Atopic eczema and allergies: Practical relevance for diagnostic work-up

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Abstract

Atopic eczema (AE) is a chronic relapsing skin disease mostly starting in childhood and often associated with allergic asthma and rhinoconjunctivitis. In the etiopathophysiology of atopic eczema skin barrier disturbance and immune deviation with a predominant Th2 response and IgE production are central. Allergy tests belong to the diagnostic work-up of AE patients, first in order to differentiate between the "extrinsic" (IgE-associated) from the "intrinsic" (non-IgE-associated) form of the disease. Essntially allergy tests are mandatory in order to find individual provocation factors for exacerbation or maintenance of eczema. These allergy tests include skin prick test (SPT), radio-allergo-sorbent test (RAST) for specific IgE antibodies against common allergens and APT. Since numerous AE patients suffer from contact allergy, therefore classical patch testing is mandatory. Although SPT and RAST have a quite high sensitivity, but the specificity of APT is much higher; revealing the fact that by APT the relevance of a certain allergen for the actual skin disease can be confidently evaluated. In suspected food allergy the gold standard is still double-blind placebo-controlled oral provocation test which should be performed in a symptom-free period after an individual elimination diet and under emergency conditions, since anaphylactic reactions may occur in AE patients. In the long-term management of AE patients educational programs "eczema school" have proved to be helpful.

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Introduction

Atopic eczema (AE), synonymous to atopic dermatitis is defined as a non-contagious chronic inflammatory itching skin disease with a typical age-related distribution of lesions [1, 2]. It continues to receive growing attention because of its increasing prevalence and the compromised quality of life in the affected patients[3, 4]. A high worldwide variation of prevalence of the disease was confirmed, with an increasing prevalence especially in industrialized countries, reaching 17% in Northern Europe [5]. The frequency in adults is low, ranging from 1% to 5,1% for a lifetime prevalence[6].

Two forms of AE are delineated, namely (1) an extrinsic form associated with IgE-mediated sensitization (80%) and (2) an Intrinsic form (20%) without IgE sensitization[7]. The common characteristic of atopic diseases as familial occurrence was first observed by Besnier[8].

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Afterwards a number of studies revealed an association between AE and subsequent development of allergic airway disease in about 30% of patients [3], especially for early-onset and severe AE [9, 10, 11] with a positive family history [12]. AE results from complex interactions between individual genetic susceptibility, environmental influence, skin barrier dysfunction, and systemic and local immune deviation [1,7,12]. As special interest has recently been focused on filaggrin[13] (a protein that is bound to keratin fibres in epidermal cells), there has been occurrence of filaggrin polymophisms. These polymophisms were found to be risk factors leading to more severe phenotypes of AE and asthma [14 -20]. Over decades there have been long discussions as to whether allergy plays a role in AE. The present paper gives an overview of the practical relevance for tests and diagnostic work-up related to AE.

Allergy tests

Atopic diseases are commonly categorized as type I IgE-mediated reactions according to the Coombs and Gell´s classification [21]. The exception is that of eczema, where type IV reactions may also be important together with type I reactions, have been categorized as type VI-b with IgE/Th2-reaction in the initiation phase, while Th1-reaction is predominant in chronic lesions.

Sensitization to many different allergens such as food and aeroallergens can trigger skin symptoms in about 30% of children with AE. Allergy testing is helpful (i) to identify specific triggering allergens, (ii) to follow up disease course and seasonal flare, as well as (iii) to compare therapeutic response and efficacy. The allergy tests commonly performed in daily practice may include

(1) Skin prick test (SPT),
(2) Atopy patch test (APT),
(3) *In vitro* allergy diagnostics, and
(4) Oral provocation test (OPT).

Skin prick test (SPT)

Skin prick test (SPT) provides inexpensive and quick results regarding the existence of an immediate-type reaction, but the Performance requires cooperation of the patient. This test is useful for determining the

Presence and significance of specific IgE antibodies to various foods and other allergens on the surface of mast cells. The predictive value of intracutaneous testing is not higher than that of SPT, but a higher risk of systemic reaction and a high false-positive rate may exist[22,23].

SPT is the second step of diagnosis after carefully taking the patients' history. It is most informative when the results are negative with a negative predictive value of over 95 %, while the positive predictive value is only about 40 % [24-26]. The sensitivity is very high but with a low specifity. A wheal of greater than 3 mm with a visible red flare analogous to the histamine control indicates a positive result. In a study of 467 children by a food-specific SPT using commercial extracts, wheal diameters of at least 8 mm for cow milk, 7 mm for hen egg and 8 mm for peanut were found to have 100 % diagnostic values for allergic reactions[27]. Testing fresh extracts of foods (Prick-to-Prick) are more reliable than the protein in commercial extracts because the latter is more prone to degradation[23]. It is noteworthy that the SPT of food allergens more frequently shows false negative results than that of aerollergens. To determine the clinical relevance of the test results it is important to have definite anamnestic evidence indicating an association of the present symptoms with the exposure to special allergens. A negative skin prick test may also be observed in cases of food intolerance and irritant responses to airborne particles[28]. Several cutoffs for SPT thresholds that highly predict food allergy have been reported with varying results. Owing to the highly selective nature of the study samples (e.g. different ages), variations in statistical methods, outcome definition, SPT devices, and allergens, these thresholds may not be generalizable to other clinical settings. A 95% positive predictive value (PPV) developed from population-based studies using standardized skin prick testing and OFC cessation criteria may overcome these limitations [29].

