

Original article

Susceptibility loci for atopic dermatitis on chromosome 21 in a Swedish population

Background: Atopic dermatitis (AD) is a hereditary, pruritic, chronic, relapsing, inflammatory skin disease resulting from multiplex interactions between genes and environmental factors. We have previously found several loci showing suggestive linkage on chromosomes 3q14, 13q14, 15q14-15 and 17q21, and weaker linkage to chromosomes 1p32, 4q24-26 and 21q21 in 109 Swedish families.

Methods: In order to confirm the linkage to chromosome 21, we carried out a replication linkage analysis with additional microsatellite markers on chromosome 21 in another set of 295 families.

Results: In the extended material, the Z-score was 2.40 ($P < 7.4 \times 10^{-4}$) in the region 21q21 for a semi-quantitative variable measurement; the severity of AD. When combining the two data sets into 404 families and stratifying according to asthma status, suggestive linkage was found only in the group of AD patients who also had asthma (Z-score 2.45, $P < 7.4 \times 10^{-4}$ and 2.69, $P < 7.4 \times 10^{-4}$) in two different regions.

Conclusions: Our results suggest that 21q21 could contain a susceptibility gene modulating the severity of AD especially in combination with asthma.

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Atopic dermatitis (OMIM 603165) (AD), also known as eczema according to the new World Allergy Organization nomenclature, is a common skin disorder characterized by itchy and inflamed skin. It often co-exists with other atopic manifestations such as asthma and allergic rhinoconjunctivitis (1, 2). The prevalence of atopic dermatitis, which is a hereditary chronic relapsing inflammatory skin disease, has increased considerably in several industrialized countries during the past three decades (3, 4). In Sweden, the prevalence of AD more than doubled between 1979 and 1991 and is now, at approximately 15%, one of the highest in the world (5). Genetic studies have demonstrated that the development of AD is due to complex interactions between genetic and environmental factors. Twin studies of AD have shown concordance rates of 0.72–0.86 in monozygotic twin pairs and 0.21–0.23 in dizygotic twin pairs (3, 6), and when both parents have AD, children have a risk of up to 75% of developing the disease (6).

During the previous few years, several genome-wide linkage analyses have been performed in different populations and several loci for AD have been found (7–9). In our previous genome-wide linkage analysis in a Swedish population (7), several susceptibility loci were found. We reported suggestive linkage to AD on chromosomes 3, 13, 15, 17 and 18; we also found weaker linkage to several other chromosomal regions including 21q21 (logarithm of

odds [LOD] 1.69, $P < 0.005$). To confirm those findings, we studied additional families and more markers focusing on each specific chromosome. Here, we report linkage to 21q21 in 404 families affected with AD.

Materials and methods

The family material

Families were recruited during 1995–1997 based on the registration of patients in the Departments of Dermatology, Karolinska Hospital and Danderyd Hospital, Stockholm, Sweden. Families with at least two siblings affected with AD and above 4 years of age were included. The family material has been described previously (10). The genotyped families were divided into two data sets. The first (D_1) consisted of 109 families and contained 470 individuals with 193 affected sib pairs and nine affected half-sib pairs. The second, replication data set (D_2) consisted of 295 pedigrees and contained 1020 individuals with 270 affected pairs and 29 affected half-sib pairs. Both data sets also contained some more distantly related affected relative pairs such as cousin pairs. The two data sets were combined into a new data set (D_{all}) summing up 404 families including 463 affected sib pairs, 38 affected half-sib pairs – a total of 1490 individuals with a mean age of 29 years in the affected siblings (Table 1).

Phenotypes

Four phenotypes were determined in the affected siblings. In one, AD was diagnosed according to the U.K. Working Party's

Table 1. Number of individuals in each data set

	Data set 1 (D ₁)	Data set 2 (D ₂)	Data set all (D _{all})	'Atopic dermatitis' + Ast	'Atopic dermatitis' - Ast	'The severity score of AD' + Ast	'The severity score of AD' - Ast
Number of pedigrees	109	295	404	286	118	286	118
Number of individuals	470	1020	1490	1095	595	1095	595
Affected sib pairs	193	270	463	359	104	359	104
Affected half-sib pairs	9	29	38	33	5	33	5
Parent-offspring pairs	127	304	431	341	90	341	90
Aunt/uncle-niece/nephew pairs	14	38	52	50	2	50	2

Table 2. Severity scoring of atopic dermatitis

Factor	Score
Age at onset <2 years	1
Hospitalization for AD	1
Number of sites* manifesting AD at examination	
0	0
1-3	1
>3	2
Raised total and/or allergen-specific serum IgE	1
Maximum score	5

*Presence of atopic dermatitis (AD) in one or both sites in bilateral structures was considered as one site.

