

## CLINICAL REPORTS

# Characterization by Phenotype of Families with Atopic Dermatitis

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The aetiology of atopic dermatitis is unknown, but is probably multifactorial, with interactions between several genetic and environmental factors. Twin studies indicate a strong genetic factor, but the susceptibility genes are unknown. This paper, describing the phenotypes of family material, forms part of a large genetic study seeking to identify susceptibility genes for atopic dermatitis by linkage analysis. We selected families with at least 2 siblings affected with atopic dermatitis (1,097 affected siblings who together form 650 affected sib pairs and 49 affected half-sib pairs). We established a phenotype database of information about the affected siblings and their relatives, in total 5,830 individuals. All siblings were diagnosed with atopic dermatitis and participated in a standardized interview covering aspects of atopy and atopic dermatitis. Of the affected siblings, 72% suffered or had suffered from asthma and/or allergic rhinoconjunctivitis and 74% had raised total and/or allergen-specific IgE serum levels. Seventeen percent of the siblings had been hospitalized for atopic dermatitis. Sixty-nine percent had 1 or both parents with atopic dermatitis. Among siblings with 1 parent with atopic dermatitis, 37% had a father with atopic dermatitis and 63% had a mother with atopic dermatitis, indicating maternal preponderance. Analysis of the occurrence of atopic dermatitis in relation to the birth order in the sibship shows an increased risk of atopic dermatitis in persons born early in a sibship. Although the families were selected for genetic sib-pair linkage analysis, we believe that this material is representative of atopic dermatitis families managed at hospitals in Stockholm. **Key words:** atopy; siblings; genetics; maternal; birth order.

(Accepted December 9, 1999.)

Acta Derm Venereol 2000; 80: 106–110.

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The aetiology of atopic disorders (atopic dermatitis, allergic asthma and allergic rhinoconjunctivitis) is unknown, but is probably multifactorial, with interactions between several genetic and environmental factors (1, 2). The rapidly increasing incidence of atopic disorders in recent decades is probably not due to genetic changes. It is more likely that other, still unrecognized, factors in the environment induce atopic disorders in genetically susceptible individuals. Previous studies have suggested that children of atopic mothers have a higher incidence of atopic disorders than those of atopic fathers (3–5). However, a recent study by Uehara et al. (6) shows no difference. It has been proposed that the order in

the sibship is important in determining the risk of developing atopic diseases (7) and that an increased number of siblings is associated with a smaller risk of atopic diseases (8–11).

Of the atopic manifestations, we have focused our investigation on atopic dermatitis. Atopic dermatitis is a hereditary, pruritic, chronically relapsing inflammatory skin disease. It has a close connection with other atopic clinical manifestations and with elevated total and/or allergen-specific serum IgE levels (4, 12). In the literature, the estimates of frequency of atopic manifestations in first-degree relatives of patients with atopic dermatitis range from 43% to 83% (13). Twin studies in atopic dermatitis support the role of a strong genetic component, with a concordance rate of 86% in monozygotic twins and 21% in dizygotic twins (14).

Our family material, of at least 2 affected siblings, is being studied to identify genes that increase the risk of developing atopic dermatitis. We are using affected sib-pair analysis, in which the differences between the number of alleles shared and not shared by affected sib-pairs at a given locus are determined. The significance is measured by likelihood of linkage. One advantage of this analytical method is that it relies only on the assumption of presence of genetic influence and does not assume any specific mode of inheritance. It is also possible to base the linkage analysis on affected individuals only, so that only patients with a definite diagnosis need to be included (15).

We purified DNA from families with affected siblings (1,787 individuals and 1,097 affected siblings) and entered their phenotype data in a database that allows for correlations between genotype and phenotype within the material. The phenotype database contains information about 5,830 individuals. In the present paper, we describe the general outline and phenotype of the family material.

## MATERIAL AND METHODS

### Recruitment of subjects

Subjects were recruited during 1995–97. We used the patient registers of the Departments of Dermatology of Karolinska Hospital and Danderyd Hospital, Stockholm. Patients diagnosed with atopic dermatitis were identified and a letter giving information about the study was sent to approximately 5,000 patients. A dermatologist (MB) then contacted all patients by telephone. They were included if they had at least 1 sibling with atopic dermatitis and were over 4 years of age. All participating subjects gave their informed consent. For children below 18 years of age, consent was also obtained from their parents. The parents were included when possible, regardless of their atopic status. The study was approved by the Karolinska Hospital Ethics Committee.

