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ORIGINAL ARTICLE

Factors associated with different results of allergy tests in children with dust mite-induced atopic dermatitis

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Abstract
Background: Atopic dermatitis (AD) is a public health problem, with an increasing prevalence worldwide. AD is a chronic inflammatory disease characterised by skin lesions and severe itching. Immunologically, AD has two forms, IgE-mediated and cell-mediated, but it may also be idiopathic. In the pathogenesis of AD, the gene mutations for filaggrin, a filament-aggregating protein present in the epidermis, are of pivotal importance, but other genetic factors are also operating, including those linked to family atopy.
Methods: We evaluated the role of family atopy, and of the results of the atopy patch test (APT) in parents, in children with mite-induced AD.

64 children, 38 males and 26 females, mean age 4.97 years, were included for the diagnosis of AD and underwent APT and skin prick test (SPT) with dust mite extracts, with evaluation of atopy and result of APT also in parents.
Results: A positive family history of atopy was shown for children with positivity to both APT and SPT compared to those with negative or only one positive result to APT or SPT ($p = 0.08$). Significant associations were found concerning APT results in children and parents. In particular, children of a positive-APT parent had an 18-fold higher risk of APT-positivity in comparison with children of negative-APT parents, while the risk was 6.6-fold higher if APT was positive in father.
Conclusion: Family atopy and a positive APT in fathers are risk factors to develop cell-mediated AD, as assessed by the APT, in children.

Abbreviations: AD, atopic dermatitis; APT, atopy patch test; SPT, skin prick test.

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Introduction

Atopic dermatitis (AD) is an important issue in public health with a rising prevalence worldwide.[1] AD is a chronic inflammatory disease, with periods of flares and remission, characterised by skin lesions of eczematous type and severe itching. It normally begins early in life and often occurs in people with a personal or family history of asthma and allergic rhinitis.[2] The prevalence of AD is estimated to be 15–30% in children and 2–10% in adults, with a mean value of 17%. Of note, the incidence of AD has increased by 2–3-fold during the past three decades in industrialised countries, and particularly in Northern Europe.[3] The disease is sustained by a complex interaction between genetic and environmental factors. AD skin is characterised by immune dysregulation and epidermal barrier defects such as abnormal terminal differentiation of keratinocytes and decreased cornification resulting in epidermal damage and altered permeability to allergens and microbes.[4] The role of genetic factors in AD is clearly demonstrated by studies on twins, showing that the concordance rate for AD is higher among monozygotic twins (77%) than among dizygotic twins (15%).[5] The importance of genetic factors in AD is further underlined by the finding that a positive parental history is the strongest risk factor for AD; the incidence rate is doubled if AD is present in one parent, and tripled if both parents are affected. Allergic asthma or allergic rhinitis in a parent appears to be a minor factor in the development of AD in their offspring, suggesting AD-specific genes.[6] Genomewide scans[7] have highlighted several possible AD-related loci on chromosomes 3q21,[8] 1q21, 16q, 17q25, 20p9[9] and 3p26.[10] Some issues were suggested as possibly being involved in AD development. The role of caesarean delivery was analysed in a systematic revision of the literature from 1966 to 2007, the conclusion was that caesarean delivery is associated with a slightly higher risk of allergic rhinitis, asthma and possibly food allergy, but was not associated with AD.[11]

Furthermore, the influence of caesarean delivery on the microbiota colonisation in the newborn and its relationship with the developing immune system do not have any clear evidence to support it.[12]

As regards breastfeeding, no apparent effect on the development of AD was demonstrated, while the introduction of foods such as cow's milk, hen's egg, wheat, hazelnut, and others may be connected to an increased risk of food allergy and AD.[13]

