

## INVESTIGATIVE REPORT

# Atopic Dermatitis – a Total Genome-scan for Susceptibility Genes

Annette HAAGERUP<sup>1,3</sup>, Torbjørn BJERKE<sup>2</sup>, Peter Oluf SCHIØTZ<sup>3</sup>, Ronald DAHL<sup>4</sup>, Helle Glud BINDERUP<sup>1</sup>, Qihua TAN<sup>5</sup> and Torben Arvid KRUSE<sup>5</sup>

<sup>1</sup>Institute of Human Genetics, University of Aarhus, <sup>2</sup>ALK Abelló, Hørsholm and Departments of <sup>3</sup>Paediatrics and <sup>4</sup>Pneumology, Aarhus University Hospital and <sup>5</sup>Department of Clinical Biochemistry and Clinical Genetics, Odense University Hospital, Odense, Denmark

**Atopic dermatitis is one of the most common chronic diseases of childhood and closely related to other clinical manifestations of allergy. The incidence is high and still increasing. The genetic contribution to disease development is substantial and complex. Only recently genetic research has begun to focus on this phenotype, and specific susceptibility genes remain to be found. To identify candidate regions holding genes for atopic dermatitis we performed a genome-scan in Danish affected sib-pair families containing sib-pairs matching a phenotype definition of both clinical atopic dermatitis and confirmed specific allergy. The scan was undertaken using 446 microsatellite markers and non-parametric linkage results were obtained from the MAPMAKER/SIBS computer program. We found evidence of linkage to three candidate regions in chromosomes 3p (MLS = 2.14), 4p (MLS = 2.00) and 18q (MLS = 2.25), one of which has not been reported previously. Eight additional regions showed weaker but positive results. *Key words: allergy; candidate; gene; genetics; region.***

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A. Haagerup, Institute of Human Genetics, Bartholin Building, Aarhus University, DK-8000 Aarhus C, Denmark. E-mail: ah@humgen.au.dk

Atopic dermatitis (AD) (MIM 603165) is a chronic relapsing dermatitis associated with high levels of IgE and often co-existing with asthma and/or rhinitis. Type I allergies develop in about one-third of patients with AD and most frequently among those who later also develop asthma or rhinitis (1). In 90% of these patients the disease presents before 4 years of age (2).

The incidence of AD during childhood has increased 5–10-fold over the past four decades to 15–20% for children born in 1980–1990 (3, 4). The exact reasons for this worrying development of the disease frequency remain to be found.

AD is a complex genetic disorder, since the disease is thought to develop in genetically predisposed individuals due to specific environmental factors. Family and twin studies estimate the genetic predisposition of AD to be considerable (5, 6) and a complex pathogenesis with polygenic inheritance is expected. As for many

diseases with complex genetics, it is generally believed that the major reasons for the increasing incidence must be found in our environment, because the genetic pool has not changed overall during the relatively limited period of time. However, molecular genetics may offer the key to better understanding of the pathogenesis of AD and, thus, in time will provide a platform from which our insight into the environmental risk factors can further expand. Therefore, identification of AD susceptibility genes is likely to facilitate future understanding, prevention, diagnosis and treatment of AD.

The AD phenotype has previously been genetically evaluated by linkage and association (see for example Refs 7–9). Three genome-scans have reported on a small number of candidate loci for AD (10–12).

Attempts to confirm atopy candidate regions unfortunately often produce conflicting results. This might be explained by variations in study design, diagnostic criteria and statistical methods and by genetic heterogeneity in the different populations studied (13). We believe that the use of a narrow allergy phenotype definition, which combines both strict clinical diagnosis and evidence of specific sensitization, defines a more aetiological homogeneous group of patients and thereby increases the chance of detecting susceptibility genes. We previously introduced and employed this strategy (14–16). To further dissect the genetics of AD, we sampled Danish affected sib-pair families by a narrow phenotype definition and carried out a genome-scan using 446 highly informative microsatellite markers. Data were analysed by non-parametric multipoint linkage analysis and revealed evidence of linkage to 11 candidate regions. Six of the regions emphasized by our study support earlier linkage reports of asthma, atopy or rhinitis candidate regions.