Table I. Severity scoring of atopic dermatitis








Factor	Score
Age at onset < 2 years	1
Hospitalization for atopic dermatitis	1
Number of sites* manifesting atopic dermatitis 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 in 1 or both sites in bilateral structures was considered as 1 site.

#### Clinical examination

All siblings were examined by the same dermatologist (MB) applying the UK Working Party's Diagnostic Criteria (16–18) and the criteria of Hanifin & Rajka (19). Siblings fulfilling the UK Working Party's Diagnostic Criteria were included as affected. The distribution of any eczema was recorded. All affected siblings participated in a standardized interview covering aspects of atopy and atopic dermatitis. This interview included information concerning age of presentation of any atopic manifestation, past or present history of food allergy or urticaria, asthma (if diagnosed by a physician, medicine intake, allergens evoking asthma), allergic rhinoconjunctivitis (if diagnosed by a physician, medicine intake) and atopic dermatitis (if previously diagnosed by a physician, hospitalization, trigger factors). A pedigree with atopic manifestations among parents, grandparents, non-participating siblings, spouses, and children of participating siblings was drawn up and the order in the sibship was noted. The grandparents' geographical origins were recorded. The parents were not examined, but answered a questionnaire using the UK Working Party's Diagnostic Criteria for atopic dermatitis.

Table II. Distribution of sib-pairs in the families

Number of affected individuals in sibship	Number of affected full-sib pairs	Number of affected half-sib pairs	Total number of sibships	Total number of affected full-sib pairs	Total number of affected half-sib pairs	Family structures
2	1	0	404	404	0	
2	0	1	27	0	27	
3	3	0	49	147	0	
3	1	2	9	9	18	
4	6	0	13	78	0	
4	2	4	1	2	4	
5	10	0	1	10	0	
		Total	504	650	49	

○ = Mother regardless of atopic manifestation; □ = father regardless of atopic manifestation; ◇ = individual regardless of sex and atopic manifestation; ◆ = affected sibling regardless of sex.

#### Quantification of IgE antibodies

IgE antibodies were quantified in all affected siblings.

1. The total IgE concentration in serum was determined using the Pharmacia CAP System, IgE FEIA (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden). The cut-off was 2 kU/l.
2. IgE antibodies to Phadiatop<sup>®</sup>, a mixture of inhalant allergens, were analysed with the Pharmacia CAP System Phadiatop<sup>®</sup> FEIA. The inhalant allergens were: *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, cat, dog, horse, birch, timothy grass, mugwort, olive, *Cladosporium herbarum* and *Parietaria judaica*. The Phadiatop<sup>®</sup> was reported as either positive or negative.
3. IgE antibodies to a mixture of relevant food allergens (fx5) were analysed with the Pharmacia CAP System RAST<sup>®</sup> FEIA. The food allergens were: hen's egg white, cow's milk, peanut, soya bean, fish and wheat flour. The RAST mixture was divided into 6 classes, where a concentration <0.35 kU/l represents a negative result (class 0).

#### Severity scoring of atopic dermatitis

An arbitrary score for the severity of the atopic dermatitis was obtained using the classification shown in Table I.

#### Statistical analysis

The phenotype database was constructed in Omnis 7 v. 3.6.4. (Blyth Software, CA, USA). The  $\chi^2$  test was used for statistical analysis. A  $p$ -value <0.05 was considered significant. The 95% confidence intervals of frequency estimations were calculated using the Bernoulli model for categorical populations with 2 categories (20). Differences in severity scores between groups were tested using the Wilcoxon-Mann-Whitney test.