Theories to explain the rise in AD include an overall improved awareness of AD, an increased exposure to air pollution, and an increased exposure to allergens.[14] Extensive research has demonstrated that a combination of food allergy, defects in the gut mucosal barrier, and increased intestinal permeability is implicated in the pathogenesis of AD.[15] Moreover, the factors grouped in the so-called ''hygiene hypothesis'', such as living in non-affluent countries or in rural areas, the number of siblings, the exposure to endotoxins as occurs in the presence of numerous animals, the kind of feeding, and others[16,17] are advocated as protective from atopy, but this theory is not universally accepted.[18–20] Indeed, up to 70% of children with AD have a spontaneous remission before adolescence (with a better prognosis when the AD onset occurs in the first year of life).[21] However, the disease can also start in adults, and in a substantial number of these patients there is no sign of IgE-mediated sensitisation.[22] In the position paper ''A revised nomenclature for allergy''[23] it is stated that, ''allergic AD would be dominated by the IgE-associated subgroup'', in which the clinical selection is based on Hanifin and Rajka's criterion, family history of or simultaneous occurrence of symptoms of atopy. Since this is the only immunologically well-defined subgroup, one should always, when appropriate, use the term IgE-associated AD. Another subgroup seems to include cell-mediated forms. It is characterised by positive atopy patch tests to aero- and food allergens or allergen-specific T cells in the peripheral blood or in skin biopsies, but in the absence of IgE sensitisation. The term allergic, T-cell-associated AD might be appropriate. The term nonallergic AD should replace the term ''intrinsic/cryptogenic variants''. In the future, all these subgroups may be better defined by immunologic characteristics. The IgE-associated form and the cell-mediated form are clinically similar but show some differences regarding the histology, the kind of cells involved, and the cytokine pattern[24,25] as well as their response to different allergy tests.[26]

We aimed this study at evaluating the factors possibly involved in the different results to allergy tests in children with house dust mite-induced AD.

Materials and methods

Patients and tests

From subjects referring to the Pediatric Allergy Service in Torremaggiore, Italy, 64 children, 38 males and 26 females (M:F ratio 1.5), mean age 4.97 ± 3.5 years, median age 4.25 years (range: 0.7–15.5 years), were consecutively included by diagnosis of AD according to Hanifin and Rajka criteria.[27] A detailed clinical history was obtained from parents to highlight the relationship between AD exacerbation and dust mite exposure as well as to exclude a causative role of food allergy. Subjects with current respiratory symptoms (rhinosinusitis, asthma) at recruitment were also excluded. Twenty-six children with rhinitis but a negative history for current or past AD served as control group.

All children were investigated using the skin prick test (SPT) and the atopy patch test (APT) with *Dermatophagoides* extracts. The SPT positivity was evaluated, using extracts from Stallergènes (Antony, France) according to the guidelines from the European Academy of Allergy and Clinical Immunology.[28] The APT was performed using material composed of inert dust mite bodies purified to 20% (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, 1:1, Merck, Milan, Italy), with white petroleum jelly (Vaseline), 20%, and white mineral oil used as excipients. The substance to be tested was applied onto intact skin of the lower back and was held firmly in position using adhesive patch tests. These patch tests were made up of aluminium Finn chambers of approximately 20 μL. The application period was 48 h. The test was read no less than 30 min after removal to avoid margin effect. Results were interpreted according to the American Academy of Dermatology[29] using a scale ranging from 1+ (weak reaction) to 3+ (very strong reaction). Only reactions of 2 and 3+ were considered positive for the purpose of this study.

Table 1 Clinical characteristics of study and control subjects.

	Study group	Control group	*p*
Number	64	26	
M:F ratio	1:5	1:6	ns
Age range	0.7–15.5 years	2.2–14.0 years	
Mean age	4.97 years ± 3.5 years	5.9 ± 2.6 years	ns
Monoparental family atopy	38 (59.4%)	8 (30.8%)	0.02
Biparental family atopy	8 (12.5%)	3 (11.5%)	ns
Negative family atopy	18 (28.1%)	15 (57.7%)	0.015
Positive SPT	14 (22%)	11 (42.3%)	ns
Positive APT	48 (75%)	4 (15.4%)	<0.001

All the parents of the children were asked about their family history of atopy. Parental atopy was defined as a positive response to the question ''has the father or the mother ever suffered from allergic asthma, rhinitis or eczema?'' The parents also underwent APT with *Dermatophagoides* spp at the same time, conducted by the same operator (N.F.).