Diagnostic Criteria (atopic dermatitis). In another, AD was combined with elevated allergen-specific serum IgE levels (sp-IgE⁺), and a third consisted of patients with a more severe phenotype (extreme AD). This group was defined as having an early age of onset (≤ 2 years) combined with a severity score ≥3. The fourth phenotype (the severity score of AD) was evaluated against a semi-quantitative standard (Table 2). The family material for the phenotype (atopic dermatitis) was divided into two subgroups according to co-existence with asthma (atopic dermatitis + Ast) or without asthma (atopic dermatitis - Ast). The same categorization was made for the semi-quantitative phenotype: (the severity score of AD + Ast) or (the severity score of AD - Ast). If one or more of the siblings in the affected pairs had concomitant asthma, the family was categorized as +Ast; and otherwise as -Ast (Table 1).

Quantification of IgE antibodies

Immunoglobulin E levels were measured at the Department of Clinical Immunology, Karolinska Hospital. The following was measured in all affected siblings: (i) the total serum IgE concentration using the Pharmacia CAP System, IgE FEIA (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden). The cut-off was 2 kU/l; (ii) IgE antibodies to Phadiatop®, a mixture of inhalant allergens (Pharmacia CAP System Phadiatop®FEIA). The Phadiatop® was reported as either positive or negative; (iii) IgE antibodies to a mixture of relevant food allergens (fx5) (Pharmacia CAP System RAST® FEIA). The RAST mixture was divided into six classes, where a concentration < 0.35 kU/l represents a negative result (class 0). In the linkage analysis, we considered increased allergen-specific serum IgE antibodies (sp-IgE⁺) to either Phadiatop® or food allergens as affected for the sp-IgE⁺ phenotype.

Genotyping

Genotyping was as described previously (7). Ten markers, D21S1432, D21S1256, D21S1437, D21S1922, D21S1884, D21S1914,

D21S1435, D21S1440, D21S1255 and D21S266, were genotyped: The mean heterozygosity was 0.71, and the mean intermarker distance was 5.83 cM. The marker map was based on the deCODE map (<http://www.ncbi.nih.gov/mapview>; accessed 16 February 2006).

Statistical analysis

As the inheritance models in complex genetic disorders such as AD are not clear, non-parametric, affected relative pair-based methods were used to detect linkage. The ALLEGRO software (11) was used for the linkage analysis for qualitative phenotypes while GENHUNTER2.0 and the command 'non-parametric' was used for the semi-quantitative trait severity score and the corresponding *P*-values were interpreted according to Kruglyak *et al.* (12) as summarized by Nyholt (13). For multi-point linkage analysis, the LOD ≥2.19 corresponding to a *P*-value of < 7.4 × 10⁻⁴ was regarded as suggestive linkage. All the families were weighted equally in a linear model.

Allele frequencies were estimated from all genotyped individuals using the zGENSTAT1.126 program (H. Zazzi, unpubl. data). All genotyped markers were checked for Mendelian incompatibilities using zGENSTAT1.126 program (H. Zazzi, unpubl. data). Either the incompatibility was resolved unambiguously, or individuals and/or pedigrees were excluded from the linkage analysis. To identify markers with allele dropout or other problems, the expected number of homozygous individuals was calculated based on the estimated allele frequencies and compared with the observed numbers of homozygotes using the Pearson chi-squared test with zGENSTAT 1.126 software (H. Zazzi, unpubl. data). Any marker deviating significantly from expected homozygosity frequency (*P* < 0.001) was reanalysed or removed from the analysis. This resulted in the removal of data from two markers (D21S1255 and D21S1446).

Results

A two-step strategy was used. First, we scanned the first data set D₁, consisting of 109 families and then the second, D₂, consisting of 295 families. Then the two data sets were combined into one including 404 families with 1490 individuals forming 463 sib pairs and 38 half-sib pairs, D_{all}. We found no evidence suggestive of any linkage to any of the phenotypes 'atopic dermatitis', 'sp-IgE⁺' or 'extreme AD' (Fig. 1).

The multipoint linkage analyses of the semi-quantitative phenotype (the severity score of AD) were also conducted in all three data sets D₁, D₂ and D_{all}. In data set D₁, the highest *Z*-score (1.96, *P* < 0.005) was close to marker D21S1914 in region 21q21.1. In the larger data