Atopy patch test (APT)

APT was first employed in the diagnosis AE in 1988 in order to include the epidermal compartment [30]. It is based on a specific cellular response to the application of allergens on the surface of healthy skin (Figure 1). The method has been standardized for aeroallergens [31-33, 45, 49, 50]. Different researchers reported that an atopy patch test with aeroallergens can elicit eczematous skin lesions in patients with AE [30, 34, 35]. This test with allergens known to elicit IgE-mediated reactions can be used for evaluation of the relevance of IgE-sensitization for eczematous skin lesions especially to house dust mites as well as other aeroallergens with a high specifity up to 91 % [36] (Table 1 & 2). In patients with AE, the most common reactions in the APT are caused by *D. pteronyssinus*, whereas controls show no reactions [31, 37, 38]. The APT can also be of great diagnostic value in patients with AE associated with *Malassezia furfur* which can induce formation of specific IgE antibodies and trigger AE [39]. Positive reactions to the recombinant allergens of *Malassezia furfur* are observed to correlate with a Th2-like peripheral blood mononuclear cell response [40].

APT is read at 48 and 72 hours according to the test criteria and reading key of the European Task Force on Atopic Dermatitis (ETFAD) [41]. Grading of the APT reactions can follow the rules of conventional patch testing [37], (with slight variations with regard to erythema, number and distribution pattern of papules [42] (Figure 2). It was shown that epicutaneous contact with aeroallergens was able to elicit eczematous skin lesions using an optimal concentration of 5000 PNU (= protein nitrogen units)/g in petrolaneum for APT [43]. APT is recommended especially in the following situations:

a. *Evaluation of clinical relevance of aeroallergen sensitizations for AE.*
b. *Clarification of food allergy in AE without identification of IgE-mediated sensitization.*
c. *Multiple IgE-mediated sensitizations without proven clinical relevance in patients with AE (42-44)*
d. *Severe or persistent AE with unknown trigger factors.*

APT is not recommended for routine diagnosis as the SPT, but the combination of APT with SPT and the determination of specific serum IgE reduce the need for oral provocation in children with AE 45. APT is also useful for diagnosis of delayed type reactions in infants with AE. The specifity of APT is higher than that of SPT or specific serum IgE level 1, 46. In a monocentric study the concordance of APT with SPT was 0,39 - 0,69 and with the specific serum IgE level was 0,42 - 0,69, dependent on the type of allergens 37. The highest rate of positive APT results was found in patients with skin lesions in the air-exposed regions with raised specific serum IgE levels 43.

Further control studies involving oral provocation tests and elimination procedures in patients with AE are necessary to elucidate the clinical relevance of APT with food allergens. The accuracy of APT was higher with fresh food substance than that with commercial food reagents in an examination of 60 children [47]. A standardization of the method for APT in patients with food allergy may lead to a replacement of oral provocating procedures in the future [45]. APT may be suitable to evaluate the actual clinical relevance of IgE-mediated sensitizations to food allergens for eczematous lesions. This test should become part of the routine diagnostic tool set for food allergies, especially to cow milk in infants and children, and should be applied before a food challenge test [48].

In vitro allergy diagnostics

It has been over 40 years since the discovery of IgE (49). Later in 1977, Hanifin and Lobitz suggested to include IgE measurement for the diagnosis of AE [50]. In 1980 the increased total serum IgE level was included as one of the minor diagnostic criteria [51]. Many children with AE have elevated serum IgE levels, mostly over 300 kU/l. The cut-off value over a high predictive value for diagnosis of allergy is not entirely clear. A recent study showed a high correlation between the total IgE and the area of involvement with oozing eczema at a 10-year follow-up [52].

Regarding specific IgE, positive predictive values of over 95 % were seen in allergy to egg, milk, peanuts and fish, while the negative predictive value is greater than 95 %, when the result of the test is negative. Correlation was found between the IgE values measured by the CAP detection system and the results of double-blinded food challenges [53]. An oral challenge test is needed to confirm the diagnosis in children with specific IgE levels falling below the 95 % predictive decision level.

Recent studies show better diagnostic results by the use of purified native or recombinant allergens, the socalled "component-resolved diagnostics", as compared to whole food allergen extracts. For example, the specific IgE for recombinant omega-5 gliadin correlates well with the oral challenge in the majority of children with immediate allergic reactions and anaphylaxis to ingested wheat [54]. Using molecular diagnosis, it has been found that

(1) *in egg allergy*, specific IgE to ovomucoid has been identified as a risk factor for persistent allergy and could indicate reactivity to heated egg. Egg-allergic patients with IgE antibodies reacting against sequential epitopes tend to have more persistent allergy [55];

(2) *in cow milk allergy*, association is observed between molecular-scale patterns and different presentations of the condition such as anaphylaxis, gastrointestinal symptoms and other severe phenotypes [56];

(3) *in peanut and legume allergy*, quantification of Ara h 2-specific IgE may accurately discriminate peanut allergy from tolerance. Patients with soybean allergy sensitized to Gly m 5 or Gly m 6 allergens may be at greater risk of experiencing severe allergic reactions. The pattern of allergenic component recognition varies in different populations or geographical areas [57];

(4) *in house dust mite allergy*, a strong correlation was found between commercially available Der p 1 and Der p 2 and IgE D. pteronyssinus. Double negative results of Der p 1 and Der p 2 IgE helped redirect the diagnosis. Der p 10 specific IgE prevalence and levels suggest different patterns in food and mite-related tropomyosin sensitization [58]. Introduction of the IgE-microarray testing is helpful in identifying additional relevant allergenic triggers and in defining the nonallergic form of atopic dermatitis through broad and comprehensive negative IgE testing [59]. Improvement of the precision in allergy diagnostic testing may decrease the need for oral food challenges and enhance the specificity of allergen immunotherapy. Nevertheless, a careful interpretation of the results in correlation with clinical findins and patient profiles is warranted to avoid false conclusions if the new technology is broadly used in general medicine.