An informed consent was obtained from children's parents, in accordance with the World Medical Association and the Helsinki Declaration.

Statistical analysis

Descriptive statistics were expressed as median and range values. Comparisons among groups were performed by the Mann–Whitney *U* test. Two-tailed χ^2 or Fisher exact test was used to evaluate differences of prevalence, as appropriate. Crude odds ratios (OR) with 95% confidence intervals (95%CI) were calculated as the measure of effect. The statistical analysis was performed using STATISTICA version 6.1 (Stat Soft, Inc., Tulsa, OK, USA).

Results

Table 1 shows the main characteristics of the 64 children included in the study and the 26 control subjects. Family history of atopy was positive in 46 children (71.9%), in eight of them (12.5%) positivity was found in both parents. In particular, the family history was positive for past or current AD and past or current rhinitis.

Seven children (10.9%) had a positive result to SPT, 41 (64.1%) had a positive result to APT, nine (14.1%) had a negative result to both tests, and 7 (10.9%) had a positive result to both tests. No difference was found according to sex and age in the prevalence of APT or SPT positivity in children.

Regarding the results to APT in parents, in 14 cases (21.8%) APT was negative in both parents, in 21 (32.8%) was positive only in father, in 13 (20.3%) only in mother and in 16 (25%) was positive in both parents. Concerning the history, when only one parent was concerned, the 11 mothers with a positive APT reported past AD (1), past AD and rhinitis (2), current AD and rhinitis (1), current rhinitis (4), and current contact dermatitis (3); the 22 fathers with a positive APT reported past AD and current rhinitis (6), current AD and current rhinitis (4), and current rhinitis (12). When both parents were concerned, mothers reported past AD and current contact dermatitis (2), current AD and current rhinitis (1), current rhinitis (4), and current contact dermatitis (1); fathers reported past AD and current rhinitis (2), current AD and current rhinitis (1), and current rhinitis (3). The three mothers with contact dermatitis had a positive patch test for nickel (two subjects) and p-phenylenediamine (one subject).

A positive family history of atopy was shown for all seven children with a positive result to both APT and SPT, but for only 39/57 children (68.4%, $p = 0.08$) with negative or only one positive result to APT or SPT. Instead, significant associations were found concerning APT results in children and parents (Table 2). In particular, children of a positive-APT parent had an 18-fold higher risk of APT-positivity in comparison with children of negative-APT parents (OR: 18.3, 95%CI: 4.3–77.3), while the risk was 6.6-fold higher if APT was positive in the father. No association

Table 2 Association between APT results and risk factors.

	Children with negative APT ($n = 16$)	Children with positive APT ($n = 48$)	OR (95%CI)	*p*
Age (years)	5.8 (0.9–12.6)	4.01 (0.6–15.5)		ns
Positive family history of atopy	10 (62.5%)	36 (75%)		ns
APT positivity in at least one parent	6 (37.5%)	44 (91.7%)	18.3 (4.3–77.3)	<0.0001
APT positivity in both parents	2 (12.5%)	14 (29.2%)		ns
APT positivity in father	4 (25%)	33 (68.7%)	6.6 (1.8–23.9)	0.003
APT positivity in mother	4 (25%)	25 (52.1%)		ns

was present between APT positivity in children and in the mother.

In the control group, 15 subjects had a positive SPT while only four had a positive APT. Nine fathers and four mothers had a positive history for rhinitis and/or asthma. APT was negative in both parents in 23 cases, positive in both parents in one case, positive only in the father in one case and positive only in the mother in one case.

