European genetic study on rheumatoid arthritis: Is there a linkage of the interleukin-1 (IL-1), IL-10 or IL-4 genes to RA?

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Abstract
The genetic predisposition for rheumatoid arthritis (RA) is only partly explained by the HLA locus and most genetic factors involved in the susceptibility (and/or severity) of the disease await further identification. The first European genome scan in RA families provided suggestive evidence for linkage with a region (3.1/3q13) on chromosome 3, but many other potential RA susceptibility genes have yet to be analysed.

Aims
To perform a linkage analysis with microsatellite markers located in the vicinity of the interleukin-1 (IL-1) gene superfamily, the IL-10 gene and the IL-4 gene cluster which might be considered putative candidate loci for RA.

Methods
107 Caucasoid European RA sibpairs from 90 nuclear families were genotyped for markers flanking the genes for the IL-1 superfamily, IL-10 and the IL-4 gene cluster. Linkage analysis based on the identity by descent (IBD) in affected siblings was analysed with the program SIBPALNA. Affected sibpairs were stratified according to the identity by state (IBS) for three markers in the HLA region (DRB1 oligotyping, D6S276 and TNFa microsatellites) and to the presence/absence of erosive disease on X-ray examination.

Results
Analysis of the whole family set showed an excess of allele sharing for markers of the IL-1 gene cluster (IBD 60%; P = 0.012) but not for IL-10 or IL-4. After stratification, the evidence of linkage to IL-1 was restricted to HLA concordant sibpairs (n = 32; IBD 70%; P = 0.006). Some evidence of linkage to IL-10 was also observed in HLA concordant sibpairs (IBD 66%; P = 0.03) and in sibpairs with erosive disease (n = 61; IBD 62%; P = 0.02).

Conclusions
We found suggestive evidence of linkage of RA to the IL-1 locus. The increased linkage to IL-1 and IL-10 in HLA-identical sibs suggests a possible interaction between these cytokines and the HLA loci. Moreover IL-10 could interact with HLA factors in predisposing to erosive disease. These results need to be tested in additional families for consistency and replication.

Key words
Rheumatoid arthritis, interleukin genetics, linkage genetics.

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Introduction
Rheumatoid arthritis (RA) is a multifactorial, polygenic disease. Genetic factors have been estimated to contribute 15% to 60% to its susceptibility (1) and it is widely accepted that certain HLA-DRB1 alleles are strongly associated with RA (2). However, the latter account for only 30% to 40% of the overall genetic risk (3-6) leaving most of the genetic component unexplained. Other RA susceptibility loci are being sought for using genome-wide screening studies (7-9). The first genome scan performed by the European Consortium on RA Families (ECRAF) confirmed the linkage with HLA and provided evidence for several other susceptibility loci outside the HLA region which are now being characterised (7,10,11).

Evidence accumulated in the last decade suggest that the balance between pro- and anti-inflammatory cytokines and Th1/Th2 patterns is disrupted in patients with RA (12-17). Several recent studies using the so-called candidate gene approach support this notion. The genes for the interleukin-1 (IL-1) superfamily consisting of IL-1 , IL-10, and the IL-1 receptor antagonist (IL-1RA) are located on chromosome 2 (2q12-q13). A number of bi- and multiallelic markers in this region (18-20) have been associated with inflammatory conditions (21-24). Previous studies on this gene cluster in RA have yielded conflicting results. No evidence for a strong association (25) or linkage (26, 27) was observed in some studies, whereas other suggested a relationship of these genes with disease severity (25,27-30).

IL-10 is an anti-inflammatory and immunoregulatory cytokine encoded by a highly polymorphic gene located on chromosome 1 (1q31-1q32). The IL-10 promoter has at least four single-basepair substitutions and two microsatellites, IL-10R and IL-10G (31,32). These combine in four haplotypes which, although not functional (33), seem to be associated with variations in the production of IL-10 ex vivo (31,34). Previous studies in RA have shown an association of the disease with the IL-10R microsatellite (35) and between the –1082 polymorphism and erosive disease (36,37). Moreover, polymorphisms in the IL-10 promoter have also been associated with certain phenotypes in other inflammatory diseases such as SLE and juvenile RA (34,38-40).

IL-4 is encoded in chromosome 5 (5q22-q32), within a cluster of genes that regulate the TH1/TH2 balance. Genes in this cluster have been associated with TH2-like reactions such as asthma and atopc dermatitis (41-45). The rare allele of a VNTR polymorphism in the third intron of the IL-4 gene is over-represented in RA patients (25) and has been associated with a less erosive course (46,47).

In the first genome-wide scan performed by the ECRAF we found some evidence for linkage to D2S160 (P < 0.05), a polymorphic marker located close to the IL-1 gene region but not to markers of the IL-10 and IL-4 genes (7). In that study disease heterogeneity was not taken into account. Recent findings from our group and others suggest that HLA and disease severity may interact with the genotype for these cytokines (25,27,36,48). We therefore performed a linkage analysis using additional markers for these genes and taking into account disease heterogeneity for HLA and erosive disease.