The detection of specific serum IgE levels may be especially beneficial for the diagnosis of patients under antihistamine therapy or with generalized skin lesions which are not allow skin testing. The predictive value of specific IgE for food allergy is higher in children than in adults [22, 60]. In a recent study of 562 children by use of specific IgE measurement, sensitization to environmental allergens was estimated at about 25 % at the age of 3 years [61]. False positive results without clinical relevance are frequently seen in screening for IgE antibodies in case of food allergy, especially in patients being sensitized to pollen or latex. A positive result has a lower specificity than a negative result. However, the significance of IgE-mediated sensitizations in the maintenance of AE is controversial and a causal relationship is often difficult to confirm. An elevated level of IgE is often found in allergological routine workup of AE, but with unclrear clinical relevance [1]. On the other hand, the sensitivity and specificity of the diagnostic criteria are not impared by exclusion of specific igE measurements, even though the detectable IgE antibodies are shown to significantly associate with AE [62]. It is worth to mention that the eosinophil count and serum eosinophilic cationic protein may correlate with the disease severity, in addition to the total and specific IgEs [63].

Oral provocation test (OPT)

According to numerous authors, the double-blind placebo-controlled OPT is the gold standard for diagnosis of food-associated exacerbation of AE [30, 64]. The test should be performed in a symptom-free period after an elimination diet. Some diagnostic diets have been proposed for OPT (Table 3) [62].

Three reaction patterns have been described for OPT (1,65-68):

a. *Immediate-type reactions appearing in 15-30 minutes, as pruritus, urticaria, angioedema, gastrointestinal symptoms, rhinoconjunctivitis, asthma or cardiovascular reactions.*
b. *Intensive generalized pruritus beginning in 30-60 minutes*
c. *Elicitation or exacerbation of eczematous lesions after 6-48 hours (late immediate-type). As relevant IgE-mediated sensitization is often not found, especially in children, allergen-specific T lymphocytes have been proposed to cause this reaction.*

Analysis of OPT in children showed that about 25% of all clinical reactions occur after two hours or later [69,70]. It is also necessary to standardize the challenge procedures for late immediate-type reactions. Repetition is needed in unclear reactions.OPT is not recommended for AE patients with serious known immediate-type reactions to food [71]. If really indicated, emergency equipment and medications must be at hand. Patients with birch pollen sensitization can also react in OPT with cross-reacting foods leading to exacerbation of eczema [72]. It is indicated that some other allergens besides the „classic“ones can exacerbate the eczema in individual patients. Whether this is caused by pseudoallergic reactions or true allergic reactions remain unclear [73].

Issues of special concern

(i) Food allergy in AE

As early as 1915, cases were reported of patients who experienced improvement of their eczematous skin lesions after avoiding specific foods [74]. Further studies described clearing of the skin lesions in series of patients after elimination of specific foods eliciting positive skin prick reactions [75, 76]. In a study of 195 adults with AE, 44% underwent a worsening of cutaneous lesions after open oral challenge with soy sauce, soybeans and other foodstuff like chocolate, cheese, coffee and yoghurt. An avoidance of these test-positive foods for 3 months led to a marked improvement in 35% and a slight improvement in 9% of these AE patients [77]. Nevertheless, it remains of paramount importance to take a careful history and to make a complete clinical examination, in order to establish the association between clinical symptoms and the potential allergens. In doubtful cases a symptom-food diary is required for at least 4-6 weeks. To get more information, the next step would be to place the patient on an oligoallergenic basic diet adapted to the age of the children. In adult patients this approach is needed very rarely in exceptional cases [78] and the components should be determined on an individual basis.
Nowadays, the prevalence of food allergy in western countries is estimated to be 2-3 % in adults and 6-8% in children in general population, while the prevalence in AE patients is much higher, with up to 20 % in children [79, 80]. Clinical manifestations of food allergy in AE are exacerbation of eczema, usually occurring after 6-48 h as a late-type reaction, or IgE-mediated urticarial or anaphylactic symptoms [81]. The relatively high incidence of food allergy in infancy, especially cow milk allergy, has been suggested to be caused by a defective mucosal barrier, increased gut permeability to large molecules and immaturity of local and systemic immunological response [82]. A series of classic experiments demonstrated that ingested food proteins readily penetrate the gastrointestinal mucosa and can be transported in an immunologically active form via the circulation to cutaneous mast cells [76,83]. In a recent prospective large population-based, age-matched, challenge-proved food allergy cohort study, it was found that filaggrin mutations increased the risk of food sensitization in the first year of life but did not play a further role in progression to clinical food allergy, suggesting the reqirement of additional factors [84].

Based on thee findings, testing for food allergy are an essential part of the management of patients with AE [81]. In general, the more severe the AE and the younger the patient, the more likely food allergy is contributing to the symptoms [85]. An increased severity of AE associated with a positive cow milk allergy has been observed [86]. The most common food allergies implicated in young children are egg, milk, peanut, fish, wheat and soya. These can be screened by a blood test for specific IgE, in which the sensitivity for specific IgE level was 97 % for hen egg, 83 % for cow milk, 79 % for wheat and 68 % for soy [69], while the specificity for hen egg was 51 %, 53 % for cow's milk, 50 % for soy and 38 % for wheat [86]. Food allergy in patients AE often improves with age [87], especially with regards to hen egg and cow milk. Approximately 20% of the young children will loose their peanut allergy by school age [79], but the allergy to peanut or shellfish may persist through life [80]. Approximately one third of children with AE and food allergy will outgrow their clinical reactivity to food over 1 to 3 years [88]. In AE patients with allergies to cow milk and hen`s egg, positive APT together with spezific IgE levels give a diagnostic accuracy of 100% for cow milk and 94% for hen egg allergy [32], therefore, the SPT is not necessary. APT alone gives the best predictive value for wheat [85]. To identify a peanut allergy a combination of SPT and APT is recommended [89]. In general, a combination of SPT with APT can significantly enhance the diagnosis of specific food allergies. It is important to realize that most children with AE will have a positive skin prick test to several kinds of foods, but only one third of these positive skin tests correlate with a positive food challenge [79].

