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Microarray-based IgE-serology improves management of severe atopic dermatitis in two children

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To the Editor,

Atopic dermatitis (AD) is one of the most common manifestations of IgE-associated allergy in childhood.(1) In the prospective German MAS cohort it was shown that AD begins before the age of 2 years and that sensitization to food allergens precedes sensitization to respiratory allergens, a process called atopic march.(2) AD patients are often sensitized to a variety of food- and aeroallergens and exhibit elevated levels of serum IgE. Identification of disease-triggering food-allergens is important when food allergies are suspected in young children so that appropriate diets can be prescribed.(3) Current approaches for the treatment of AD are based on elimination diet, topical therapies, and systemic immunosuppression, while data on efficacy of AIT in AD are contradictory. In this respect, allergy diagnosis in patients with AD is highly relevant, as it allows disease management by antigen-specific forms of treatment such as allergen-avoidance and AIT.

Molecular allergy diagnosis, earlier termed component-resolved diagnostics (CRD), is based on determining sensitization with purified natural or recombinant allergen molecules.(4) CRD allows differentiation between genuine and cross-sensitizations in

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polysensitized patients, assessing the risk of systemic reactions in food allergies and supports accurate prescription of AIT.(5) Chips containing a broad panel of allergen molecules are particularly useful early in childhood because they allow to identify IgE sensitization towards a comprehensive set of allergens with small serum volumes.(6) In this article we exemplify for two cases of severe AD in children how molecular allergy diagnosis can profoundly improve disease management.

Case 1: CRD-guided dietary management of AD

A 6 year old boy suffering from severe AD who was treated with systemic cyclosporine came to our department in June 2015 because of an AD exacerbation and eczema herpeticum (Figure 1A, Supplemental Figure 1A). Born in 2009, he was breastfed from birth till 6 months. At the age of 3 months he began to develop atopic dermatitis (dryness, redness, itching of skin). Before the age of one year, consumption of goat's and cow's milk, fresh vegetables, fruits, chicken, egg, soy and wheat led to worsening of AD. At 4 years he experienced severe angioedema and urticaria immediately after consumption of a small piece of pork which had been boiled in pea soup. Since 2013 the boy was on strict diet eating only pork, buckwheat and cooked vegetables.

Initially he was treated with emollients and low-dose topical corticosteroids with good efficacy. Until August 2013 (age 4 years), AD was mild but then exacerbated. Since then the course was severe and increasing doses of topical and systemic steroids as well as antihistamines were given with only short-term efficacy. In 2014 the boy started to suffer from extremely severe symptoms of AD. At that time the first allergy

diagnosis was performed revealing strongly elevated levels of total IgE (>2000 U/ml) and poly-sensitization to most food- and aeroallergens as detected by a multiplexed extract-based test (MAST) comprising 35 allergen-extracts from food and respiratory allergen-sources. Systemic therapy with cyclosporine was initiated. Starting July 2014, 75 mg per day was given, yielding moderate control of AD symptoms. When the dose was reduced to 50 mg per day, treatment became ineffective, so administration of 75 mg was resumed and increased to 100 mg per day with modest improvement. In May 2015 the boy started to suffer from severe Herpes Simplex infection (Kaposi's varicelliform eruption, Figures 1A, Supplemental Figure 1A), and was treated with acyclovir (800 mg 5 times per day for 7 days). In June 2015 he was again referred to our department with generalized erythrodermia, severe dryness, erythema, multiple papules, excoriations, lichenification, infiltration of widespread skin areas, multiple postherpetic spots (Figure 1A, Supplemental Figure 1A) and a SCORAD index of 82. Peripheral lymphnodes were enlarged up to 2-4 cm, the abdomen was bloated (Supplemental Figure 1B), liver as well as spleen were enlarged.

