostic caveats to consider with regard to this study's assessment of vitiligo. First, vitiligo clinically is diagnosed as lesions with loss of pigmentation. Any condition that destroys the melanocytes of the skin without leaving a scar or other evidence of a different process will, to the eye of the ordinary clinical examiner, be indistinguishable from vitiligo. In this study, our ascertainment of vitiligo relied on the reports of routine medical examinations. To our knowledge, no skin biopsies were done to confirm the diagnosis of vitiligo. Furthermore, in some of these cases the lesions are being assessed remotely in time, long after the active process that created them. A skin biopsy done at this time would not likely be informative beyond indicating the absence of melanocytes.

Second, in SLE, the discoid rash, particularly in pa-

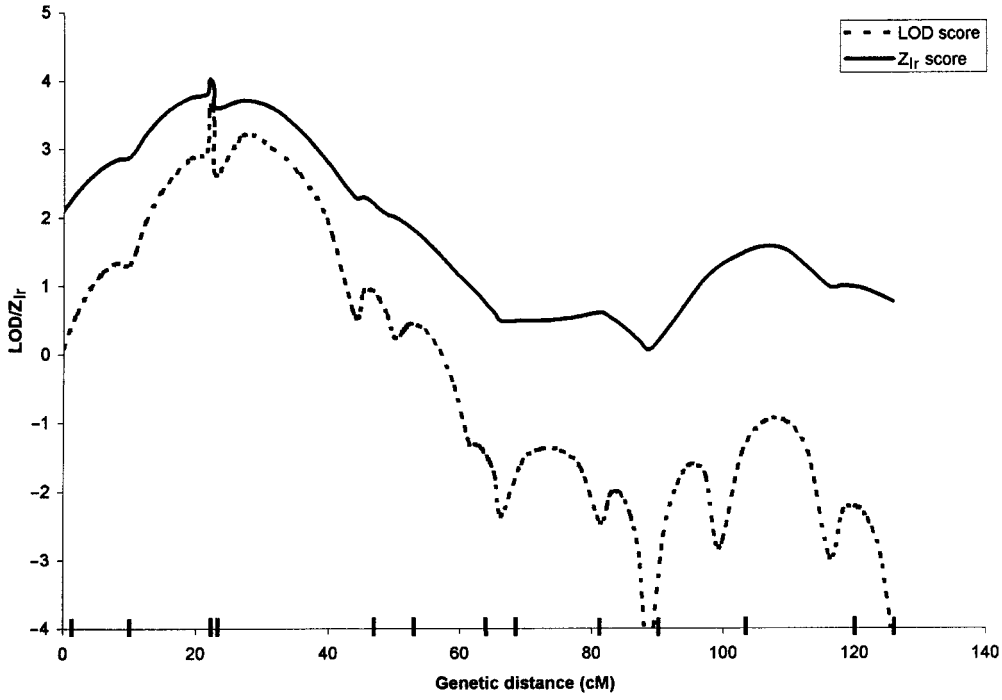

Figure 1 Multipoint model-free (Z_{lr} score) and model-based (LOD score) linkage, for chromosome 17 in families with vitiligo-related SLE. Marker positions are denoted by the vertical tic marks; the marker order, from left to right, is D17S1303-D17S1998-D17S974-D17S1303-D17S2196-D17S1294-D17S1299-D17S2180-D17S1290-D17S2193-D17S1301-D17S784-D17S928.

tients requiring profound immunosuppression, may resolve, leaving hypopigmented lesions that cannot be distinguished from vitiligo and that might be referred to as "vitiligo" by the physician. The pathology of the discoid rash is fundamentally different than that of ordinary vitiligo, and so this is a potentially confounding ascertainment issue. Among the 20 patients affected with vitiligo, only 1 was diagnosed with discoid lesions. Since discoid rash is much more prevalent in African Americans with SLE than in European Americans with SLE, one would expect that African-American pedigrees may be more susceptible by this problem. Indeed, we did not find any linkage effects in the six African-American pedigrees multiplex for SLE and containing an individual affected with vitiligo when they were evaluated as described above for the European-American pedigrees multiplex for SLE (data not shown). However, there are probably too few pedigrees to allow us make a definite conclusion.

Our finding suggesting genetic commonality between some cases of SLE and some cases of vitiligo brings the molecular mechanisms of two quite different autoimmune processes together. SLE is thought of as a largely humoral autoimmune process, particular in its effector mechanisms, and is a systemic autoimmune disorder. Much of the disease specificity and abnormalities focus on the B lymphocyte. Indeed, there are even data suggesting a role for Epstein-Barr virus as an environmental risk factor in this disease (James et al. 1997, 2001). Patients with SLE are always symptomatic and, if left untreated, usually have a life-threatening immune-destructive disease process. On the other hand, the melanocyte destruction of vitiligo proceeds asymptomatically, except for the hypopigmentation. This is a prototypic organ-specific autoimmune disorder. There is a report that vitiligo may be triggered by a cytomegaloviral infection in some patients (Grimes et al. 1996). Although autoantibodies have been found, the current paradigm for vitiligo as found in the general population is that it is mediated by melanocyte-specific T lymphocytes (Ogg et al. 1998; Li et al. 2000). Whether, in these pedigrees multiplex for SLE, the individuals who are affected with vitiligo evidence a hypopigmentation mechanism similar to that described for the ordinary sporadic cases of vitiligo is not yet known.

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 that are particularly related to either immune response or dermatologic traits; these include the gene for retinitis pigmentosa 13 (*RP13*), the gene for T-cell immunodeficiency, the gene for congenital alopecia (*WHN*), the gene for non–insulin-dependent diabetes mellitus (*SLC2A4*), the gene for myasthenia gravis (*MGI*), and the gene for homeobox B9 related to breast cancer. Any one of these candidate gene(s) has the potential for causal mutation(s).

In summary, we have identified a potentially informative genomic region at 17p13, which may contain the putative gene, *SLEV1*, for vitiligo-related SLE. Here we would like to emphasize that the main phenotype used for the linkage analysis was SLE; so, the linkage to the 17p region reflects a gene predisposing to SLE. Nevertheless, since the presence of vitiligo in the family was used as a pedigree-ascertainment criterion, and since vitiligo and SLE may be associated, it is possible that SLE and vitiligo may have some common autoimmune genetic determinant(s). Alternatively, we may assert that there is a gene that leads primarily to developing SLE and that, at least among the ascertained families, also modifies the risk for vitiligo. From this study, it is also evident that, regardless of the actual number of genes involved in SLE, decreasing the sample heterogeneity by subgrouping the families on the basis of common associated traits has increased the likelihood that genes for SLE will be identified; in fact, to our knowledge, this result is one of the few more-convincing SLE-linkage results published to date.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Center for Medical Genetics, Marshfield Medical Research Foundation, http://research.marshfieldclinic.org/genetics/

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 152700] and vitiligo [MIM 193200])

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