Patients showing an oral allergy syndrome (OAS) associated with AE can be diagnosed based on the clinical findings and sensitization patterns, and often do not require oral food provocation testing [67], when labial provocation is positive. The application of component-resolved diagnosis for the differential diagnosis between sensitization and real allergy in apple-mediated OAS in birch pollen allergy seems promising but further studies are needed to confirm its clinical relevance Some studies investigated the prognosis of AE with IgE sensitization. A sensitization to hen egg, cow milk and peanut appeared correlated with the severity and persistence of the disease [86], which could not be confirmed in a similar study [93].
Also non-immune hypersensitivity reactions "pseudo-allergic reactions" can be elicited in AE by additives in foods (eg sulfites, benzoates, colourings); they can only be diagnosed by provocation tests [1].

(ii) Aeroallergens in AE

Elevated levels of specific serum IgE levels against aeroallergens are very common in AE patients [94 -96]. It is known that patients with AE improve when they were placed in a dust-free environment or at high altitude climate [97 -100]. Both seasonal and perennial allergens should be first tested with SPT in every patient with AE, followed by measurement of the specific serum IgE levels if necessary (Table 4). Dust mites are the most frequently encountered aeroallergens in temperate climates. Patients with histories of mite allergy were significantly more likely to have persistent AE beyond school age [92].

Dermatophagoides genus were identified as the most important allergen in house dust [101] .In which *D. farinae* are the species mostly found in North America while *D. pteronyssinus* are mostly seen in Europe. The most allergenic material is the mite faecal matter, which induces sensitization and clinical disease [102]. Dust mites have the capability of acting as irritant contact haptens and IgE antigens.

The allergic manifestations are mainly eczematous dermatitis, especially in children, followed by rhinitis and asthma, and very rarely anaphylaxis [103 -105]. In two small hospital-based longitudinal studies the risk of allergic respiratory disease is especially increased in patients with IgE-associated AE versus non-IgE associated AE [93, 106]. But no correlation could be found between AE before age 2/3 and aeroallergen sensitivity at the age of 12 months and 3 years [107, 108]. Recent data showed that in a group of 1700 children respiratory allergy was associated with both

D. ptenoyssinus and *D. farinae* whereas AE was only associated with exposure to *D. farinae* [109] . Sensitivity to dust mite is demonstrated in the majority of adult AE patients, including elderly adults, by immunoglobulin E-mediated tests and/or atopy patch tests [110].

(iii) Allergic contact Dermatitis in AE

Contact dermatitis should always be included in the diagnostic work-up for unclear flare-ups of AE (103). Patients with AE are considered to be less prone to contact allergies [111]. however, many studies show that they are sensitized to many common allergens such as nickel, lanolin and fragrances as frequently as normal individuals [112,113]. There's no general association between atopy and fragrance allergy in a recent comprehensive review [114,115]. On the contrary, the prevalence of contact allergy in adult atopic patients is around 41 %, preferably against nickel, thiomersal and wool wax alcohol [113,116]. It is recently found in German patients with occupational irritant contact dermatitis, as compared to controls (vocational school apprentices), that both filaggrin mutations and AE increase the risk of irritant contact dermatitis [117]. Children with atopic dermatitis are as frequently sensitized to nickel, cobalt, thimerosal, and fragrance as children with no history of atopic dermatitis, and there are no differences associated with sex [118]. Patch testing in children resulted positive in 55.3% (50% relevant) of AE compared with 76.9% (77.5% relevant) of the children without AE, while the most frequent allergens detected were nickel (16.3%), cobalt (6.9%), isothiazolinone (5.4%) and potassium dichromate (5.1%)[119]. In adult AE, a significantly higher prevalence of contact sensitization to fragrance chemicals has been found in many studies, and the association seems unrelated to the filaggrin mutations [120].

Conclusion

Over the last decades there has been major progress in understanding the etiopathophysiology of AE. However, in the diagnostic management of AE the dermatological diagnosis using clinical criteria is the basis which needs to be followed by diagnostic steps to detect individual provocation factors, namely by allergy tests. Hence in most of the cases, allergy testing remains mandatory to analyse the role of food or aero-allergens. The benefit of a positive allergy test reaction in AE may not be as apparent as in allergic rhinitis, where effective allergen-specific immunotherapy (ASIT) is routinely available. In a recent randomized multicenter study in chronic AE, ASIT with house dust mite showed a significant benefit after one year [121]. Through allergy testing it is possible to identify the clinically significant individual provocation factors and to plan rational avoidance strategies, which are possible only after testing.

