**ELECTRONIC LETTER**

**Linkage mapping of systemic lupus erythematosus (SLE) in Finnish families multiply affected by SLE**

S Koskenmies, P Lahermo, H Julkunen, V Ollikainen, J Kere, E Widén

**PARTICIPANTS AND METHODS**

**Patient recruitment**

The patients were recruited as described previously. The study was approved by the local ethical committees according to applicable regulations. Patients treated at Kuopio and Helsinki University hospitals, patients registered by the Lupus Foundation of Finland, and patients who answered an advertisement published in patient bulletins were recruited in 1995. We identified patients who had been treated in the two university hospitals during 1992–1995 from the corresponding hospital registries and contacted all patients personally or by mail. A second recruitment phase started in autumn 1996. We contacted doctors (mainly rheumatologists) from 17 central hospitals in Finland and four other major hospitals that treated patients with SLE. A letter was sent to all patients with the clinical diagnosis of SLE who had been treated in these hospitals during 1993–1996. All patients were asked whether they had relatives or family members diagnosed with SLE or a connective tissue disease similar to SLE. We asked patients with a positive family history to participate and obtained informed consent. According to the prevalence of SLE in Finland, we succeeded in contacting roughly 1200 out of 1500 available patients personally (by phone or by mail) during the two phases of recruitment; this accounted for about 85% of all Finnish patients with SLE who needed...
hypothesis of no linkage. The simulations consisted of 200 iterations, as described previously. In each iteration, we drew founder alleles from the estimated allele frequency distributions and introduced missing genotypes to exactly the same positions as in the real data. As a result, we obtained an empirical distribution for the non-parametric linkage scores, which corresponded to suggestive and significant linkage observed under the null hypothesis in the entire genome scan.17

RESULTS
Demographic, clinical, and laboratory features of families with SLE
Seventy-three patients with SLE and 96 healthy relatives from 35 multiply affected families were identified. Their clinical features have been described previously.19 In 32/35 families, two family members were affected. The most common combination was sister and sister (16 families); this was followed by aunt and niece (eight families). Three families had three patients with SLE. In two of these families, the affected family members were first degree relatives; in the third family, a second degree relative (a cousin) and first degree relatives (mother and son) were involved. Seven (9.6%) of the 73 familial cases were men, which corresponded well with the sex distribution of SLE in the general population.20

Genome scan
We used the marker set MD-10 to capture 50–60% of the maximum inheritance information throughout the genome. Loss of information was mainly caused by the pedigree structure (that is, missing parents). Table 1 shows the highest non-parametric linkage scores obtained and the corresponding information content (chromosome 3, chromosome 5, and chromosome 6).

We used simulations to establish the empirical thresholds for suggestive and significant linkage (that is, non-parametric linkage scores reached at random once per one or

Data analysis and simulations
As the mode of inheritance of SLE is not known, we carried out linkage analysis with non-parametric analysis with Genehunter software (version 2.0 for chromosomes 1–22 and version 1.3 for the X chromosome) (Fred Hutchinson Cancer Research Center, Seattle, WA, USA). Genehunter multipoint analyses the degree of identity by descent sharing among all affected pedigree members at each location of the genome. To get an estimate of the global p value, we performed allele dropping simulations under the null hypothesis of no linkage. The simulations consisted of 200 iterations, as described previously. In each iteration, we drew founder alleles from the estimated allele frequency distributions and introduced missing genotypes to exactly the same positions as in the real data. As a result, we obtained an empirical distribution for the non-parametric linkage scores, which corresponded to suggestive and significant linkage observed under the null hypothesis in the entire genome scan.17

Table 1 Non-parametric linkage scores in genomewide scan of families with systemic lupus erythematosus (linkage mapping set MD-10)

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Marker</th>
<th>Non-parametric linkage score</th>
<th>p value</th>
<th>Information content</th>
</tr>
</thead>
<tbody>
<tr>
<td>3q</td>
<td>D3S1278</td>
<td>1.78</td>
<td>0.03</td>
<td>0.64</td>
</tr>
<tr>
<td>5p</td>
<td>DSS418</td>
<td>1.74</td>
<td>0.04</td>
<td>0.61</td>
</tr>
<tr>
<td>HLA region</td>
<td>D6S276</td>
<td>1.22</td>
<td>0.11</td>
<td>0.52</td>
</tr>
<tr>
<td>6q</td>
<td>D6S441</td>
<td>2.35</td>
<td>0.01</td>
<td>0.57</td>
</tr>
<tr>
<td>8</td>
<td>D8S560</td>
<td>0.62</td>
<td>0.26</td>
<td>0.44</td>
</tr>
<tr>
<td>14q</td>
<td>D14S288</td>
<td>1.51</td>
<td>0.07</td>
<td>0.55</td>
</tr>
</tbody>
</table>

Table 2 Non-parametric linkage scores after addition of markers to linkage mapping set MD-10 in families with systemic lupus erythematosus