Patients and methods
The diagnosis ascertainment, genotyping and analytical methods used in the first European genome scan have been described (7) (http://www.genethon.fr/projets/genflu/PR/RES_PR/PR_REA_DME). Briefly, 90 Caucasian families including 83, 6 and 1 families with 2, 3 and 4 affected sibs respectively (107 affected sibpairs or ASP) were studied. All of the affected sibs in these families fulfilled the 1987 ACR criteria for RA (49).

DNA extracted from blood by standard techniques was genotyped using fluorescence-based microsatellite marker analysis and the programs GENESCAN ANALYSIS 2.0.0 and GENOTyper 1.1. A total of 18, 18 and 12 markers with a mean spacing of 12 centiMorgans (cM) was initially used to
cover chromosomes 2, 1 and 5, respectively. In the present study we used 12 additional markers, with an intermarker distance ranging from 0 to 4 cM for the IL-1 locus and 2 additional markers for the IL-10 and the IL-4 loci, respectively. HLA-DRB1 genotyping was performed using the Inolipa kit (Murex, Chatillon, France). Bipoint non-parametric linkage analysis was performed with J. Terwilliger’s ANALYZE package using the program SIBPALNA 1.1. Sharing of alleles identical-by-descent (IBD) in affected sibpairs was calculated and compared with the random expectation of IBD = 50%. For the purpose of this study, in families with more than two affected sibs one sibpair was chosen at random. Potential interactions of cytokine genes with the HLA locus were investigated by stratifying the sibpairs as HLA concordant (i.e. identical genotype) or discordant according to the identity by state (IBS) for three markers in the HLA region (TNFa, D6S276 and HLA-DRB1 typing). Data were analysed according to the presence of erosive disease assessed in X-rays of the hands and feet by the rheumatologist caring for each patient. Sibpairs were classified as “erosive” if this was the case for all affected sibs and “non-erosive” if at least one affected sib lacked erosive disease.

**Results**

Linkage analysis with additional markers in the overall family set confirmed an excess of allele sharing (IBD > 50%) for all the markers flanking the IL-1 locus. Besides D2S160 (P = 0.045), the marker used in the first genome scan, the evidence for linkage was suggestive at markers D2S1888 (P = 0.012), D2S1896 (P = 0.017) and D2S2269 (P = 0.013) located proximally to the IL-1A gene (20). The genome-wide scan had shown no linkage to markers located close to the IL-10 and IL-4 loci and this was confirmed on the whole family set using additional polymorphic markers for these genes (Table I).

Analysis after stratification for HLA markers yielded 32 sibpairs with identical and 58 with discordant HLA genotypes, respectively. An excess of allele sharing for markers of the IL-1 locus was observed only in the HLA-identical affected sibpairs (IBD 70%; P = 0.006), but not in the HLA non-identical sibpairs (Table II). Among HLA-identical siblings, the evidence for linkage did not reach the threshold for significant linkage settled in the first genome scan (P < 10^{-4}), but was suggestive for linkage at two contiguous markers (D2S121; P = 0.018 and D2S1895; P = 0.006) and reached a P value of < 0.05 in five additional markers (Table II). The effect of the HLA locus on the sharing at the IL-1 locus was significant (P = 0.03).

HLA stratification showed also a deviation from the randomly expected 50% IBD for all markers for the IL-10 locus in HLA-identical siblings. Among the latter, the evidence for linkage was suggestive at D1S1725 (mean IBD 66%; P = 0.03; Table II). In 61 out of 90 sibpairs, the erosive phenotype was present in both siblings, whereas 29 encompassed at least one affected sib without erosions. Using this stratification we observed a deviation from the randomly expected 50% IBD only for D1S1725, a marker for

**Table I.** Non-parametric linkage analysis for interleukin-1 (IL-1), IL-10 and IL-4 genes in the whole family set.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Locus</th>
<th>AFM marker</th>
<th>Position to telomere(^a) (cM)</th>
<th>Alleles (no.)</th>
<th>Heterozygosity (%)</th>
<th>No. alleles IBD Shared</th>
<th>Not shared (%)</th>
<th>IBD (%)</th>
<th>P</th>
</tr>
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<td>IL-1</td>
<td>D2S1897</td>
<td>a046ya9</td>
<td>121.9</td>
<td>17</td>
<td>88</td>
<td>71.1</td>
<td>65.2</td>
<td>51</td>
<td>NS</td>
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<tr>
<td></td>
<td>D2S340</td>
<td>277wc9</td>
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<td>9</td>
<td>70</td>
<td>67.7</td>
<td>54.2</td>
<td>56</td>
<td>NS</td>
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<td></td>
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<td>77</td>
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</tr>
<tr>
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<td>b007wc5</td>
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<td>12</td>
<td>79</td>
<td>81</td>
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<td>55</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>D2S160</td>
<td>220ze3-b</td>
<td>127.4</td>
<td>10</td>
<td>78</td>
<td>82.5</td>
<td>61.3</td>
<td>57</td>
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<td>12</td>
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<td>81.2</td>
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<td></td>
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<td></td>
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<td>65.3</td>
<td>52</td>
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<td>69.9</td>
<td>74.3</td>
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<td>a102wc5</td>
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<td>9</td>
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<td>59.8</td>
<td>62.4</td>
<td>50</td>
<td>NS</td>
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</table>