References:

Ring J, Przybilla B, Ruzicka T. (eds): Handbook of Atopic Eczema. 2nd ed. Springer Verlag Berlin Heidelberg New York 2006

1. *Aoki T, Fukuzumi T, Adachi J et al. Re valuation of skin lesion distributing in topic dermatitis: analysis of cases 0-9 ears of age. Acta Derm Venerol 992;176: 19-23.*
2. *Spergel JM, Paller AS. Atopic dermatitis and the atopic march. J Allergy Clin Immunol 2003;112:118-27.*
3. *Björkstén B. The environment and sensitisations to allergens in early childhood. Pediat All Immunol 1997;8:32-9.*
4. *Williams H, Robertson C, Stewart A et al. Worldwide variations in the prevalence of symptoms of atopic eczema in the International Study of Asthma and Allergies in Childhood. J Allergy Clin Immunology 1999;103:125-38.*
5. *Wahn, U, Wichmann, H. Special report on allergies. In: Health monitoring of the Federation. Metzler-Poeschel 2000, Stuttgart.*
6. *Novak N, Bieber T, Leung DY. Immune mechanisms leading to atopic dermatitis. J Allergy Clin Immunol 2003;112:128-39.*
7. *Besnier E. Première note et observations préliminaire pour servir d´introduction à l´étude diathésique. Ann Dermatol Syphiligr 1892 ;4:634.*
8. *Salob SP, Atherton DJ. Prevalence of respiratory symptoms in children with atopic dermatitis attending pediatric dermatology clinics. Pediatrics 1993;91:8-12.*
9. *Aberg N, Engström I. Natural history of allergic diseases in children. Acta Paediatr Scand 1990; 79:206-11.*
10. *Linna O, Kokkonen J, Lahtela P et al. Ten-year prognosis for generalized infantile eczema. Acta Paediatr 1992;81:1013-6.*
11. *Kjellman B, Hattevig G. Allergy in early and late onset atopic dermatitis. Acta Paediatr 1994;83:229-31.*
12. *Sandilands A, Sutherland C, Irvine AD et al. Filaggrin in the frontline: role in skin barier function and disease. J Cell Sci 2009;122:1285-94.*

13. *Cork MJ, Robinson DA, Vasilopoulos Y et al. New perspectives on epidermal barrier dysfunction in atopic dermatitis: gene-environment interactions. J Allergy Clin Immunol 2006;118:3-21.*
14. *Palmer CN, Ismail T, Lee SP et al. Filaggrin null mutations are associated with increased asthma severity in children and young adults. J Allergy Clin Immunol 2007;120:64-8.*
15. *Weidinger S, O'Sullivan M, Illig T. et al. Filaggrin mutations, atopic eczema, hay fever, and asthma in children. J Allergy Clin Immunol 2008;121:1203-9.*
16. *Weidinger S, Baurecht H, Wagenpfeil S et al. Analysis of the individual and aggregate genetic contrutions of previously identified serine peptidase inhibitor Kazal type 5 (SPINK5), kallikrein-related peptidase 7 (KLK7), and filaggrin (FLG) polymorphisms to eczema risk. J Allergy Clin Immunol 2008;122:560-8.*
17. *Rodríguez E, Illig T, Weidinger S. Filaggrin loss-of-function mutations and associations with allergic diseases. Pharmacogenomics 2008;9:399-413.*
18. *Weidinger S, Illig T, Baurecht H. et al. Loss-of-function variations within the filaggrin gene predispose for atopic dermatitis with allergic sensitizations. J Allergy Clin Immunol 2006;118:214-9.*
19. *Rodríguez E, Baurecht H, Herberich E. et al. Meta-analysis of filaggrin polymorphisms in eczema and asthma: robust risk factors in atopic disease. J Allergy Clin Immunol 2009;123:1361-70.*
20. *Coombs RRA, Gells PGH. The classification of allergic reactions underlying disease. In: Clinical aspects of immunology, second edition. R.R.A. Coombs, P.G.H. Gells (Eds.). Oxford: Blackwell Scientific: Davis, Philadelphia 1963, 317.*
21. *Sampson HA. Food allergy. Part 2: diagnosis and management. J Allergy Clin Immunol 1999;103:981-9.*
22. *Ortolani C, Ispano M, Pastorello EA. Comparison of results of skin prick tests (with fresh foods and commercial food extracts) and RAST in 100 patients with oral allergy syndrome. J Allergy Clin Immunol 1989; 83:683-90.*
23. *Sampson HA, McCaskill CC. Food hypersensitivity and atopic dermatitis: evaluation of 113 patients. J Pediatr 1985;107:669-75.*
24. *Bock SA, Buckley J, Holst A et al. Proper use of skin tests with food extracts in diagnosis of food hypersensitivity. Clin Allergy 1978; 8:559-64.*