## MATERIALS AND METHODS

### *Patients and participants*

A total of 424 individuals from 100 Danish nuclear families selected for clinical atopy were enrolled in the Danish allergy project ITA (Inheritance of Type I Allergy). The families were recruited from the western part of Denmark through one adult and four paediatric outpatient allergy clinics in Aarhus, Herning, Viborg and Aalborg. All participants (200 parents and 224 children) were clinically examined and questionnaire-tested by one doctor. They had blood drawn

for DNA analysis and serum measurement of total IgE and specific IgE, RAST, to 11 common allergens. Among the offspring the mean age was 10.8 years and the male/female sex ratio was 1.2, equivalent to random distribution ( $p=0.35$ ). The ITA project was carried out in accordance with the Helsinki declaration and approved by the Danish scientific ethical committee system. All participants and/or their parents gave informed consent.

#### Phenotype definition

In the ITA project we have chosen to evaluate five distinct phenotypes of type I (immediate type) allergy: atopy, allergic asthma, allergic rhinitis, AD and increased total IgE. All phenotypes include, in addition to the specific clinical phenotype character, increased specific IgE measurements against at least one of the tested allergens, i.e. RAST  $\geq 1+$ . The results of the genome-scans for allergic rhinitis (15) and allergic asthma, atopy and increased total IgE (16) were reported elsewhere.

In the present study we report the results of the genome-scan for AD defined by the phenotype: clinical AD and increased specific IgE against at least one of the tested allergens (RAST  $\geq 1+$ ).

This phenotype is equivalent to the IgE-associated atopic eczema/dermatitis syndrome, AEDS, proposed in EAACI recommendations (17). Families holding at least two full siblings with the above outlined phenotype were selected. The specific IgE measurements (CAP RAST FEIA, Pharmacia, Uppsala, Sweden) were increased when  $\geq 0.35$  kUA/l ( $\geq$  class 1) and determined for 11 allergens: grass, birch, mugwort, olive, *Pariaetaria*, cat, dog, horse, mite (*D. pteronyssinus* and *D. farinae*) and *Cladosporium herbarum*. AD was diagnosed according to the Hanifin & Rajka criteria (18) and determined by two individual doctors – first by one doctor, who did all the interviews and examinations of the allergy families, and secondly by another doctor, who evaluated the questionnaires. The rate of agreement on the clinical diagnosis was high (>98%); in the few cases of discrepancy the questionnaires were re-evaluated by the same two physicians and consensus was obtained. Twenty-three ITA sib-pair families qualified for the AD genome-scan (Table I). The total number of offspring was 57, of which 51 had the correct phenotype and thus participated in the statistical analysis. Five of the families contained three affected siblings, adding up to a total of 33 affected sib-pairs, of which 28 pairs were independent.

#### Molecular genetics

A comprehensive set of 446 highly informative microsatellite markers (<http://www.marshmed.org/genetics/>) scattered with a mean distance of 7.84 cM (range 0–16.27 cM) throughout the 22 autosomal chromosomes and the X chromosome was selected. The markers were purchased as scan primers (DNA Technology, Aarhus Denmark). PCR conditions were optimized to make all primers perform in a multiplex PCR with 1–6 (mean=3.5) primer sets. Products were analysed in 47 panels of 4–14 primer sets; the separation and genotyping were carried out on an ABI 310 Genetic Analyzer (Perkin Elmer, Foster City, CA, USA).

#### Statistical analysis

The data from the AD-affected sib-pair families were analysed by non-parametric multipoint linkage analysis using the maximum likelihood score (MLS) approach (19). The computer program MAPMAKER/SIBS (20) was used for

Table I. Numbers and clinical features of the atopic dermatitis sib-pair families

	Parents (n=46)	Offspring (n=57)
Males/females	23/23	27/30
Age, mean (years)	41.0	11.4
Atopic dermatitis	10	52
RAST $\geq 1+$	16	51
Asthma	9	41
Rhinitis	17	44
Total IgE $\geq 100$ kU/l	21	46
Glucocorticoid medication	18	50

RAST  $\geq 1+$  indicates specific allergy to at least one of the 11 allergens tested. The use of glucocorticoid medication is local and/or systemic and stated at the time of the clinical examination.

calculation of maximum likelihood IBD (identical by descent) scores and information content. Maximum likelihood and exclusion calculations allowed for dominance variance. An increment step setting of five facilitated computing of LOD scores in a total of 2345 positions. In order to evaluate the significance of the findings obtained genotypes of the 446 markers were simulated in the 23 families under the null hypothesis of no linkage. One thousand genome scans were simulated and for each MLS scores were calculated as described for the empirical data set. As a comparison, we also estimated the multipoint NPL scores (21) and Zlr statistics (22) on all the chromosomes using the Allegro computer program (23).