Extract-based testing was inconclusive because IgE antibodies to all 35 tested allergen sources were found. Molecular allergy diagnosis using ImmunoCAP ISAC (Thermofisher, Uppsala, Sweden) (Table 1, Supplemental Table 1, case 1) showed that the boy was sensitized to certain types of food (egg: Gal d 1, 2, 3; milk: Bos d 4, 5, 8; storage proteins from several nuts; peanut: Ara h 1, 2, 3, 6; soybean; Gly m 5, 6; wheat: Tri a 11, 14, 19) while marker allergens for other food (e.g., fish, beef, pork, chicken meat, shrimp) were negative. We found broad cross-sensitization to lipid transfer proteins, especially from wheat which showed the highest IgE reactivity (Tri a

14: 35 ISU-E) (Table 1, Supplemental Table 1). Interestingly, sensitizations towards only few clinically relevant respiratory allergens, i.e., Bet v 1 from birch and other PR-10 proteins, Asp f 6 from *Aspergillus*, Hev b 1 and 6 from latex, were found. The broad reactivity with all allergen-extracts was thus most likely caused by IgE to clinically irrelevant carbohydrate epitopes because the carbohydrate marker MUXF3 was positive (Table 1, Supplemental Table 1). Since sensitization to birch pollen and mold were the only respiratory sensitizations the severe perennial skin symptoms could thus be only explained by sensitization to certain food allergens.

It was thus decided to stop cyclosporine treatment and to continue only with topical therapy (hydrocortisone, emollients), antihistamines and amino acid nutrition (Neocate up to 200-400g per day). Under this treatment skin symptoms improved promptly (Figure 1B). Based on the results from molecular allergy diagnosis fish, chicken, pork and beef, together with rice products and cooked potatoes as carbohydrate-sources could be gradually reintroduced into the diet.

Case 2: Molecular allergy diagnosis allows accurate prescription of AIT for AD treatment

The second case is a 14 year old boy suffering from severe AD, moderate persistent asthma, allergic rhinoconjunctivitis and food allergy. He was born in 2001 and developed the first mild symptoms of AD at 1 month of age. At 3 months, severe aggravation and generalization of AD were reported. Since then the boy suffered from severe and persistent AD without stable remission and with exacerbations after consumption of cow's milk and wheat. He was treated with emollients and long-term

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courses of topical corticosteroids without positive effect. At the age of 2 years his first asthma-attack occurred after eating fish, followed by recurrent asthma-attacks after respiratory viral infections and physical activity. Asthma was treated with steroid inhalers and long-acting β_2 -agonists. At the age of 3 years he started to suffer from persistent allergic rhinitis and received courses of nasal steroids for 1 month, 3-4 times per year, without achieving full symptom-control. At the age of 7 years the boy obtained disability status due to severe course of atopic dermatitis and asthma, leading to social isolation, inability to attend school and impairment in quality of life. At the age of 9 years he experienced a further severe exacerbation of AD and asthma and had to take systemic steroids but with only short-time positive effects.

In 2011, at the age of 10, the boy was referred to our department, continuing anti-inflammatory therapy until 2013. Between 2011 and 2013, SCORAD was always above 60, the boy suffered from persistent rhinitis, showed blood eosinophilia >14% and total IgE levels of 4000-5000 U/ml. Skin prick tests were positive for HDM, cat, dog, tree-, grass- and weed pollen, extract-based IgE serology was positive for HDM, cat, fish, birch, soy, potato, cow's milk, beef, carrot, wheat, *Alternaria*, egg white, chicken and grass pollen. A diet avoiding milk, fish, egg, chicken, fruits and cereals was not successful.

At the end of 2013, molecular allergy diagnosis was performed by ImmunoCAP ISAC, showing high levels of specific IgE against group 1 grass pollen allergens (Cyn d 1, Phl p 1), Bet v 1 from birch pollen and cross-reactive PR-10 proteins, allergens from dog (Can f 1, 2, 5), cat (Fel d 1, 4), horse (Equ c 1), mouse (Mus m 1)

and house dust mite (Der p 1, 2 as well as Der f 1, 2) (Table 1, case 2). Sensitization to Asp f 6 from *Aspergillus* was also found. Interestingly, the only food allergen recognized was the major fish allergen Gad c 1. ISAC data thus demonstrated a lack of IgE sensitization to allergens from cow's milk, egg, wheat, nuts and LTPs. Thus we reintroduced milk, peas and wheat into the diet, omitting only fish and histamine-liberating food. ISAC data confirmed sensitization to major HDM allergens and thus we performed 2 consecutive courses of HDM AIT in 2013 and 2014.

