Linkage and association of an interleukin 4 gene polymorphism with atopic dermatitis in Japanese families

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Abstract
We examined linkage between markers at and near the IL4 gene and atopic dermatitis (AD) in 88 Japanese nuclear families. Affected sib pair analysis suggested linkage between the IL4 gene and AD (SPLINK lod=2.28). Transmission disequilibrium testing showed a significantly preferential transmission to AD offspring of the T allele of the -590C/CT polymorphism of the IL4 gene (p=0.001). A case-control comparison suggested a genotypic association of the TT genotype with AD (odds ratio=1.88, p=0.01). Since the T allele was reported to be associated with increased IL4 gene promoter activity compared with the G allele, our data indicate that genetic differences in transcriptional activity of the IL4 gene influence AD predisposition, particularly in Japanese, because of a high frequency of the T allele. (J Med Genet 1998;35:502-504)

Keywords: interleukin-4 gene; atopic dermatitis; linkage; transmission disequilibrium test

IL4 plays an important role in IgE synthesis by activating the pre-T helper cells to Th2 cells that trigger isotype switching from IgM/IgG to IgE in B cells. This process induces the expression of vascular cell adhesion molecule-1 (VCAM1), an adhesion molecule involved in the migration of mononuclear cells and eosinophils into sites of tissue inflammation. IL4 has also been shown to inhibit production of interferon-γ (IFN-γ), which inhibits IgE synthesis, and downregulates the differentiation of Th1 cells. Lymphocytes from patients with AD are reported to secrete increased amounts of IL4. A recent study indicated that IL4 mRNA expression is increased in AD skin lesions as compared with normal skin or unin-}

Table 1 Results of linkage analyses in affected pairs of sibs with atopic dermatitis

<table>
<thead>
<tr>
<th>Marker</th>
<th>Km from</th>
<th>SPLINK</th>
<th>GENEHUNTER</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lod</td>
<td>p</td>
<td>NPL p</td>
</tr>
<tr>
<td>IL4</td>
<td>144</td>
<td>2.28</td>
<td>0.001</td>
</tr>
<tr>
<td>IL9</td>
<td>148</td>
<td>0.69</td>
<td>0.05</td>
</tr>
<tr>
<td>D5S399</td>
<td>152</td>
<td>0.16</td>
<td>0.25</td>
</tr>
<tr>
<td>D5S436</td>
<td>159</td>
<td>0.39</td>
<td>0.12</td>
</tr>
<tr>
<td>D5S434</td>
<td>164</td>
<td>0.09</td>
<td>0.33</td>
</tr>
</tbody>
</table>

†More than two affected sibs were weighted by 2/n (n=the number of affected sibs).
Atopic dermatitis and interleukin 4 gene

cells from the mouth using standard phenol extraction techniques. DNA samples from AD families were genotyped for five microsatellite DNA markers on chromosome 5q using PCR primers and conditions described elsewhere. All DNA samples were tested for the -590C/T IL4 gene polymorphism by the PCR-RFLP (restriction fragment length polymorphism) method. The region of interest was amplified by PCR with a primer pair (5'-TAAACTTGGGAGAACATGGT and 5'-TGGGGAAAGATAGAGTAATA) at 93°C for five minutes, followed by 36 cycles of melting at 93°C for 60 seconds, annealing at 48°C for 60 seconds, and an extension of 72°C for 60 seconds, followed by 72°C for three minutes. The PCR product is 195 bp and spans positions -562 to -756 in the IL-4 promoter sequence. The PCR products were digested with AatII, denatured, and run on a urea 6% polyacrylamide gel at 60 W for three hours. The gels were dried and autoradiographed.

Non-parametric affected sib pair linkage analysis using the SPLINK (version 1.07) and multitip non-parametric analysis using the GENEHUNTER (version 1.2) programs were performed. Sibships containing more than one pair were weighted in SPLINK. Linkage results were interpreted according to the guidelines proposed by Lander and Kruglyak. The transmission disequilibrium test (TDT) was performed using the ASSTDT program in the Genetic Analysis System (GAS version 2.0; A Young, University of Oxford, 1993-1995) available at http://users.ox.ac.uk/~ayoung/gas.html. In case-control comparisons, the oldest offspring with AD from each unrelated family was selected and compared with unrelated control subjects, since at least two AD offspring per family were probands.

Suggestive evidence for linkage between AD and the polymorphic IL4 marker was obtained with SPLINK (lod score 2.28) and GENEHUNTER (NPL score 2.37) programs (table 1). No evidence for linkage was obtained with markers at IL9, D5S399, D5S436, or D5S434. The calculated information content by GENEHUNTER was from 0.7 to 0.85.

TDT indicated that no alleles of the microsatellite polymorphic markers at IL4, IL9, D5S399, D5S436, or D5S434 significantly deviated from the expected ratio of 1:1 transmission to AD offspring (p>0.05, data not shown). The T allele of the -590C/T IL4 gene polymorphism was significantly preferentially transmitted to the AD offspring: 86 T alleles were transmitted and 50 T alleles were not transmitted (p=0.0014, based on binomial distribution, one sided). Since preferential transmission of the T allele to AD offspring was observed, we examined the allele distribution in the control subjects. Comparisons of genotypic and allelic distribution between the AD patients and controls showed a significant increase in the number of T allele homozygotes (p=0.01) and a non-significant increase in the T allele (p=0.08) in the AD cases compared with the controls (table 2).

To date, studies on linkage between gene markers on 5q31-33 and atopy have yielded conflicting results. An association between the -590C/T polymorphism and serum total IgE levels was reported by Rosenwasser et al., but this association was not replicated by Walley and Cookson. However, the latter observed a weak association of the polymorphism with specific IgE to house dust mite and with wheezing and a non-significant trend of increased T allele frequency in asthmatics compared to controls. Therefore, we feel that our results are not inconsistent with those of Walley and Cookson, though the degree of the association differs: linkage between atopy and 5q31-33 was not observed in the families Walley and Cookson examined, while linkage between the IL4 gene and AD was observed in our families. This may result from racial differences in the IL4 allele frequencies, which are significant between whites and Japanese: the T allele frequency was 0.7 in Japanese controls, 0.26 in Australian controls, and 0.27 in white UK controls. In the present study, homozygosity for the T allele was associated with AD, indicating the greater importance of the -590C/T polymorphism in AD in Japanese than white populations. Since the T allele is reported to be associated with higher IL4 gene promoter activity than the C allele, we speculate that genetic differences in transcriptional activity of the IL4 gene influence AD predisposition in the Japanese.

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