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

Tomoko Kawashima, Emiko Noguchi, Tadao Arinami, Kimiko Yamakawa-Kobayashi, Hideki Nakagawa, Fujio Otsuka, Hideo Hamaguchi

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 -590CT 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 C 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.

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 uninflamed skin of patients with AD.

Linkage between total serum IgE levels, multi-allergen IgE antibody, or bronchial hyper-responsiveness and several polymorphic gene markers in the cytokine gene cluster, including the ILA gene on chromosome 5q31-33, has been reported, though subsequent studies failed to find evidence for linkage between 5q markers and atopic phenotypes. Rosenwasser et al reported that the IL4 promoter polymorphism, a C to T change at position -590 counting from the first ATG codon, is associated with total serum IgE levels and asthma.

There have been only a few linkage studies in AD. Chromosome 11q13 was excluded as a major susceptibility locus for AD. Weak association between genetic variants of the mast cell chymase gene and eczema has been reported in the Japanese. No studies of the linkage between AD and the 5q region and the IL4 promoter polymorphism have been reported. In this study, we investigated linkage and association between gene markers on 5q31-3q3 and AD in Japanese AD families.

A panel of 377 subjects from 88 families was ascertained through at least two AD sibs undergoing treatment for AD at clinics in Ibaraki and Tokyo, Japan. The diagnosis of AD was made based on the appearance of active skin disease, distribution of skin lesions, and clinical course of the disease. All of the sibs analysed met the diagnostic criteria of Hanifin and Rajka. The AD sibs included 93 males and 108 females aged from 1 to 38 years (mean 10 years). The control subjects were 215 children from Ibaraki, Japan aged 11 years with no history of AD. All of the control subjects were unrelated Japanese. All subjects gave informed consent. This study was approved by the Committee of Ethics at the University of Tsukuba.

Genomic DNA was extracted from peripheral blood leucocytes or mucous membrane

<table>
<thead>
<tr>
<th>Marker</th>
<th>KCM from pos*</th>
<th>SPLINK†</th>
<th>GENEHUNTER</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Log <em>p</em></td>
<td>NPL  <em>p</em></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>DSS309</td>
<td>152</td>
<td>0.16</td>
<td>0.25</td>
</tr>
<tr>
<td>DSS436</td>
<td>159</td>
<td>0.39</td>
<td>0.12</td>
</tr>
<tr>
<td>DSS434</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).
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.1

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'-
TAAAACGTGGAGAACATGGT and 5'-
TGGGGAAAGATAGATGTAATA) 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)17
and multipoint non-parametric analysis using the GENEHUNTER (version 1.2)18 programs were performed. Sibships containing more than one pair were weighted in SPLINK. Link- age results were interpreted according to the guidelines proposed by Lander and Kruglyak.19

The transmission disequilibrium test (TDT) was performed using the ASSTDT program in the Genedosage 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 old-
est offspring with AD from each unrelated family was selected and compared with unre-
lated 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 GENE-
HUNTER (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 GENE-
HUNTER was from 0.7 to 0.85.

TDT indicated that no alleles of the micro-
satellite 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 dis-
btribution, one sided). Since preferential trans-
mision of the T allele to AD offspring was
observed, we examined the allele distribution in the control subjects. Comparisons of geno-
typic and allelic distribution between the AD
patients and controls showed a significant increase in the number of T alleles 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-q33 and atopy have yielded conflicting results. An association between the
-590C/T polymorphism and serum total IgE
levels was reported by Rosenwasser et al,13 but
this association was not replicated by Walley
and Cookson.20 However, the latter observed a
weak association of the polymorphism with
specific IgE to house dust mite and with
wheeling 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,20 though the degree of the association differs: linkage between atopy
and 5q31-q33 was not observed in the families
Walley and Cookson examined,11 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, homogygosity
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 transcrip-
tional activity of the IL4 gene influence AD
predisposition in the Japanese.

Table 2 Genotypic and allelic distributions of the IL4 -590C/T polymorphism in Japanese unrelated controls and patients
with atopic dermatitis

<table>
<thead>
<tr>
<th></th>
<th>Genotype</th>
<th>Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>CC</td>
</tr>
<tr>
<td>Controls</td>
<td>215</td>
<td>17 (0.08)</td>
</tr>
<tr>
<td>AD parents*</td>
<td>122</td>
<td>7 (0.06)</td>
</tr>
<tr>
<td>AD offspring</td>
<td>88</td>
<td>8 (0.09)</td>
</tr>
<tr>
<td>Odds ratio</td>
<td>1.16</td>
<td>0.48</td>
</tr>
<tr>
<td>95% confidence interval</td>
<td>0.52-2.78</td>
<td>0.29-0.83</td>
</tr>
<tr>
<td>p value</td>
<td>0.73</td>
<td>0.007</td>
</tr>
</tbody>
</table>

C corresponds to the -590 C allele and T corresponds to the -590 T allele.
*Parents with history of atopic diseases were excluded.

p-values were calculated using x2 chi square test. p values of genotypes were based on one genotype + all other genotypes combined.
The genotype distributions did not deviate significantly from those based on Hardy-Weinberg equilibrium in each group (x2=0.91, p=0.34 in the controls; x2=2.14, p=0.14 in the AD patients; x2=3.70, p=0.054 in the AD offspring).


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