The new treatment strategy led to improvement of AD, rhinitis and asthma symptoms (Figure 2A, B). We were able to decrease topical steroid application for skin treatment and switched to topical calcineurin inhibitors (tacrolimus) for face and neck. Dosages of steroids and long-acting β_2 -agonists were gradually reduced, and since 2012 asthma treatment was not required. Antihistamines were given occasionally on demand. Requirement for nasal steroids was reduced and no systemic steroids were needed in 2013-2015.

Discussion

This study exemplifies how molecular allergy diagnosis can have a profound effect on the management of children suffering from severe AD and other allergic co-morbidities (i.e., food allergies, asthma). In fact it is well established that certain food- and respiratory allergens can be potent triggers of AD (7) and it is therefore very important to accurately determine the sensitization profile in patients. According to extract-based IgE-measurement, the patient of case 1 appeared to exhibit an extremely broad sensitization since IgE to all tested respiratory and food allergen

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sources was detected. Restrictive diet, anti-inflammatory treatment for several years and, finally, systemic immunosuppression for almost one year could not stop disease-progression. Molecular allergy diagnosis revealed that the boy had only one relevant respiratory sensitization, i.e., to birch which flowers only in spring and therefore cannot account for severe perennial AD-symptoms. Positive IgE test-results with all other respiratory allergen-extracts in the MAST-test were most likely due to IgE-recognition of cross-reactive carbohydrate epitopes because marker allergens for sensitization to mites, animals and pollen were negative in ISAC. Analysis of IgE-reactivities to food allergens allowed identifying the disease-triggering components. In fact, prompt remission of AD was observed when the boy received amino acid nutrition and it is now possible to reintroduce certain foods into his diet according to ISAC test results, including chicken, pork, rice and boiled potatoes.

The patient from case 2 suffered from severe AD and respiratory allergy (asthma, rhinoconjunctivitis). It was not possible to achieve proper disease control for several years despite massive anti-inflammatory drug treatment. By CRD disease-triggering allergens could be identified. Based on the reactivity profile, AIT to HDM was prescribed and indeed led to an almost complete remission of respiratory symptoms, allowing discontinuation of asthma-therapy. There is increasing evidence that AIT is also effective in AD. Several strategies for AIT to food allergies are currently being developed and in clinical evaluation including oral immunotherapy approaches and the use of recombinant hypoallergens for food allergy (8, 9).

Furthermore, essential foods such as milk and wheat which had been withdrawn based on extract-based testing could be reintroduced into the boy's diet. Since IgE-serology was negative to major wheat food allergens in ISAC, positive extract-based test-results are likely due to cross-reactive carbohydrate epitopes, like in case 1. IgE reactivity to milk was most likely due to sensitization to albumin which does not cause relevant symptoms because it is readily digested.

Our two cases are representative for patients with complex conditions of polysensitization for whom the disease-triggering allergens cannot be unambiguously defined with extract-based tests and, since no generally applicable guidelines exist, a patient-tailored approach must be considered.⁽¹⁰⁾ In our two patients we exemplified that CRD can indeed profoundly change disease-management by unravelling the disease triggering allergens. Control of symptoms was achieved by antigen-specific forms of treatment, i.e., elimination-diet and AIT, which allowed reduction of immunosuppressive and anti-inflammatory therapies.

Molecular allergen-testing requires profound knowledge of biological features and immunological properties of individual allergens which cannot always be replaced by diagnostic guidelines. It is also clear that IgE-serology only allows to detect allergen-specific antibodies and therefore provocation testing will still be needed to confirm clinically relevant sensitizations. However, our results indicate that IgE-testing by allergen micro-arrays may be considered for first line diagnosis, especially in cases of polysensitization, in order to accurately define preventive and treatment strategies.