25. *Sampson HA, Albergo R. Comparison of results of skin tests, RAST, and double-blind, placebo-controlled food challenges in children with atopic dermatitis. J Allergy Clin Immunol 1984; 74:26-33.*
26. *Hill DJ, Heine RG, Hosking CS. The diagnostic value of skin prick testing in children with food allergy. Pediatr Allergy Immunol 2004;14:435-41.*
27. *King MJ, Lockey RF. Allergen prick-puncture skin testing in the elderly. Drugs Aging 2003;20:1011-7.*
28. *Peters RL, Gurrin LC, Allen KJ. The predictive value of skin prick testing for challenge-proven food allergy: a systematic review. Pediatr Allergy Immunol 2012;23:347-52.*
29. *Ring J, Darsow U, Gfesser M, Vieluf D. The 'atopy patch test' in evaluating the role of aeroallergens in atopic eczema. Int Arch Allergy Immunol 1997 ;113:379-83.*
30. *Darsow U, Vieluf D, Ring J. Evaluating the relevance of aeroallergen sensitization in atopic eczema with the atopy patch test: a randomized, double-blind multicenter-study. J Am Acad Dermatol 1999;40:187-93.*
31. *Langeveld-Wildschut EG, Thepen T, Bihari IC et al. Evaluation of the atopy patch test and the cutaneus late-phase reaction as relevant models for the study of allergic inflammation in patients with atopic eczema. J Allergy Clin Immunol 1996; 98:1019-27.*
32. *Wistokat-Wülfing A, Schmidt P, Darsow U. Atopy patch test reactions are associated with T lymphocyte-mediated allergen-specific immune responses in atopic dermatitis. Clin Exp Allergy 1999;29:513-21.*
33. *Mitchell EB, Crow J, Chapman MD et al. Basophils in allergen-induced patch test sites in atopic dermatitis. Lancet 1982;1:127-30.*
34. *Clark RA, Adinoff AD. Aeroallergen contact can exacerbate atopic dermatitis: patch test a a diagnostic tool. J Am Acad Dermatol 1989;21:863-9.*
35. *Fuiano N, Fusilli S, Incorvaia C. House dust mite-related allergic disease: role of skin prick test, atopy patch test, and RAST in the diagnosis of different manifestations of allergy. Eur J Pediatr 2010;169:819-24.*
36. *Darsow U, Vieluf D, Ring J. Atopy patch test with different vehicles and allergen concentrations-an approach to standardization. J Allergy Clin Immunol 1995;95:194-201.*
37. *Darsow U, Vieluf D, Ring J. The atopy patch test: an increased rate of reactivity in patients who have an air-exposed pattern of atopic eczema. Br J Dermatol 1996;135:182-6.*
38. *Tengvall Linder M, Johansson C, Scheynius A, Wahlgren C. Positive atopy patch test reactions to Pityrosporum orbiculare in atopic dermatitis. Allergy 2000;30:122-31.*
39. *Johansson C, Eshaghi H, Linder M-T. Positive atopy patch test reaction to Malassezia furfur in atopic dermatitis correlates with a T helper 2-like peripheral blood mononuclear cells response. J Invest Dermatol 2002;118:1044-51.*
40. *Vieluf D, Kunz B, Bieber T. et al. "Atopy patch test" with aeroallergens in patients with atopic eczema. Allergo J 1993;2:9-12.*
41. *Darsow U, Ring J. Airborne and dietary allergens in atopic eczema: a comprehensive review of diagnostic tests. Clin Exp Dermatol 2000; 25:544-51.*
42. *Darsow U, Abeck D, Ring J. Allergy and atopic eczema: on the value of the "atopy patch test". Hautarzt 1997;48:528-35.*
43. *Turjanmaa K, Darsow U, Niggemann B. EAACI/GA(2)LEN Position paper: Present status of the atopy patch test. Allergy 2006;61:1377-84.*
44. *Roehr CC, Reibel S, Ziegert M et al. Atopy patch tests, together with determination of specific IgE levels, reduce the need for oral food challenges in children with atopic dermatitis. J Allergy Clin Immunol;107:548-53.*
45. *Darsow U, Lübbe J, Taïeb A et al. Position paper on diagnosis and treatment of atopic dermatitis. J Eur Acad dermatol Venerol 2005;19:286-95.*
46. *Canani RB, Ruotolo S, Auricchio L et al. Diagnostic accuracy of the atopy patch test in children with food allergy-related gastrointestinal symptoms. Allergy 2007;62:738-43.*
47. *Pustisek N, Jaklin-Kekez A, Frkanec R et al. Our experiences with the use of atopy patch test in the diagnosis of cow´s milk hypersensitivity. Acta Dermatovenerol Croat 2010;18:14-20.*
48. *Bennich HH, Ishizaka K, Johansson SG et al. Immunoglobuline E: a new class of human*