Table III. Age at presentation of the 1,097 atopic dermatitis siblings

Age	<2 years	2–5 years	6–10 years	>10 years	Unknown
No. individuals	853	122	52	67	3
% of total	78%	11%	5%	6%	0.3%

## RESULTS

## Siblings

The focus of this study is the affected siblings, since they allow genetic linkage analysis. The number of affected siblings in the families ranged from 2 to 5 individuals (Table II). The 1,097 siblings belonged to 481 different families. Of these families, 21 contained 2 or more different sibships, since the siblings were recruited from more than 1 generation. There were 650 affected sib pairs and 49 affected half-sib pairs (Table II). The median age of the 1,097 siblings at examination was 29 years (the 25th percentile was 18 years and the 75th percentile was 39 years). There were 667 females (median age 31 years, range 4–84 years with the 25th percentile at 20 years and the 75th percentile at 40 years). There were 430 males (median age 27 years, range 4–58 years, with the 25th percentile at 15 years and the 75th percentile at 39 years). Table III shows the age at presentation of atopic dermatitis among the siblings. Seventeen percent of the siblings had been hospitalized for atopic dermatitis. Fig. 1 shows the distribution of the arbitrary atopic dermatitis severity score, which approximates a normal distribution. Half the siblings reported at least 1 period of urticaria. Asthma and/or allergic rhinoconjunctivitis (AR) had occurred in 72% (only atopic dermatitis in 28%, atopic dermatitis and asthma in 6%, atopic dermatitis and AR in 33%, atopic dermatitis, AR and asthma in 33%). Of the affected siblings, 74% were positive in Phadiatop® and/or the mixture of relevant food allergens (fx5) and/or had raised total IgE serum levels (Fig. 2). Past or

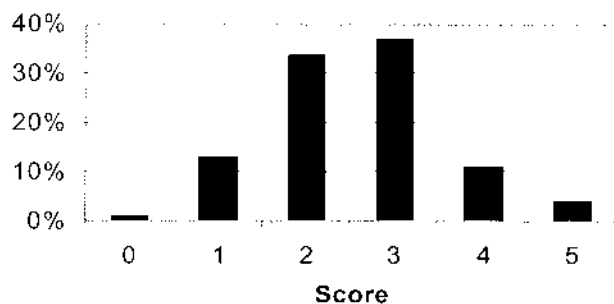


Fig. 1. Distribution of atopic dermatitis severity scores among affected siblings (n=1,097).

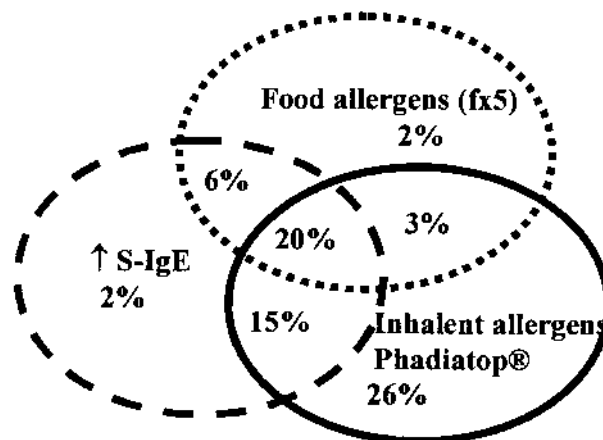


Fig. 2. Prevalence of elevated serum IgE levels and/or allergen-specific IgE levels in the 1,097 affected siblings. (See Material and Methods for food allergens and Phadiatop®).

present food allergy was reported in 67% of the atopic dermatitis siblings, with skin symptoms in 74%, respiratory symptoms in 61% and gastrointestinal symptoms in 6%.

## Parents

Information about both parents was obtained from all siblings. Table IV shows the atopic disorders among the parents. Significantly more parents had a history of atopic dermatitis than of asthma ( $p < 0.0001$ ) or allergic rhinoconjunctivitis ( $p < 0.0001$ ). Among the siblings that had only one parent with an atopic manifestation, this manifestation was distributed as described in Table V. Thus for atopic dermatitis the affected parent was a father in 37% of the siblings and a mother in 63% of the cases. This difference was significant ( $p < 0.01$ ).