\(^a\)Genetic distances to telomere are based on the Genethon linkage map (51). The number of IBD alleles in the affected sibpairs and the associated P value were calculated by the program SIBPALNA. P ≥ 0.05 indicated by NS.
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Table II. Non-parametric linkage analysis for interleukin-1 (IL-1), IL-10 and IL-4 after stratification for HLA-DR status.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Locus</th>
<th>HLA-identical sibpairs (n = 32)</th>
<th>HLA-discordant sibpairs (n = 58)</th>
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<td></td>
<td>No. alleles IBD</td>
<td>IBD (%)</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td>Shared</td>
<td>Not shared</td>
<td></td>
</tr>
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<td>D2S1897</td>
<td>24.9</td>
<td>22.4</td>
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<td>28.6</td>
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</tr>
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<td></td>
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<td>31.4</td>
<td>18.3</td>
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<td>30.1</td>
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<td>25</td>
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<td>17.6</td>
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</table>

*Genetic distances to telomere are based on the Genethon linkage map (51).
The number of IBD alleles in the affected sibpairs and the associated P value are calculated by the program SIBPALNA.

P ≥ 0.05 indicated by NS.

the IL-10 gene. Sibpairs with the erosive phenotype in both affected sibs had a mean IBD of 62% (P = 0.021), whereas “non-erosive” sibpairs showed no excess of allele sharing (IBD 50%). No linkage to markers in the IL-4 gene cluster was observed either in the whole family set (Table I) or after stratification for HLA (Table II) or erosive disease (data not shown).

Discussion

The data presented in this study provide evidence suggestive of RA linkage to the IL-1 gene cluster interacting with HLA. As far as linkage is concerned, the genetic contribution of this locus to the susceptibility of RA would be much weaker than that of HLA (7). This is sustained by two previous studies using the Arthritis Research Campaign’s National Repository of family material which excluded a major contribution of this gene to RA susceptibility (26, 27). Our data, however, suggest that the IL-1 locus may be relevant to certain patient subsets. This is illustrated by the observed interaction between IL-1 and HLA. In our family set, the linkage to IL-1 came only from HLA-identical sibpairs, which suggests that genes within this locus play an additional role in the susceptibility for RA in families where the disease is also linked to HLA.

An interaction of the IL-1 locus with HLA has also been suggested in two recent studies. In one of them, Cantaegril et al. reported the lack of a direct association between IL-1 markers and RA or erosive disease. Nevertheless, the combination of HLA-DR alleles containing the shared epitope with certain IL-1b alleles yielded a high risk of erosive RA (25). Although we did not observe a separate interaction between IL-1 and erosive disease, HLA-DR identity itself may be associated with erosive disease.

In contrast to our findings, the results of Cox et al. suggest an effect of IL-1 markers within affected sibs not sharing HLA-DRB1 alleles (27). The definition of HLA-DRB1 allele-sharing status and thus the proportion of HLA-identical sibpairs between their study and ours differed (53% versus 35%; respectively). This and other differences in demographic or genotypic characteristics might explain the divergent results.

Concerning the IL-10 locus, we found weak evidence of linkage and only in HLA-identical affected sibpairs and in those with erosive disease. A relationship between certain IL-10 haplotypes and an erosive course has been suggested in some longitudinal association studies (36), but has not been corroborated by other authors (25). A possible linkage or association of the IL-10 gene to disease susceptibility or to the prognosis in RA is of interest since genetic variations in IL-10 haplotypes correlate with the production capacity for IL-10 (31, 37).

We did not find any evidence for linkage of the IL-4 gene cluster to either RA or to specific patient subsets. This rules out a major contribution of the gene to the susceptibility to RA, but is not in contradiction with the reported association between RA and IL-4 (25) since weak genetic effects cannot be ruled out by linkage analysis (1, 50). Taken together we present suggestive evidence of the linkage of RA to the
IL-1 locus. This and the IL-10 locus interact with HLA and might be of importance in patient subsets where the disease has a strong HLA component. Moreover, our results suggest a possible role of the IL-10 locus in erosive RA. Several association studies support these notions. The contribution of these genes to the disease susceptibility and prognosis is expected to be much weaker than that of HLA. Therefore, additional linkage and association studies are needed in other patient populations are confirmed. To confirm these findings, the ECRAF enquires transmission disequilibrium testing in a large number of European single-case families.

Acknowledgements

The authors are indebted to S. Moin- droul, V. Decaulne, N. Prina and A. Delaye for the technical work and to J. Weissenbach for his co-operation.

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