The –590C/T and –34C/T interleukin-4 promoter polymorphisms are not associated with atopic eczema in childhood

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Susceptibility to the development of asthma and other atopic diseases is known to have a genetic component. To date, several studies have linked chromosome 5q31 to asthma and atopy in human beings. This region harbors a cluster of cytokine and growth factor genes, IL-4 presenting as a prime atopy candidate gene, inasmuch as it plays a pivotal role in the atopy pathway. Our approach was to identify polymorphisms within the promoter regions of IL-4 and test their association with atopic eczema. Polymorphisms were typed in a cohort of 76 small nuclear families and 25 triads with childhood atopic eczema. The genotypes were used to test for linkage in the presence of association with atopic eczema. A new polymorphism, –34C/T, was identified and studied with a known polymorphism, –590C/T. On its own, each polymorphism showed no association with atopic eczema. The 2 polymorphisms were used to generate haplotypes, and a significant result was found for the –590C/–34C haplotype. However, after Bonferroni correction for multiple testing, the association became nonsignificant. Neither polymorphism predisposes to early-onset atopic eczema by itself, but suggestive linkage was found for the –590C/–34C haplotype in this study. (J Allergy Clin Immunol 2001;108:285-7.)

Key words: *Atopy, eczema, dermatitis, asthma, genetic, IL-4, promoter, polymorphism, association, linkage disequilibrium*

Atopy, often manifested clinically as asthma, eczema, allergic rhinitis (hay fever), or food allergy, is recognized as a genetically complex disease because its analysis is complicated by the combined effects of genetic heterogeneity, phenocopy, gene interactions, and incomplete penetrance.[1]

Of the atopic disorders, eczema offers the best model for identifying genetic factors, inasmuch as it shows a higher concordance in monozygotic twins, has a closer correlation with elevated serum IgE, and is easier to diagnose than other atopic conditions.

Several studies have linked chromosome 5q23-31 to elevated serum IgE asthma, eczema, and atopy in general.[1] Harbored within this region is a cluster of cytokine genes, including IL-4.[1]

IL-4 plays an important role in IgE synthesis by activating the pre–T helper cells that trigger isotype switching from IgM/IgG to IgE in B cells by promoting T_H2 cell development. IL-4 has also been shown to inhibit production of IFN-γ, which inhibits IgE synthesis and the development of T_H1 cells.[2] Lymphocytes from children with atopic dermatitis are reported to secrete increased amounts of IL-4,[3] and studies have shown that IL-4 mRNA expression is increased in acute skin lesions from patients with atopic eczema in comparison with normal skin.[4]

It is logical to expect that increased expression of IL-4 might lead to atopic diseases; therefore, the IL-4 promoter region would seem the most likely site for polymorphism. One of the IL-4 promoter polymorphisms identified thus far, –590C/T,[5] is close to the glucocorticoid response element that positively stimulates IL-4 expression.

Abbreviation used
df: Degree(s) of freedom

METHODS

Our approach was to screen the IL-4 promoter region for additional polymorphisms in control subjects. We then confirmed the presence of the –590C/T polymorphism and identified a second polymorphism, –34C/T, in a cohort of families affected with atopic eczema. We tested the association of these polymorphisms with atopic eczema in a cohort of 101 families consisting of 76 affected sibling pairs and their parents and 25 triads of mother, father, and affected child, identified from families attending clinics at the Allergy Centre, Dermatology and Immunology Departments at the Royal Children's Hospital, Parkville, and the Westmead Hospital, Sydney.

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TABLE I. Linkage in the presence of association between the –590C/T and –34C/T IL-4 promoter polymorphisms and atopic eczema

	Transmitted	Untransmitted	
–590			Freq: C = 59%, T = 41%
C	25	28	$\chi^2 = 0.17$, $P = .68$
T	28	25	
–34			Freq: C = 46%, T = 54%
C	34	25	$\chi^2 = 1.37$, $P = .24$
T	25	34	

Freq, Frequency.

TABLE II. Linkage in the presence of association between the –590C/T and –34C/T IL-4 promoter haplotypes and atopic eczema

–590, –34	Transmitted	Untransmitted	
CC	23	10	$\chi^2 = 5.12$, $P = .023$
CT	12	21	$\chi^2 = 2.45$, $P = .117$
TC	3	6	$\chi^2 = 1.00$, $P = .317$
TT	14	15	$\chi^2 = 0.03$, $P = .852$

RESULTS

Initially, each polymorphism was tested individually. At the –590C/T polymorphism we could find no evidence of excess transmission of either allele to the affected children ($\chi^2 = 0.17$ with 1 degree of freedom [df]; $P = .68$). We observed a similar result with the –34C/T polymorphism ($\chi^2 = 1.37$ with 1 df; $P = .24$); there was no transmission disequilibrium with respect to the affected children (Table I). When the polymorphisms were scored for association with each other (independently of disease), we found a high degree of linkage disequilibrium: the –590C and –34C alleles and the –590T and –34T alleles are strongly associated with each other ($\chi^2 = 18.74$ with 1 df; $P = .000015$)—as expected, given their close proximity (556 base pairs).