The relatively small sample of sib-pair families available to this study limited the application of further statistical analysis. Therefore, evaluation of clinical subgroups or parent of origin effects were not pursued, so as not to lose statistical power. However, in the future such calculations may become relevant for the analysis of identified specific susceptibility gene variants and their implication in disease severity. This most likely will require a larger sample.

## RESULTS

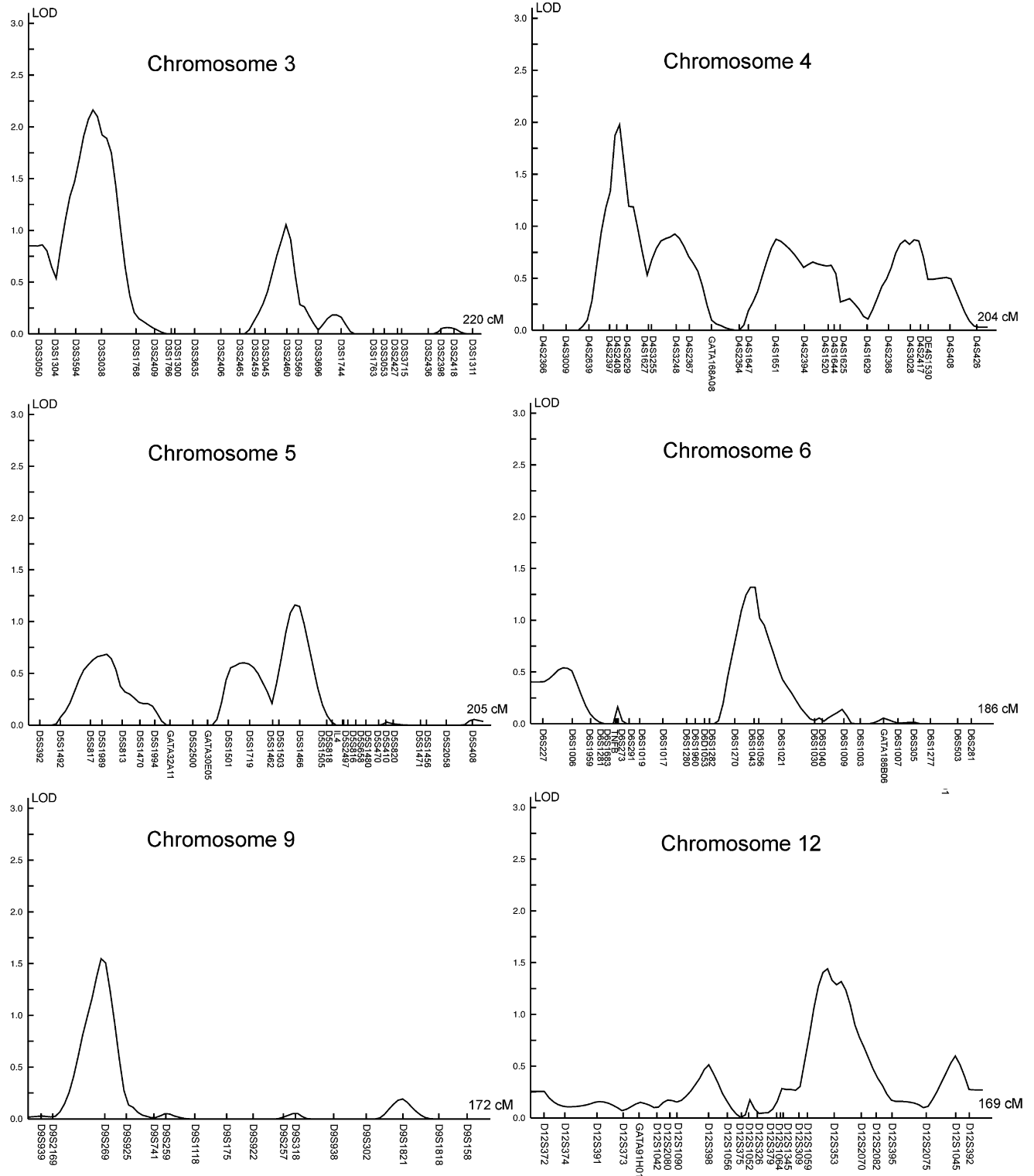
We found linkage of AD to three regions defined by  $MLS \geq 2$ . The regions were 3p26–3p24 ( $MLS=2.14$ ), 4p15–4p14 ( $MLS=2.00$ ) and 18q11–18q12 ( $MLS=2.25$ ). Another eight regions (5q21–5q23, 6q13–6q14, 9p23–9p22, 12q23, 18p11, 22q11–22q12, Xp22–Xp21 and Xp11) showed evidence of linkage characterized by MLS from 1–2. Results of the multipoint linkage analysis for the nine chromosomes with  $MLS > 1$  are shown as plots by chromosome (Fig. 1). Specific data from the 11 regions with  $MLS > 1$  are given (Table II).

