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Association and Linkage of Atopic Dermatitis with Chromosome 13q12–14 and 5q31–33 Markers

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Atopic dermatitis is a chronic inflammatory skin disease that affects 10–20% of the population. Linkage of atopy, asthma, allergic rhinitis, and total serum IgE levels to several different chromosomal regions have been described extensively, but little is known about the genetic control of atopic dermatitis. We tested for the association and linkage between atopic dermatitis and five chromosomal regions: 5q31–33, 6p21.3, 12q15–24.1, 13q12–31, and 14q11.2/14q32.1–32.3. Marker analysis was performed in two Caucasian populations: (i) 192 unrelated German children with atopic dermatitis and 59 non-atopic children from a German birth cohort study (MAS '90), parental DNA was tested in 77 of 192 children with atopic dermatitis; (ii) 40 Swedish families with at least one family member with atopic dermatitis selected from the International Study of Asthma and Allergy in Children. Evidence for linkage and allelic association for atopic dermatitis was observed for markers on chromosome 13q12–14 and 5q31–33. ***Key words: genetics/marker analysis. J Invest Dermatol 115:906–908, 2000***

Twin and family studies have demonstrated the importance of genetic factors in contributing to the development of atopic dermatitis (AD), which is a common skin disease (Schultz-Larsen *et al*, 1986; Küster *et al*, 1990). A number of studies on the genetics of atopy, IgE responsiveness, and asthma have been published (Marsh *et al*, 1994; Meyers *et al*, 1994; Postma *et al*, 1995; Barnes *et al*, 1996; The Collaborative Study on the Genetics of Asthma 1997; Daniels *et al*, 1996), but few investigations have focused on AD (Fölster-Holst *et al*, 1998; Mao *et al*, 1996; Cox *et al*, 1998). Identification of susceptibility genes for AD should lead to a better understanding of pathogenetic mechanisms and this could be the basis for future preventive studies.

The objective of this study was to test for the association and linkage of AD and markers in candidate gene regions in children from two Caucasian populations.

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[1]Professor David G. Marsh was actively involved in the design and implementation of this study. He passed away on March 29, 1998, after a heroic battle against brain cancer. We wish to dedicate this paper to his memory.

Abbreviations: MAS '90, German birth cohort study; ISAAC, International Study of Asthma and Allergy in Children; TDT, transmission disequilibrium test; HMG1, high-mobility group protein 1; CLEO, conserved lymphokine element-0.

SUBJECTS AND METHODS

One hundred and ninety-two children of bilateral German ethnicity diagnosed with AD were recruited from the MAS '90 cohort along with 59 non-atopic children. Genomic DNA was collected from one or both parents in 77 of the 192 children with the diagnosis of AD. The MAS '90 cohort initially comprised 1314 unrelated, healthy, mature newborns born in 1990 (Bergman *et al*, 1994). Parents participating in the study gave their written informed consent as approved by the Ethics Committee (Berlin, Germany). Yearly follow-up visits from birth until present were conducted. the diagnosis of AD was defined by the criteria of MAS '90 (Bergmann *et al*, 1997): a combination of "dry skin" and at least three of nine typical signs (e.g., itching, erythema, red papules) at more than three of 26 typical anatomic sites (e.g., cheeks, retroauricular, extensor and flexural areas).

In addition, 40 Swedish families in which at least one member had AD were recruited from the International Study of Asthma and Allergy in Children (ISAAC) cohort (3537 children, aged 13–14 y) in Linköping. Recruited families had at most one atopic parent and at least three children, at least one of whom had a history of allergy as defined by the criteria in the ISAAC (Asher *et al*, 1995). The ISAAC questionnaire included five questions related to skin symptoms, including AD (Williams *et al*, 1999). A diagnosis of AD was based on the criteria suggested by Hanifin and Rajka (1980).

Genomic DNA was purified from blood (Qiagen Blood DNA Kit) or jugal epithelial cells (Aron *et al*, 1994). Microsatellite markers were analyzed as described before (Marsh *et al*, 1994). Markers were analyzed for linkage and allelic association on the following chromosomes: 5q31–33 (*D5S642, D5S666, D5S1972, D5S643, D5S436, D5S207, D5S210, D5S2090, CSF1R*), 6p21.3 (*D6S291, DQCAR*, and *D6S273*), 12q15–24.1 (*D12S1052, D12S379, D12S1064, D12S351, D12S95, PAH*), 13q12–31 (*D13S219, D13S894, D13S1491, D13S218, D13S1288, D13S1248, D13S263, D13S291, D13S328, D13S270, D13S284, D13S318*), 14q11.2, and 14q32.1–32.3 (*TCRD, D14S34, D14S260, D14S542*). All primers were supplied by Research Genetics (Huntsville, AL).