## Other family members

The total number of individuals from which any data was collected was 5,830 (2,929 men and 2,901 women). The number of individuals studied in each family ranged from 8 to 37 with a median of 11. Of the 2,004 grandparents of the

Table IV. Atopic disorders among parents of siblings with atopic dermatitis

Parent's atopic manifestation	Proportion of 1,097 siblings having		
	0 parent with atopic manifestation (%)	1 parent with atopic manifestation (%)	2 parents with atopic manifestations (%)
Atopic dermatitis	31	61	8
Asthma	68	29	3
Allergic rhinoconjunctivitis	50	42	8
Any atopic manifestation	17	63	20

Table V. Gender of parents of atopic dermatitis siblings where only 1 parent was affected

Affected parent	Atopic dermatitis	Allergic rhinoconjunctivitis	Asthma
Father	37%	44%	45%
Mother	63%	56%	55%
	$p < 0.01$	<i>n.s.</i>	<i>n.s.</i>

The null hypothesis of equally affected status irrespective of gender. *ns* = not significant.

affected siblings, 96.8% were Caucasian and 3.2% non-Caucasian. Fig. 3 shows the occurrence of atopic dermatitis in relation to birth rank order among all sibships in the material (i.e. affected siblings plus non-affected siblings and children of the siblings). It also shows a decrease in frequency of atopic dermatitis among siblings with higher birth rank, at least in families with 2, 3 or 4 siblings.

## DISCUSSION

The families participating in this study were chosen to fit the design of our genetic study of atopic dermatitis. In genetic studies it is important to characterize the phenotype carefully, as it will be correlated to the genotype. It is also of value to know whether the phenotype of the patients is representative of the disease studied. Since we are studying many patients it is reasonable to assume that they are representative of patients with atopic dermatitis who are managed at hospitals in Stockholm. However, epidemiological implications of data must be interpreted with caution, since the families were not selected randomly, and we have no control group.

There is a close connection between atopic dermatitis, asthma and allergic rhinoconjunctivitis. In general, 50–80% of patients with atopic dermatitis develop asthma and/or allergic rhinoconjunctivitis (21). This was so for 72% of our siblings. The majority of our siblings (88.4%) were over 10 years of age, by which age most patients who will develop asthma and/or allergic rhinoconjunctivitis have done so (22). This allowed us to separate the patients with atopic dermatitis alone from those with additional atopic manifestations. In

genetic studies, this is of value since one explanation for the connection between atopic dermatitis and asthma/allergic rhinoconjunctivitis could be sharing of disease susceptibility genes.

There is no good biochemical marker for atopic dermatitis and the diagnosis has usually been based on the criteria of Hanifin & Rajka (19). However, the UK Working Party modified these criteria and achieved higher specificity of the diagnosis (16–18). We used the latter as our inclusion criterion. Eighty-seven percent also fulfilled the criteria of Hanifin & Rajka. The reason for not all of them fulfilling the latter criteria was that some siblings had no current eczema. In the former criteria, raised IgE level or a positive skin prick test is not included and in those of Hanifin & Rajka they are classified as minor criteria. However, there is an ongoing discussion as to whether an increased allergen-specific IgE level and/or positive skin prick test should become an obligatory criterion of atopic dermatitis (23). In our study, 72% of the affected siblings were sensitized to common inhalant and/or food allergens on the basis of a positive Phadiatop® and/or a RAST mixture of common food allergens. This agrees with previous studies (12).

When studying the genetics of complex diseases it is of importance to estimate the severity of the disease, because variations in severity might be explained by the degree of genetic burden. To this end, we used a score system that combined findings at the time of examination with the patient's medical history. The scores appeared to be normally distributed in the population studied. The fact that so many of our patients (17%) had been hospitalized for eczema might reflect a more severe variant of the disease than in the general population. It could also reflect the high proportion of adults participating, since the threshold for hospitalization was lower previously than currently.

Urticaria is common, affecting 10–20% of the general population at some time during life (24). In our material the figure was 50%. This probably reflects the higher prevalence of urticaria in patients with atopic dermatitis than in a non-selected population, but again, this must be interpreted with caution since no control group was included.