The reported results were obtained by computing data using the conservative *independent sib-pairs* facility from the MAPMAKER/SIBS program. From simulations it was estimated that the likelihood of finding three or more loci with  $MLS \geq 2$  in one genome-scan by chance was  $p=0.08$ . Consequently the probability that at least one of the three loci harbours an AD susceptibility gene is 92%.

Both NPL and Zlr statistics approximately follow the standard normal distribution when the sample is

sufficient. However, when the IBD information provided by the data is incomplete (missing data, low heterozygosity or sparse markers), the NPL approach will become conservative when compared to the Zlr approach, and in that case different conclusions can be expected. Our results showed that both the NPL and the Zlr statistics captured the three highest peaks found

by the MLS approach. The NPL scores were 3.13 ( $p=0.0009$ ), 2.84 ( $p=0.0023$ ), 2.49 ( $p=0.006$ ) and the Zlr results were 3.08 ( $p=0.001$ ), 2.24 ( $p=0.0126$ ), 2.73 ( $p=0.004$ ) for the peaks found on chromosomes 3, 4 and 18, respectively. Thus, these results indicate that, although our sample is limited, the data are sufficiently informative.



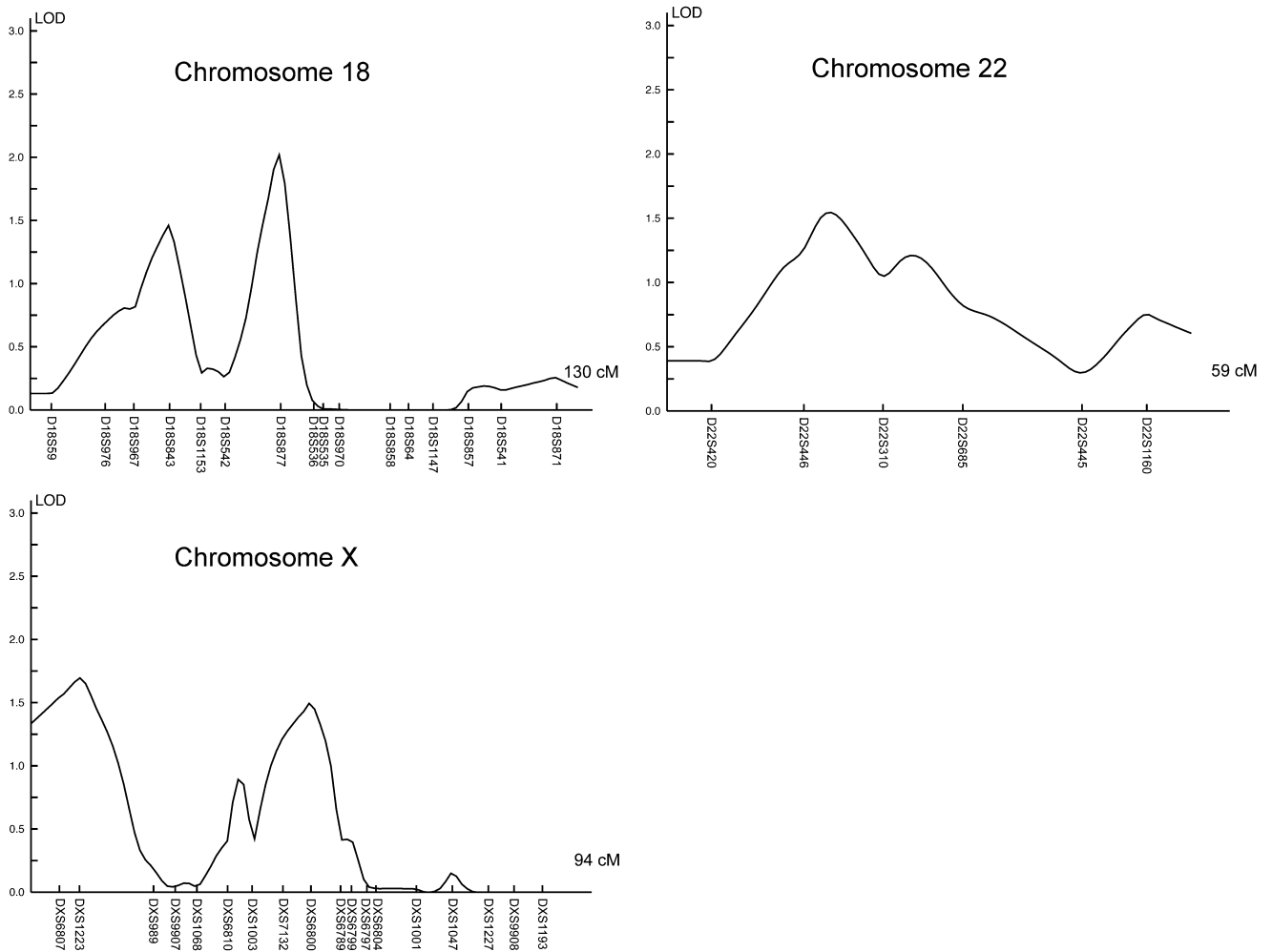


Fig. 1. Maximum likelihood IBD (MLS) curves with specific microsatellite markers for the nine chromosomes with MLS >1. The LOD scores were computed at all markers and five times between every two markers, increment steps of 5.

Three general atopy candidate genes were tested for linkage. For the TNF- $\beta$  (chromosome 6) and Fc $\epsilon$ R1 $\beta$  (chromosome 11) genes the MLSs were 0.23 and 0.19, respectively. The IL4 receptor (IL4R) alpha-subunit region between marker D16D769 and D16S409 on chromosome 16 gave maximum MLS of 0.03.