Discussion

AD is basically caused by trans-epidermal water loss, although its pathophysiology is, as explained above, very complex. AD may be idiopathic but more often the altered permeability (related to skin barrier defects and especially in mutations of the filament aggregating protein filaggrin) may facilitate sensitisation of the skin to environmental allergens.[30] This in turn may elicit immune responses in the skin itself, as well as in other target organs such as the respiratory tree and lungs.[31] Recent research highlighted the important role played by house dust mites (HDM) as a cause of AD, which is sustained by the ability of their allergens to penetrate into the epidermis and worsen AD severity through three mechanisms: inherent proteolytic enzyme activity of the major allergens Der p 1 and Der f 1[32,33]; activation of proteinase-activated receptors-2 (PAR-2)[34]; and immunoglobulin E (IgE) binding, which leads to inflammation. Concerning the latter mechanism, the airborne proteins from mites bind to specific IgE antibodies and elicit the release of histamine and other inflammatory mediators from mast cells and basophils, which result in tissue damage and exacerbation of the itch-scratch cycle, which can further aggravate AD.[35] As far as allergy testing is concerned, the SPT or the measurement of specific IgE antibodies to HDM in serum is used to indicate sensitisation. However, they show only type-I IgE-mediated allergic responses to a protein, without assessing the ability of the antigen to induce inflammation, which is instead demonstrated by the APT. When biopsy is performed from allergen-induced eczematous APT site, a sequence of immunological events occur, including: the generation of allergen-specific T cells, with an initial TH2 cytokine pattern and a subsequent TH1 pattern; an early influx of dendritic epidermal cells that capture the allergen through the IgE receptor and present it to specific T cells; the T cell-mediated inflammatory reaction in the skin site of testing, with macroscopic and microscopic similarities with the lesional skin in AD.[36]

We have previously found in a group of 297 children with different clinical expression (current AD, current AD and respiratory symptoms, past AD and respiratory symptoms, and respiratory symptoms with neither current nor past AD) and tested by APT and SPT with mite extracts, that in all subjects with past or current AD the rate of positivity was significantly higher for APT. At the same time, in subjects with exclusive respiratory symptoms the most frequently positive test was the SPT. Indeed, the patients with AD showed two different patterns of allergic response to allergens, one IgE-mediated, as evaluated by positive SPT, and the other cell-mediated, as evaluated by positive APT,[37] this suggesting reconsideration of the significance of APT.[38]

We aimed the present study at evaluating AD in its IgE-mediated, cell-mediated, or not immunologically mediated (i.e. not associated to any test positivity), presentations by the relationship between positivity to SPT and APT and gender, age, family atopy. In addition, we looked at the association between APT positivity in the study subjects and in their parents, and the relationship between APT positivity and age of father and mother. The results confirm that in children with AD, the APT is the test most frequently positive; in fact, 11% of subjects had a positive result to SPT, while 64% had a positive result to APT, and 11% had a positive result to both tests. The novel aspect of the study is the finding that there is a relationship between the positive result of the APT in children and their parents. In fact, a positive family history of atopy, which is quite common in allergic children[39,40] was not significantly associated to positive or negative results of the APT. However, the association between a positive APT in children and a positive APT in one parent was highly significant (<0.0001). In particular, the most significant association appeared to be between children and fathers. The APT positivity in mothers, though it was double in mothers of children with positive APT compared with mothers of children with negative APT, did not reach statistical significance. Of interest, in the control group formed by children with rhinitis but a negative history for AD, the rate of positive APT was significantly lower (15% vs. 75% in the study group). These observations highlight the importance of the previously described genetic background in AD[5–10] and warrants further investigation on the factors related to skin response to aeroallergens underlying the development of a positive APT.

In conclusion, this study confirms the important role of the APT with dust mites in the diagnosis of AD in children. It also adds a new observation as regards the positive results of APT in parents, particularly in fathers, as being a factor in the development of a cell-mediated response to mites, as assessed by a positive APT, in children.

Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the responsible Clinical Research Ethics Committee and in accordance with those of the World Medical Association and the Helsinki Declaration.

Confidentiality of data. The authors declare that they have followed the protocols of their work centre on the publication of patient data and that all the patients included in the study have received sufficient information and have given their informed consent in writing to participate in that study.

Right to privacy and informed consent. The authors have obtained the informed consent of the patients and/or subjects mentioned in the article. The author for correspondence is in possession of this document.

Conflict of interest

The authors declare they have no conflict of interest.

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