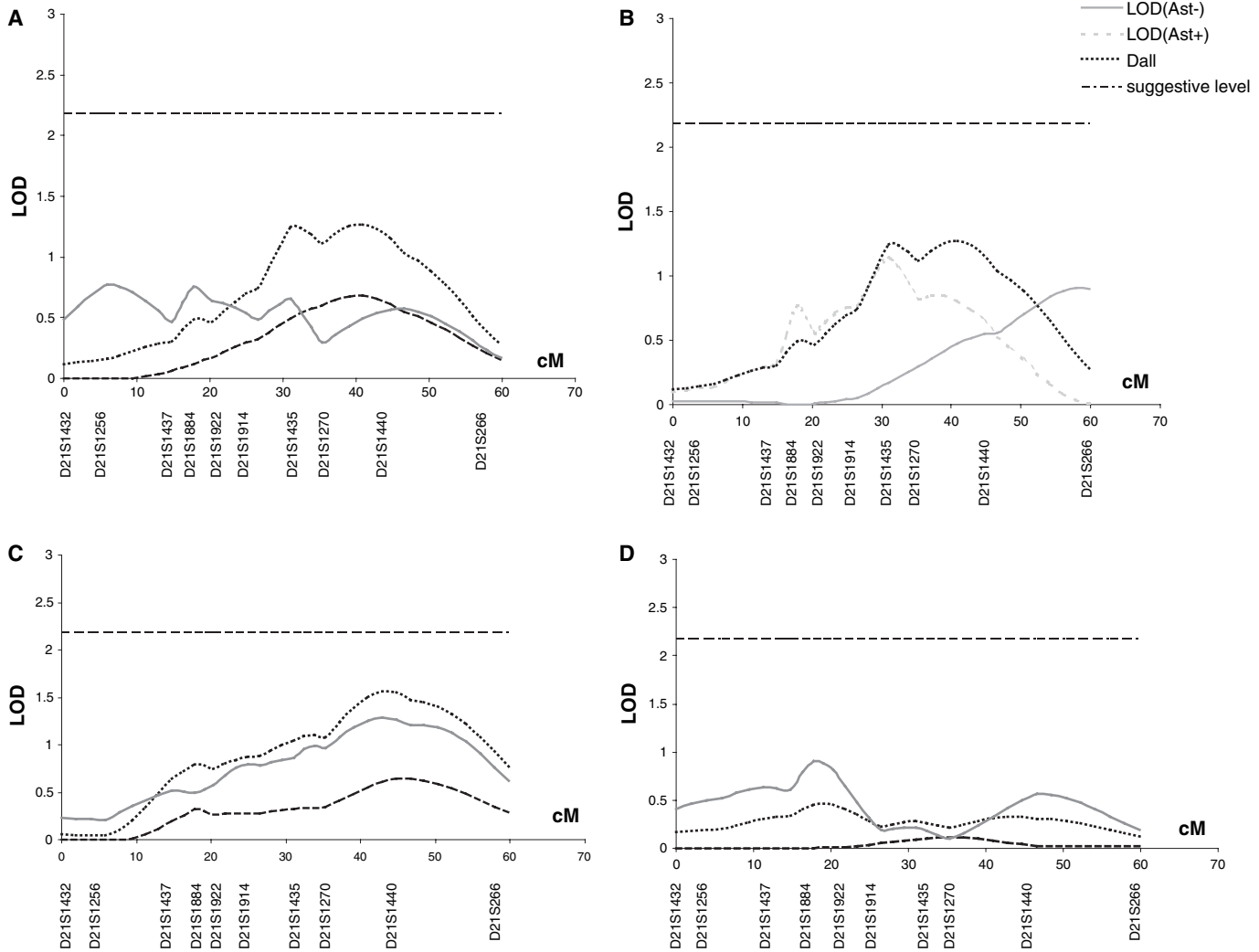


Figure 1. Results of multipoint linkage analysis in three data sets D_1 , D_2 and D_{all} for phenotypes: (A) 'atopic dermatitis', (B) 'atopic dermatitis' with asthma (Ast+) and without asthma (Ast-), (C) 'extreme AD' and (D) 'sp-IgE⁺'. The upper horizontal line represents the threshold for suggestive linkage, $P < 7.4 \times 10^{-4}$. The LOD score is given on the y-axis and the recombination distance in centimorgans (cM) from p-ter to q-ter on the x-axis in linear scale. The linkage analysis was performed in ALLEGRO.

set, D_2 , the highest Z-score was 2.68, close to marker D21S1884 in the same region. In data set D_{all} , one peak was close to marker D21S1884 on region 21q21.1 (Z-score 2.40, $P < 7.4 \times 10^{-4}$), (Fig. 2).

The combined data set D_{all} was then divided into two groups ('the severity score of AD' + Ast) and ('the severity score of AD' - Ast). In the former, two peaks were achieved, one with Z-score 2.45 close to marker D21S1922 in region 21q21.1, the other with Z-score 2.69 close to marker D21S1440 in region 21q21.3 (Fig. 2).

Discussion

In our previous genome-wide linkage analysis, several loci, 3q14, 13q14, 15q14-15 and 17q21, showed suggestive

linkage to AD while other loci, including 21q21, showed weaker linkage to AD (LOD 1.69, $P < 0.005$) (7). To confirm these findings, we carried out replication linkage analyses with additional microsatellite markers in another 295 families focusing on each specific chromosome.