Information content reflects both marker density and marker heterozygosity and is useful in predicting the marker map quality. The information content was high throughout the genome and the mean value in the 11 appointed regions (Table II) was 0.85 (range 0.61–0.97).

## DISCUSSION

Over the past decade intensive research into the complex genetics of allergy has generated numerous reports of linkage and association. The genetic model for allergic disease still remains to be determined but a combination of general allergy genes and organ-specific genes has been suggested (24). In the present study we evaluated the clinical phenotype AD combined with

documented type I allergy, RAST  $\geq 1+$ . The narrow phenotype definition was used in an attempt to decrease the genetic heterogeneity and thereby increase the power of the study (24). We obtained the highest LOD scores in three regions on chromosomes 3p, 4p and 18q (Table II).

The region on 3p26–3p24 was (Fig. 1) identified in our study by the flanking markers D3S3594 and D3S3038. Previously suggestive linkage of AD to marker D3S1768 on chromosome 3p21 has been found (12). For the same marker we did not find linkage (MLS=0.17). The distance between the peak markers of the two studies is approximately 13 cM. Our result may be seen as support for and refinement of the AD candidate region on chromosome 3p.

On chromosome 18q11–18q12 our peak marker D18S877 is located almost 30 cM proximal to the marker D18S851 (MLS=0) that also suggested linkage of AD in the Swedish population (12). These two different observations were both based on evaluation of an AD phenotype of combined clinical symptoms and increased specific IgE.

Table II. Specified linkage results from 11 potential atopic dermatitis candidate regions