To test for association in the German population the frequencies of various alleles were compared among children affected with AD and

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Table I. Association and linkage to a polymorphic marker (*D13S218*) on chromosome 13q12–14 in the German population[a]

Locus	Dist (cM)	χ^2 Global ×2(df)	χ^2 Global p	TDT Global Ewens ×2 (df)	TDT Global Ewens p	TDT Global Pearson ×2 (df)	TDT Global Pearson p
D13S1491	0	7.5 (10)	NS	8.1 (9)	NS	20.1 (24)	NS
D13S218[b]	2	22 (8)	0.005	12.8 (6)	0.046	23.5 (12)	0.008
D13S1288	3	12 (13)	NS	6.3 (10)	NS	18.2 (22)	NS

[a]Association- and TDT analysis for AD and chromosome 13q12–31 markers in the German population. Only the significant and adjacent markers in the region 13q12–14 are shown. All other analysed markers in a region over 30 cM were not significant.
[b]Allele-by-alele association analysis ×21(1 df)=8.9, p=0.0029 for allele 189 where 61 were counted in affected and 33 in unaffected children. Allelic TDT=4.0, p=0.046 for allele 193 where four were transmitted and 12 were not transmitted and allelic TDT=6.7, p=0.009 for allele 195 where 27 were transmitted and 11 were not transmitted to the affected child.

unaffected controls using a χ^2 test for a 2×N (alleles) contingency table (GAS, Version 2.0, routine ASSCOMPARE). Aditionally, a χ^2 test for an allele of interest is performed by collapsing the 2×N table into a 2×2 table comparing a given allele of interest with all others. In the 77 German trios and 40 Swedish nuclear families, parental transmission of marker alleles was analyzed using results from two transmission disequilibrium test (TDT) procedures (SIB-PAIR, David L.Duffy, Version 0.93, 1995/1996; Duffy 1996). The first is a multiallalic test for complete symmetry in the full N×N transmission table, where N is the number of alleles at the locus (Duffy *et al*, 1990). The null hypothesis is tested with a Pearson χ^2 statistic proposed by Ewens and Spielman (1995), the "global (symmetry)" TDT. The second analysis is performed using each allele in turn with the comparison group being all other alleles, an "allele-by-allele" TDT (Spielman *et al*, 1993). Affected sib pairs from the Swedish nuclear families were used in an affected sib-pair analysis using the program SIBPAL 2.7 included in the SAGE 2.2A suite (SAGE, 1994).

RESULTS

AD is associated with chromosome 13q12–14 and chromosome 5q31–33 markers in the German population In the German population comparison of 192 unrelated AD children with the unaffected control group showed a significant association between AD and *D13S218* on chromosome 13q12–14 using both the global and allele-by-allele statistics (**Table I**). Using the TDT, parental transmission of marker alleles showed significant evidence for linkage to the same marker (**Table I**). On chromosome 5q31–33 evidence for association for the markers *D5S436* and *D5S643* (1 cM upstream of *D5S436*) were also found by both analysis (**Table II**).

AD is associated with chromosome 13q12–14 markers in the Swedish population In the Swedish families, evidence for association and linkage was also found for *D13S218* using the global and allelic TDT and the affected sib-pair analysis (**Table III**). The only other evidence for association was found with an allele at marker *D5S436* (allelic TDT=4.0, p=0.046 for allele 250 where four were transmitted and 12 were not transmitted to the affected child; Ewens TDT=13.5 (12 df), Ewens p=NS). None of the investigated markers on chromosome 6p, 12q, and 14q showed significant evidence for association with AD in either study population (data not shown).

DISCUSSION

Using the candidate gene approach, linkage of bronchial hyperresponsiveness and high total serum IgE to chromosome 5q31.1–q33.3 has been described (Marsh *et al*, 1994; Meyers *et al*, 1994; Postma *et al*, 1995). Interestingly, Meyers *et al* (1994) and Postma *et al* (1995) found their most significant finding for the marker

Table II. Association and linkage to two adjacent markers (*D5S436*, *D5S643*) on chromosome 5q31–33 in the German population[a]

Locus	Dist (cM)	χ^2 Global ×2(df)	χ^2 Global p	TDT Global Ewens ×2 (df)	TDT Global Ewens p	TDT Global Pearson ×2 (df)	TDT Global Pearson p
D5S666	0	7.8 (14)	NS	9.6 (11)	NS	22.3 (26)	NS
D5S643[b]	10	20 (9)	0.017	9.9 (9)	NS	17.8 (14)	NS
D5S436[c]	11	30 (14)	0.008	18.3 (13)	NS	56.3 (41)	0.007
D5S207	13	1.9 (6)	NS	6.0 (10)	NS	9.9 (10)	NS

[a]Association- and TDT-analysis for AD and chromosome 5q31-33 markers in the German population. Only the significant and adjacent markers are shown. All other analysed markers in the region over 21 cM were not significant.
[b]Allele-by-allele association analysis ×21(1 df)=10, p=0.0014 for allele 146 where none were counted in affected and 3 in unaffected children and ×2(1 df)=7.3, p=0.007 for allele 164 where six were counted in affected and seven in unaffected children.
[c]Allele-by-allele association analysis ×2(1 df)=10, p=0.0016 for allele 254 where none were counted in affected and three in unaffected children. Allelic TDT=8.2, p=0.0043 for allele 238 where 19 were and 5 were not transmitted to the affected child.