Because of the significant linkage disequilibrium, we analyzed the haplotype transmissions. Each untransmitted parental haplotype was scored, and we observed the following control frequencies: –590C/–34C (47%), –590C/–34T (23%), 590T/–34C (6%), and –590T/–34T (24%; Table I).

We found significant association of the –590C/–34C haplotype with atopic eczema ($\chi^2 = 5.12$, $P = .023$) but not with the other 3 haplotypes (Table II). However, correcting for multiple tests (a total of 6 tests, comprising 2 separate loci and 4 haplotypes) through use of the Bonferroni method reduces the *P* value threshold to .008, and the CC haplotype *P* value does not reach this adjusted *P* value threshold.

The Bonferroni correction is the strongest multiple testing correction because it assumes independent tests; our 6 tests are not independent, so this correction produces a very conservative *P* value threshold. It is unclear how to adjust for these 6 tests correctly.

DISCUSSION

To date, studies on linkage of atopy to 5q31 have yielded conflicting results.[1] The reasons for this most likely pertain to subject selection when the cohorts were chosen for study. Most studies have focused on individuals with asthma or elevated serum IgE, both of which can be exclusive symptoms of atopy, leading to phenotypic variation. Therefore it is hardly surprising that many conflicting reports of genetic linkages have been published for what is already recognized as a genetically complex disease. We selected individuals with an early age of onset, a family history of atopic eczema, and a clearly defined phenotype, all of which enhance the likelihood of the disease's having a genetic origin.

In 1995, Rosenwasser et al[5] first reported an association between the IL-4 promoter polymorphism –590C/T and serum total IgE levels. Since then, several papers have reported studies of the association of this polymorphism with atopy. In subjects in the United Kingdom, Walley and Cookson[6] found a weak association with asthma and atopy but not with elevated serum IgE. Noguchi et al[7] and Kawashima et al[8] reported similar findings: increased transmission of the T allele to Japanese children affected with asthma or atopic dermatitis.

Using our cohort of 101 families, we obtained no evidence of association of atopic eczema with –590C/T.[5] In Australian families, Dizier et al[9] also failed to find association of this polymorphism with elevated serum IgE, despite weak evidence of linkage. The reported inconsistencies between different populations might result from racial differences in the IL-4 allele frequencies between Caucasians and Japanese. For example, the T allele frequency is 70% in Japanese controls,[7] 26% in Australian controls,[9] and 27% in white controls in the United Kingdom.[6] Though predominantly Caucasian, our cohort contains families from ethnically diverse origins. These observations suggest that IL-4, a molecule with which it interacts, or both of these play a more significant role in allergic disease in the Japanese population.

The second polymorphism, –34C/T, was recently reported by Takabayashi et al[10]; it was found to be in strong linkage disequilibrium with –590C/T, the –590T/–34T haplotype being prevalent. In a manner consistent with their findings, we observed strong linkage disequilibrium between the –590C and –34C alleles. Takabayashi et al[10] did not study the association of this polymorphism with any disease, and our study is the first to test this polymorphism in a clinical context. Using a transmission disequilibrium test, we found no linkage or association of this polymorphism with atopic eczema, suggesting that neither the –590C/T polymorphism nor the –34C/T polymorphism on its own is playing a functional role in the expression of atopic eczema. When the polymorphisms were sorted into 4 haplotypes, no significant association was detected with 3 of them; the fourth haplotype appeared to be associated, but it did not meet the significance levels after correction for multiple testing. However, the multiple testing correction assumed independence of tests, an assumption that is incorrect here, and it thus represents a conservative *P* value threshold.

Although this study showed no association with each single nucleotide polymorphism separately, it did show association with a particular haplotype, the –590C/–34C haplotype. It is possible that the single nucleotide polymorphisms separately lack power to detect linkage whereas the haplotype is sufficiently polymorphic (4 alleles) to detect a suggestive linkage. This study thus provides further tentative evidence that IL-4 might play a role. To investigate this further, the haplotype should be retested for linkage in another cohort with similar selection criteria and preferably with a larger sample size. By testing for the –590C/–34C haplotype only, it is possible to avoid the multiple-testing issue.

The sample size in this study was quite small once the criterion for the transmission disequilibrium test (heterozygous parents) was met. Furthermore, the strict clinical criteria might have compromised the sample size. However, power is gained by reducing heterogeneity. Setting stringent criteria is important in analyzing the genetics of complex diseases, such as eczema, in which the maximum number of variables should be eliminated to optimize the likelihood of finding true linkage or association. Phenocopies are also greatly reduced by selecting small nuclear families with more than 1 occurrence of eczema. Unfortunately, this also reduces the sample size.

Despite the numerous reports of linkage to the IL-4 region of chromosome 5 and the obvious candidacy of IL-4 as an atopy gene, there still remains a surprising lack of significant association of variations in the IL-4 gene with atopy. This study provides further tentative evidence that this region of 5q31 contains 1 or more loci influencing the predisposition to atopy, but it is still not possible to assign a major role to the IL-4 gene in the genetic predisposition to atopic disease.

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