immunoglobulin. Bull World Health Organ 1968; 38:151-2.
49. *Hanifin JM, Lobitz WCJr. Newer concepts of atopic dermatitis. Arch Dermatol 1977;113:663-70.*
50. *Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. Acta Derm venerol 1980;92:44-7.*
51. *Katoh N, Hirano S, Kishimoto S. Prognostic factor of adult patients with atopic dermatitis. J Dermatol 2008; 35:477-84.*
52. *Sampson HA, Ho DG. Relationship between food specific IgE concentrations and risk of positive food challenges in children and adolescents. J Allergy Clin Immunol 1997;100: 444-51.*
53. *Palosuo K, Varjonen E, Kekki OM et al. Wheat omega-5 gliadin is a major allergen in children with immediate allergy to ingested wheat. J Allergy Clin Immunol 2001;108:634-8.*
54. *Caubet JC, Kondo Y, Urisu A, Nowak-Węgrzyn A. Molecular diagnosis of egg allergy. Curr Opin Allergy Clin Immunol 2011;11:210-5.*
55. *Fiocchi A, Bouygue GR, Albarini M, Restani P. Molecular diagnosis of cow's milk allergy. Curr Opin Allergy Clin Immunol 2011;11:216-21.*
56. *Nicolaou N, Custovic A. Molecular diagnosis of peanut and legume allergy. Curr Opin Allergy Clin Immunol 2011;11:222-8.*
57. *Bronnert M, Mancini J, Birnbaum J et al. Component-resolved diagnosis with commercially available D. pteronyssinus Der p 1, Der p 2 and Der p 10: relevant markers for house dust mite allergy. Clin Exp Allergy 2012;42:1406-15.*
58. *Mari A, Scala E, Alessandri C. The IgE-microarray testing in atopic dermatitis: a suitable modern tool for the immunological and clinical phenotyping of the disease. Curr Opin Allergy Clin Immunol 2011;11:438-44.*
59. *Werfel T, Kapp A. Environmental and other major provocation factors in atopic dermatitis. Allergy 1998;53:731-9.*
60. *Kjaer HF, Eller E, Andersen KE et al. The association between early sensitization patterns and subsequent allergic disease. The DARC birth cohort study. Pediatr Allergy Immunol 2010;20:726-34.*
61. *Diepgen TL, Sauerbrei W, Fartasch M. Development and validation of diagnostic scores for atopic dermatitis incorporating criteria of data quality and practical usefulness. J Clin Epidemiol 1996;49:1031-8.*
62. *Cheung PF, Wong CK, Ho AW et al. Activation of human eosinophils and epidermal keratinocytes by Th2 cytokine IL-31: implication for the immunopathogenesis of atopic dermatitis. Int Immunol 2010;22:453-67.*
63. *Bindslev-Jensen C, Ballmer-Weber BK, Bengtsson U et al. Standardization of food challenges in patients with immediate reactions to foods -position paper from the European Academy Allergology and Clinical Immunology. Allergy 2004;59:690-4.*
64. *Breuer K, Kapp A, Werfel T. IgE-vermittelte Reaktionen auf Nahrungsmittel bei Neurodermitis. Akt Dermatol 2000;26:19-22.*
65. *Majamaa H, Moisio P, Holm K et al. Cow´s milk allergy: diagnostic accuracy of skin prick test and specific IgE. Allergy 1999;54:346-351.*
66. *Isolauri E, Turjanmaa K. Combined skin prick and patch testing enhances identification of food allergy in infants with atopic dermatitis. J Allergy Clin Immunol 1996;97:9-15.*
67. *Niggemann B, Kleine-Tebbe J, Saloga J et al. Standardisierung von oralen Provokationstests bei IgE-vermttelten Nahrungsmittelallergien. Allergo J 1998;7:45-50.*
68. *Celik-Bilgili S, Mehl A, Verstege A. The predictive value of specific immunoglobilin E levels in serum for the outcome of oral food challenges. Clin Exp Allergy 2005;35:268-73.*
69. *Breuer K, Wulf A, Constien A et al. Birch pollen related food as provocation factor of allergy symptoms in children with atopic eczema/dermatitis syndrome. Allergy 2004;59:988-94.*
70. *Bahna SL. Blind food challenge testing with wide-open eyes. Ann Allergy 1994;72:235-8.*
71. *Breuer K, Heratizadeh A, Wulf A et al. Late eczematous reactions to food in children with atopic dermatitis. Clin Exp Allergy 2004;34:817-24.*
72. *Worm M, Ehlers I, Sterry W et al. Clinical relevance of food additives in adult patients with atopic dermatitis. Clin Exp Allergy 2000;30:407-14.*
73. *Schloss OM. Allergy to common foods. Trans Am Pediatr Soc 1915;27:62-8.*
74. *Talbot FB. Eczema in childhood. Med Clin N Am 1918;1:985-96.*
75. *Blackfan KD. A consideration of certain aspects of protein hypersentiveness in children. Am J Med Sci 1920;160:341-50.*
76. *Uenishi T, Sugiura H, Uehara M. Role of foods in irregular aggravation of atopic dermatitis. J Dermatol 2003;30: 91-7.*
77. *Werfel T, Erdmann S, Fuchs T et al. Approach to suspected food allergy in atopid dermatitis. J Dtsch Dermatol Ges 2009;7:265-71.*
78. *Eigenmann PA, Sicherer SH, Borkowski TA et al. Prevalence of IgE-mediated food allergy among children with atopic dermatitis. Pediatrics 1998;101:E8.*
79. *Osterballe M, Andersen KE, Bindslev-Jensen C. The diagnostic accuracy of the atopy patch test in diagnosing hypersensitivity to cow´s milk and hen´s egg in unselected children with and without atopic dermatitis. J Am Acad Dermatol 2004;51: 556-62.*
80. *Behr-Völtzer C, Hamm M, Vieluf D, Ring J. Diät bei Nahrungsmittel-Allergien und –Intoleranzen. Urban & Vogel, 4. Aufl., München 1979.*
81. *Høst A, Halken S. Can we apply clinical studies to real life? Evidence-based recommendations from studies on development of allergic diseases and allergy prevention. Allergy 2002;57:389-97.*
82. *Brunner M, Walzer M. Absorption of undigested proteins in human beings: the absorption of unaltered fish protein in adults. Arch Intern Med 1928; 42:172 -9.*
83. *Tan HT, Ellis JA, Koplin JJ et al; HealthNuts Study Investigators. Filaggrin loss-of-function mutations do not predict food allergy over and above the risk of food sensitization among infants. J Allergy Clin Immunol 2012;130:1211-3.*
84. *Guillet G, Guillet MH. Natural history of sensitizations in atopic dermatitis. Arch Dermatol 1992;128:187-92.*
85. *Pourpak Z, Farhoudi A, Mahmoudi M et al. The role of cow milk allergy in increasing the severity of atopic dermatitis. Immunol Invest 2004;33:69-79.*
86. *Iida S, Kondo N, Agata H. Differences in lymphocyte proliferative responses to food antigens and specific IgE antibodies to foods with age among food-sensitive patients with atopic dermatitis. Ann Allergy Asthma Immunol 1995;74:334-40.*