Table III. Association and linkage to a polymorphic marker (*D13S218*) on chromosome 13q12–14 in the Swedish population[a]

Locus	Dist (cM)	TDT Global Ewens ×2 (df)	TDT Global Ewens p	TDT Global Pearson ×2 (df)	TDT Global Pearson p	Sib-pair-analysis p
D13S1491	0	8.5 (5)	NS	18.1 (13)	NS	NS
D13S218[b]	2	18.4 (6)	0.005	18.0 (9)	0.014	0.05
D13S1288	3	10.9 (11)	NS	18.2 (22)	NS	NS

[a]TDT- and sib-pair-analysis for AD and chromosome 13q12–31 markers in the Swedish population. Only the significant and adjacent markers in the region 13q12–14 are shown. All other analysed markers in the region over 30 cM were not significant.
[b]Allelic TDT=4.5, p=0.034 for allele 187 where one were and seven were not transmitted, allelic TDT=6, p=0.014 for allele 189 where six were and 18 were not transmitted and allelic TDT=4.4, p=0.036 for allele 191 where 33 were and 18 were not transmitted to the affected child.

D5S436, the same marker showing evidence for association with AD in this study. Chromosome 5q31.1–q33.3 is a region of special interest as several candidate genes for atopy and atopy-related phenotypes map to this region: interleukin (IL)-13, IL-4, IL-5, interferon regulatory factor 1, IL-3, granulocyte–macrophage colony-stimulating factor, IL-9, and granulocyte–macrophage colony-stimulating factor 1 receptor (Marquet *et al*, 1996). Interestingly, D5S436 maps in the region centromeric of CSF1R and telomeric of CSF2 (Marquet *et al*, 1996), where the glucocorticoid receptor is located (Huebner *et al*, 1990). Abnormalities in glucocorticoid receptor binding have been observed in patients with steroid-resistant asthma (Sher *et al*, 1994) and in patients with AD (Clayton *et al*, 1995).

One candidate gene that maps in the region of chromosome 13q is the IgE-dependent histamine-releasing factor (MacDonald *et al*, 1999). One nearby marker yielding evidence of linkage to atopy in two distinct populations (Australian, UK) (*D13S270*) was also used in our analysis (Daniels *et al*, 1996). This marker showed no evidence for linkage to AD in our two study populations. Recently, a genome-wide scan described evidence for linkage to asthma in Caucasian sib-pairs in 13q21.3-qter using multipoint analysis (The Collaborative Study on the Genetics of Asthma,

1997); however, we did not observe evidence for linkage nor significant association to AD with marker *D13S318*. Few known candidate genes for AD map in region of *D13S218*, e.g., the high-mobility group protein 1. High-mobility group protein 1 plays a part in gene regulation as a transactivator or quasi-transcription factor (Aizawa *et al*, 1994). High-mobility group protein 1 binds to conserved lymphokine element-0 in the IL-5 promoter (Marrugo *et al*, 1996). It also binds to the conserved lymphokine element 0 of IL-4, granulocyte–macrophage colony-stimulating factor and granulocyte colony-stimulating factor genes. Polymorphisms in the high-mobility group protein 1 gene may lead to interference in the transcription of IL-4 and IL-5.

Many AD patients will develop asthma or allergic rhinitis later in life. It seems that similar genetic and immunologic mechanisms are involved in these associated atopic diseases. So it is likely that a region on chromosome 5q31–33 is associated with and linked to bronchial hyperresponsiveness, total IgE, and AD. Asthma, atopy, and elevated IgE levels are unlikely to be caused by single gene alterations but rather by a complex interaction of both major and minor disease susceptibility genes and environmental factors. Although there is an overlap in these phenotypes, differences do occur. We hypothesize that there are both common and distinct areas on chromosomes that are associated with one of these phenotypes. Unlike chromosome 5, on chromosome 13 it seems that the different phenotypes are linked not to the same, but to different regions; also, on chromosome 12, evidence for linkage was shown to both, asthma and total IgE (Barnes *et al*, 1996; Nickel *et al*, 1997), but not to AD.

In conclusion, we have identified two chromosomal regions showing evidence for both association and linkage with AD. Further linkage studies using a genome-wide screen would provide further insight in the genetic basis of AD.

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