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Marker</th>
<th>Non-parametric linkage score</th>
<th>p value</th>
<th>Information content</th>
</tr>
</thead>
<tbody>
<tr>
<td>3q</td>
<td>D3S1278</td>
<td>1.55</td>
<td>0.06</td>
<td>0.71</td>
</tr>
<tr>
<td>5p</td>
<td>DSS418</td>
<td>2.03</td>
<td>0.02</td>
<td>0.63</td>
</tr>
<tr>
<td>HLA region</td>
<td>D6S273</td>
<td>2.17</td>
<td>0.02</td>
<td>0.78</td>
</tr>
<tr>
<td>6q</td>
<td>D6S960</td>
<td>2.47</td>
<td>0.008</td>
<td>0.67</td>
</tr>
<tr>
<td>8</td>
<td>D8S560</td>
<td>0.48</td>
<td>0.31</td>
<td>0.46</td>
</tr>
<tr>
<td>14q</td>
<td>D14S288</td>
<td>2.20</td>
<td>0.02</td>
<td>0.62</td>
</tr>
</tbody>
</table>
20 genome scans, respectively\(^{15}\)). The best observed non-parametric linkage score under the null hypothesis of no linkage showed that non-parametric linkage = 1.7 is reached once per genome scan (suggestive linkage) and that the global p value = 0.05 corresponds to non-parametric linkage = 3 (significant linkage). Three loci exceeded the threshold for suggestive linkage; none was significant (table 1). To verify the highest non-parametric linkage scores and to cover gaps in the map, we genotyped more markers at regions with non-parametric scores \(>1.7\) (chromosome 3q, chromosome 5p, and chromosome 6q) and regions with gaps \(>20\) cM if the corresponding non-parametric linkage score was \(>1\) (chromosome 6p (that is the HLA region), chromosome 8, and chromosome 14). In all regions with suggestive

![Figure 1](attachment:image.png)
linkage, non-parametric linkage scores increased with additional markers (table 2). The most striking change in non-parametric linkage scores was seen in the HLA region, in which the non-parametric linkage score increased from 1.2 to 2.1. Figure 1 shows the overall non-parametric linkage scores, including all genotyped markers.

DISCUSSION

This was the first nationwide study in Finland to map genetic factors that confer susceptibility to SLE. We recruited >80% of patients with SLE in Finland, which has a population of 5 million. Our results suggest that no single major gene contributes to the risk of the disease in Finland, despite its population structure. Our genome scan showed three loci that exceeded the threshold of 1.7; this corresponded to the definition of suggestive linkage genomewise.25 Even after we increased the amount of information with additional markers, no locus reached the threshold for significant linkage. Ideally, the results should be verified in an independent dataset. Unfortunately, we cannot increase the amount of information with additional markers.

We identified two previously reported regions (the HLA region and chromosome 14q21–q23) and two novel regions on chromosome 5p and chromosome 6q as possibly linked to SLE. Linkage to HLA was in accordance with previous studies. Association between SLE and the class II and III genes in the human leucocyte antigen complex was seen in studies in the 1970s.26 Studies of patients of European descent consistently show an association between SLE and especially HLA DR2 and DR3.27–29 The HLA region also has been implicated in more recent linkage mapping studies. Gaffney and colleagues reported strong linkage to the HLA region; this was supported by results from Shai and colleagues and Lindqvist and colleagues in patients of Swedish pedigree.30–32

Chromosome 14q21–q23 also has been implicated repeatedly in previous linkage studies, although no study alone has reported a significant linkage result. Evidence for linkage to chromosome 14q has been reported in three independent data sets: Gaffney and colleagues (LOD 2.81, p<0.00016), Shai and colleagues (non-parametric linkage 2.02, p=0.02) and Lindqvist and colleagues in patients of Swedish pedigree (LOD 1.15) but not in the combined Minnesota cohort or the Oklahoma dataset.31 The data support locus heterogeneity between the different datasets. Our study adds to the evidence that a susceptibility gene for SLE exists in the chromosome 14q.

The biological relevance of the observed excess sharing of alleles in the novel regions on chromosomes 5p and 6q is more difficult to evaluate. Interestingly, the same region on chromosome 6q25–q27 has been implicated in other autoimmune diseases. Several studies support a locus that confers susceptibility to insulin dependent diabetes in this chromosomal region (IDDM5).30–33 Supportive evidence for the existence of a common autoimmune susceptibility locus in the region comes from a study by Myerscough and colleagues.32 They report evidence for linkage disequilibrium between rheumatoid arthritis and the markers D6S311 and D6S440. As the highest observed Z score in our study resided in the same region, chromosome 6q25–q27 may harbour a gene that influences the predisposition to autoimmunity.

Recently, Rioux and colleagues showed the power of linkage disequilibrium analysis in complex disease mapping.34 By genotyping polymorphic markers at 0.35 cM intervals in a region initially identified by linkage mapping, they successfully identified linkage disequilibrium and an ancestral haplotype that spanned 250 kb in patients with inflammatory bowel disease in an outbred Canadian population. A similar approach should be feasible among Finnish patients and controls in an expanded study. In a population characterised by local founder effects, linkage disequilibrium mapping in selected geographical areas might be a useful strategy for identifying relatively rare susceptibility alleles.35 We intend to use the power of linkage disequilibrium to identify common ancestral chromosomes among a larger cohort of patients with SLE and controls by high resolution mapping.

ACKNOWLEDGEMENTS

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Authors' affiliations

Koskenmies, J Kere, Department of Medical Genetics, University of Helsinki
Lohermo, V Ollikainen, J Kere, E Widén, Finnish Genome Center, University of Helsinki
J Julkunen, Department of Internal Medicine, Peijas Hospital, Helsinki, University Hospital
V Ollikainen, CSC-Scientific Computing Ltd, Espoo, Finland
J Kere, Department of Biosciences at Novum and Clinical Research Centre, Karolinska Institutet, Stockholm

Correspondence to: Professor J Kere, Karolinska Institutet, Department of Biosciences at Novum, and Clinical Research Centre, 141 57 Huddinge, Sweden; juha.kere@biosci.ki.se

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