87. *Sicherer SH, Leung DY. Advances in allergic skin disease, anaphylaxis, and hypersensitivity reactions to foods, drugs, and insects. J Allergy Clin Immunol 2005;116:153-63.*
88. *García-Ara MC, Boyano-Martínez MT, Díaz-Pena JM. Cow's milk-specific immunoglobulin E levels as predictors of clinical reactivity in the follow-up of the cow's milk allergy infants. Clin Exp Allergy 2004;34:866-70.*
89. *Ebo DG, Bridts CH, Verweij MM et al. Sensitization profiles in birch pollen-allergic patients with and without oral allergy syndrome to apple: lessons from multiplexed component-resolved allergy diagnosis. Clin Exp Allergy 2010;40:339-47.*
90. *Wolkerstorfer A, Wahn U, Kjellman NI et al. Natural course of sensitization to cow´s milk and hen´s egg in childhood atopic dermatitis. Clin Exp Allergy 2002;32:70-3.*
91. *Horwitz AA, Hossain J, Yousef E. Correlates of outcome for atopic dermatitis. Ann Allergy Asthma Immunol 2009;103:146-51.*
92. *Novembre E, Cianferoni A, Lombardi E et al. Natural history of intrinsic atopic dermatitis. Allergy 2001; 56:452-63.*
93. *Leung DYM, Rhodes AR, Geha RS. Atopic dermatitis (atopic eczema). In: Fitzpatrick TB, Eisen AZ, Wolff K, Freedberg IM, Austen KF (Eds.). 1993, 1543-63.*
94. *Rostenberg A, Sulzberger MD. Some results of patch tests. Arch Dermatol 1937;35:433-54.*
95. *Rowe AH. Dermatitis of the hand fue to atopic allergy to pollen. Arch Dermatol Syph 1946;54:683-703.*
96. *Kumei A. Investigation of mites in the houses of atopic dermatitis (AD) patients, and clinical improvements by mite elimination. Arerugi 1995; 44:116-27.*
97. *Okada K, Sakai A, Hidaka K et al. Systematic cleaning of the mite antigens in home environment and its effects on atopic dermatitis. Nippon Koshu Eisei Zasshi 1994; 41:165-71.*
98. *Tan BB, Weald D, Strickland I et al. Double-blind controlled trial of effect of housedust-mite allergen avoidance on atopic dermatitis. Lancet 1996;347:15-8.*
99. *Tuft LA. Importance of inhalant allergen in topic dermatitis. J Invest Dermatol 1949;12:211-19.*
100. *Arlian LG, Bernstein D, Bernstein IL et al. Prevalence of dust mites in the homes of people with asthma living in eight different geographic areas of the United States. J Allergy Clin Immunol 1992;90:292-300.*
101. *Zock JP, Heinrich J, Jarvis D et al. Distribution and determinants of house dust mte allergens in Europe: the European Community Respiratory Health Survey II. J Allergy Clin Immunol 2006;118:682-90.*
102. *Tupker RA, de Monchy JG, Coenraads PJ. House-dust mite hypersensitivity, eczema and other non pulmonary manifestations of allergy. Allergy 1998;53:92-6.*
103. *Gaffin JM, Phipatanakul W. The role of indoor allergens in the development of asthma. Curr Opin Allergy Clin Immunol 2009;9:126-35.*
104. *Edston E, van Hage-Hamsten M. Death in anaphylaxis in a man with house dust mite allergy. Int J Legal Med 2003;117:299-301.*
105. *Wüthrich B, Schmid-Grendelmeier P. Natural course of AEDS. Allergy 2002;57:267-8.*
106. *Nickel R, Kulig M, Forster J, et al. Sensitisation to hen´s egg at the age of twelve months is predictive for allergic sensitization to common indoor and outdoor allegens at the age of 3 years. J Allergy Clin Immunol 1997; 99:613-7.*
107. *Rhodes HL, Sporik R, Thomas P. Early life risk factors for adult asthma: a birth cohort study of subjects at risk. J Allergy Clin Immunol 2001;108:720-5.*
108. *Krämer U, Lemmen C, Bartusel E. Current eczema in children is related to Der f1 exposure but not to Der p1 exposure. Br J Dermatol 2006;154:99-105.*
109. *Katsarou A, Armenaka M. Atopic dermatitis in older patients: particular points. J Eur Acad Dermatol Venereol 2011;25:12-8.*
110. *DeGroot AC. The frequency of contact allergy in atopic patients with dermatitis. Contact Dermatitis 1990; 22:273-7.*
111. *Lever R, Forsyth, A. Allergic contact dermatitis in atopic dermatitis. Acta Derm-Venerol 1992;176:95-8.*
112. *Enders F, Przybilla B, Ring J et al. Epicutaneous testing with a standard series. Results in 12,026 patients. Hautarzt 1988;39: 779-86.*
113. *Buckley DA, Basketter DA, Kan-King-Yu D et al. Atopy and contact allergy to fragrance: allergic reactions to the fragrance mix I (the Larsen mix). Contact Dermatitis 2008;59:220-5.*
114. *Schnuch A, Uter W. Allergic contact dermatitis and atopic eczema. In: Handbook of atopic eczema, 2nd edition, Ring J, Przybilla B, Ruzicka T (Eds.). Springer: Berlin Heidelberg 2005, 176-99.*
115. *Pónyai G, Hidvégi B, Németh I et al. Contact and aeroallergens in adulthood atopic dermatitis. J Eur Acad Dermatol Venereol 2008;22:1346-55.*
116. *Visser MJ, Landeck L, Campbell LE et al. Impact of loss-of-function mutations in the filaggrin gene and atopic dermatitis on the development of occupational irritant contact dermatitis. Br J Dermatol 2012 Oct 5. doi: 10.1111/bjd.12083.*
117. *Simonsen AB, Deleuran M, Johansen JD, Sommerlund M. Contact allergy and allergic contact dermatitis in children - a review of current data. Contact Dermatitis 2011;65:254-65.*
118. *Schena D, Papagrigoraki A, Tessari G et al. Allergic contact dermatitis in children with and without atopic dermatitis. Dermatitis 2012;23:275-80.*
119. *Thyssen JP, Linneberg A, Engkilde K et al. Contact sensitization to common haptens is associated with atopic dermatitis: new insight. Br J Dermatol 2012;166:1255-61.*
120. *Werfel T, Breuer K, Ruéff F et al. Usefulness of specific immunotherapy in patients with atopic dermatitis and allergic sensitization to house dust mites: a multi-centre, randomized, dose-response study. Allergy 2006;61:202-5.*