The reported cumulative incidence of food allergy was 67%. However, a positive RAST to the panel of food allergens was observed in 31% of the affected siblings. The explanation of

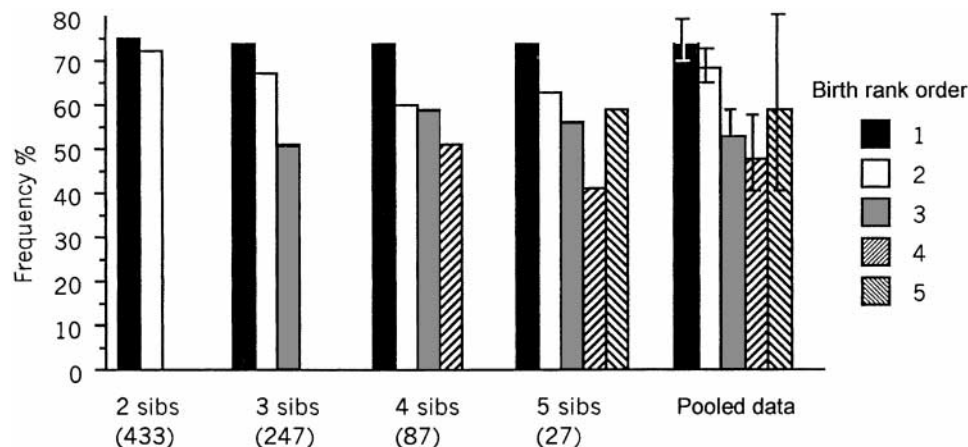


Fig. 3. Occurrence of atopic dermatitis in relation to the birth order in the sibship among 2,090 siblings. Number of sibships in brackets. Bars (pooled data) represent 95% confidence intervals.

this discrepancy is most likely that the IgE levels to previous food allergens had been normalized in adults, or that the food allergy reported was a non IgE-mediated intolerance reaction.

Children are considered more likely to develop the same atopic manifestation as their parents (25). Similarly, atopic manifestations among the parents in this study were significantly more often atopic dermatitis than asthma or allergic rhinoconjunctivitis. For those patients with only 1 parent with atopic dermatitis, it was more common that this

parent was the mother, which is also consistent with some previous studies (3–5) but not with others (6). Such a difference was not shown for asthma or allergic rhinoconjunctivitis. Several factors might explain the maternal preponderance. Mothers with atopic dermatitis may give birth to children prone to atopic dermatitis because of genomic imprinting or environmental factors *in utero* or during nursing. This tendency could also be due to a bias in selection, so that affected mothers were better at persuading the family to participate in the study. It is also possible that the threshold for developing atopic dermatitis is higher among men than among women. This would imply that men need more genetic factors to develop clinical symptoms than women do. Consequently, those men that have atopic dermatitis because of their relatively high genetic disposition would have a greater risk of fathering children with severe atopic dermatitis and a higher proportion of atopic dermatitis-affected children. However, we found no difference in atopic dermatitis scores between children born to affected fathers and those born to affected mothers. Nor did the proportion of atopic dermatitis in children born to affected fathers differ from that in children born to affected mothers.

Analysis of the occurrence of atopic dermatitis in relation to the birth order in the sibships showed an increased risk of atopic dermatitis in those born early in a sibship. This tallies with other studies (9, 11). One explanation could be that children in larger families are exposed to more infections early in life, which may prevent the development of atopic disorders. However, other authors have shown a higher risk for the second child to develop atopic dermatitis than for the first to do so (7), thus further studies are required to resolve the discrepancy.

In conclusion, the families in our genome-wide linkage analysis of susceptibility genes show phenotypes in good agreement with previously published data, indicating that our material is representative of atopic dermatitis families managed at hospitals in Stockholm. We are now using the family material to localize and, it is hoped, identify susceptibility genes for atopic dermatitis. This will also permit the study of gene-to-gene and gene-to-environment interactions, and may assist the development of new therapies based on the aetiological mechanisms underlying atopic dermatitis.

#### ACKNOWLEDGEMENTS

This paper is dedicated to the late Professor Sture Lidén, who initiated this project. The skilful assistance of Anneli Eriksson, Stina Gustavsson and Anneli Holmsten is gratefully acknowledged. This research was supported by grants from The Swedish Asthma and Allergy Association, The Swedish Foundation for Health Care Science and Allergy Research, Swedish Strategic Funds (SSF) and the Edward Welander-Finsen Foundation.

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