Cytogenetic position	Marker map position (cM)	Marker	Info <sup>a</sup>	MLS <sup>b</sup>
3p26–3p24	17.90	D3S3594	0.85	1.51
	25.37		0.61	<b>2.14</b>
	30.35	D3S3038	0.80	1.93
4p15–4p14	29.81	D4S2397	0.90	1.29
	33.04	<b>D4S2408</b>	0.95	<b>2.00</b>
	37.60	D4S2629	0.96	1.26
5q21–5q23	115.62		0.83	1.16
	117.51	D5S1466	0.92	1.14
6q13–6q14	86.80	D6S1043	0.95	1.33
	88.70	D6S1056	0.93	1.07
9p23–9p22	24.20	D9S269	0.87	1.54
12q23	106.60		0.72	1.42
	108.60	D12S353	0.81	1.28
18p11	28.10	D18S843	0.78	1.04
18q11–18q12	54.50	<b>D18S877</b>	0.91	<b>2.25</b>
22q11–22q12	10.40	D22S446	0.74	1.26
	13.96		0.64	1.53
	19.30	D22S310	0.97	1.04
	22.90		0.76	1.21
Xp22–Xp21	0.00	DXS6807	0.86	1.54
	3.50	DXS1223	0.92	1.69
Xp11	39.20	DXS7132	0.85	1.22
	44.10	DXS6800	0.95	1.50

Regions presenting MLS > 1 are listed both by cytogenetic and genetic map positions.

<sup>a</sup>Information content (Info) is given for all suggestive positions.

<sup>b</sup>Increased maximum likelihood IBD score (MLS) scores obtained between markers are included to place a potential candidate gene in relation to a close marker. MLS > 2 and relating markers are shown in bold.

Thus, of the three identified susceptibility candidate regions for AD the locus on chromosome 4p is new, whereas our finding on 3p and 18q provides support for previous results in these regions. When observing the allele sharing patterns in the three regions the genetic contributions on 3p and 4p were most consistent with recessive inheritance, whereas the pattern in the 18q region clearly was compatible with dominant inheritance.

We have previously reported evidence in favour of linkage of atopy to the locus on chromosome Xp11 (16). The same locus has shown suggestive linkage by a single-point analysis for an extreme AD phenotype (12). Our present study confirms both the locus as a strong candidate locus for AD as well as our previous finding in the region. The three studies all identified DXS6800 as peak marker in the region, which also harbours the gene for the Wiskott-Aldrich syndrome (WAS) characterized by eczema, thrombocytopenia, proneness to infection and bloody diarrhoea. The WAS gene has been suggested as an AD candidate gene (25).

Our results in another five of the identified regions, although insignificant by themselves, supported earlier reports evaluating asthma, rhinitis, atopy or other

allergy-related phenotypes; i.e. chromosomes 3p (26), 4p (15, 16), 5q (15, 16, 26–30), 9p (31) and 12q (32–34).

Four new regions (6q, 18p, 22q and Xp22–Xp21) showed weak but positive results, MLS 1.04–1.69. Interestingly a case of late-onset AD in a patient with complex glycerol kinase deficiency caused by a micro-deletion on Xp21 was recently reported (35). On the basis of this report and our result on Xp22–Xp21 we suggest the gene for glycerol kinase or a closely linked gene as a candidate gene for AD.

Genetic studies evaluating the AD phenotype have investigated the regions 5q31 (IL4), 6p21 (TNF- $\beta$ ), 11q13 (Fc $\epsilon$ R1 $\beta$ ), 12q, 13q, 14q11 (MCC), 16p (IL4R $\alpha$ ), 17q11 (RANTES). Our study revealed some support for the regions 5q and 12q as described above. No evidence of linkage was found to the atopy candidate genes TNF- $\beta$  (MLS=0.23) and Fc $\epsilon$ R1 $\beta$  (MLS=0.19) or to the IL4R region (MLS=0.03).

Our study neither replicated nor excluded the findings in two previous AD genome-scans (10, 11), just as they did not confirm or exclude each other. Overall the three studies varied in sample and marker panel sizes, and they applied different statistics and phenotype definition to different populations. We used

a higher marker density in a more limited sample size and criteria for the clinical diagnosis similar to Lee et al. (10), and we strengthened our phenotype definition to gain power.

In conclusion, we sampled a clinically thoroughly described set of allergy sib-pair families and conducted a comprehensive genome-scan in a search for AD candidate genes. A narrow phenotype definition, 446 highly informative markers and conservative statistical analysis were used. Three AD candidate regions were identified by LOD scores  $>2$ . Moreover, we found evidence supporting an additional eight regions. The region on chromosome 4p was a new locus for AD, and the three regions, 3p, 18q and Xp11, represented confirmation of previously reported AD loci. Also, five of the 11 regions were confirmatory to reports suggesting linkage of other allergy phenotypes. Our study supported the theory of a heterogeneous inheritance of AD and contributed to the few known AD candidate loci.

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