Here, we report the results from chromosome 21. The phenotype ('the severity score of AD') showed evidence in favour of linkage to 21q21.1 in the first data set D_1 (Z-score 1.69, $P < 0.005$). A second, larger, data set D_2 confirmed this linkage (Z-score 2.68, $P < 7.4 \times 10^{-4}$). These data suggest that 21q21.1 could be a region harbouring a susceptibility gene for AD.

It is, however, very common for AD to coexist with allergic asthma and allergic rhinoconjunctivitis. In general, 50–80% of AD patients also have asthma and/or allergic rhinoconjunctivitis (14). In the family material

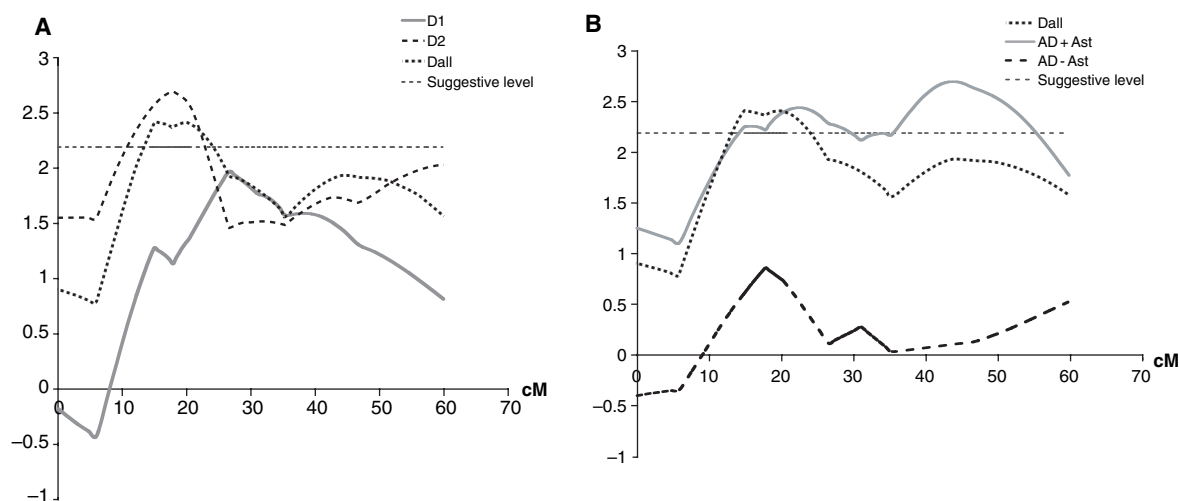


Figure 2. Result of the multipoint linkage analysis in three data sets D_1 , D_2 and D_{all} ; (A) 'The severity score of AD'; (B) 'The severity score of AD' with asthma and without asthma. The horizontal line represents the threshold for suggestive linkage, $P < 7.4 \times 10^{-4}$. The linkage analysis was performed in GENEHUNTER2.0.

used in the study, 72% of the affected siblings suffered or had suffered from asthma and/or allergic rhinoconjunctivitis and 74% had raised total and/or allergen-specific serum IgE levels (10). As different phenotypes could have different susceptibility loci, and the phenotypes can confound each other, we stratified our data D_{all} into families having asthma and those not having asthma. In the phenotype with asthma ('the severity score of AD' + Ast), we found two regions showing suggestive linkage; 21q21.1 (Z-score 2.70) and 21q21.3 (Z-score 2.72). However, in the phenotype without asthma ('the severity score of AD' - Ast) no region showed suggestive linkage.

Two genome-wide linkage analyses have previously shown that 21q21 is linked to asthma (15, 16). The susceptibility loci 21q21.1 and 21q21.3 linked to AD found in this study could either be the result of a confounding effect and be an asthma locus and not an AD locus, or could be linked to both the phenotypes

asthma and AD. The susceptibility loci could also be linked to more severe AD phenotype in which asthma often coexists. Asthma is one of the factors contributing to the severity score used in this study.

To elucidate this question we also tested for a distribution difference in the AD severity scores (according to Table 1) between the two groups ('the severity score of AD' + Ast) and ('the severity score of AD' - Ast). The chi-squared test showed that the phenotype ('the severity score of AD' + Ast) included significantly more patients with higher scores than the phenotype ('the severity score of AD' - Ast) ($\chi^2 = 16.05$, $df = 5$, $P < 0.0067$) did. This could reflect the fact that the loci on 21q21 are linked to more severe AD, or that patients with a higher severity score have a stronger genetic background. To conclude, we report for the first time a linkage among AD patients with asthma to chromosome 21q21 in a Swedish population.

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