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Figures and Tables

Figure 1. Patient 1 with exacerbation of AD and an eczema herpeticum during systemic cyclosporine treatment (A) and after diet (B).

Figure 2. Case 2 in 2010 with AD exacerbation (A) and in 2014, after 2 courses of AIT to HDM (B).

Table 1. Results from molecular allergy diagnosis testing. IgE results sorted by allergen source for the two cases. Only results which were positive for at least one of the two cases are shown.

Supplementary Figure 1. Patient 1 with exacerbation of AD and an eczema herpeticum during systemic cyclosporine treatment (A) and swollen abdomen and increased inguinal lymphnodes (B).

Supplementary Table 1. Results from molecular allergy diagnosis testing. IgE results sorted by allergen source for the two cases as in Table 1 but all results are shown.

Source	Allergen	Protein family	Case 1		Case 2	
			slgE (ISU)	Level *	slgE (ISU)	Level *
Mainly species-specific food components						
Egg white	nGal d 1	Ovomucoid	17 ISU-E	Very high	<0.3 ISU-E	Undetectable
	nGal d 2	Ovalbumin	25 ISU-E	Very high	<0.3 ISU-E	Undetectable
	nGal d 3	Conalbumin/Ovotransferrin	>100 ISU-E	Very high	<0.3 ISU-E	Undetectable
Egg yolk/chicken meat	nGal d 5	Livetin/Serum albumin	1.4 ISU-E	Moderate	<0.3 ISU-E	Undetectable
Cow's milk	nBos d 4	Alpha-lactalbumin	55 ISU-E	Very high	<0.3 ISU-E	Undetectable
	nBos d 5	Beta-lactoglobulin	66 ISU-E	Very high	<0.3 ISU-E	Undetectable
	nBos d 8	Casein	51 ISU-E	Very high	<0.3 ISU-E	Undetectable
Cow's milk/meat	nBos d 6	Serum albumin	1.5 ISU-E	Moderate	<0.3 ISU-E	Undetectable
Cod	rGad c 1	Parvalbumin	<0.3 ISU-E	Undetectable	39 ISU-E	Very high
Cashew nut	rAna o 2	Storage protein, 11S globulin	80 ISU-E	Very high	<0.3 ISU-E	Undetectable
Hazelnut	rCor a 1.0401	PR-10 protein	34 ISU-E	Very high	22 ISU-E	Very high
	rCor a 8	Lipid transfer protein (nsLTP)	15 ISU-E	Very high	<0.3 ISU-E	Undetectable
	nCor a 9	Storage protein, 11S globulin	60 ISU-E	Very high	<0.3 ISU-E	Undetectable
Walnut	nJug r 2	Storage protein, 7S globulin	3.4 ISU-E	Moderate	<0.3 ISU-E	Undetectable
	nJug r 3	Lipid transfer protein (nsLTP)	15 ISU-E	Very high	<0.3 ISU-E	Undetectable
Peanut	rAra h 1	Storage protein, 7S globulin	83 ISU-E	Very high	<0.3 ISU-E	Undetectable
	rAra h 2	Storage protein, Conglutin	>100 ISU-E	Very high	<0.3 ISU-E	Undetectable
	rAra h 3	Storage protein, 11S globulin	3.6 ISU-E	Moderate	<0.3 ISU-E	Undetectable
	nAra h 6	Storage protein, 2S albumin	52 ISU-E	Very high	<0.3 ISU-E	Undetectable
	rAra h 8	PR-10 protein	30 ISU-E	Very high	10 ISU-E	High
	rAra h 9	Lipid transfer protein (nsLTP)	15 ISU-E	Very high	<0.3 ISU-E	Undetectable
Soybean	rGly m 4	PR-10 protein	<0.3 ISU-E	Undetectable	17 ISU-E	Very high
	nGly m 5	Storage protein, Beta-conglycinin	34 ISU-E	Very high	<0.3 ISU-E	Undetectable
	nGly m 6	Storage protein, Glycinin	70 ISU-E	Very high	<0.3 ISU-E	Undetectable
Wheat	rTri a 19.0101	Omega-5 gliadin	9.9 ISU-E	High	<0.3 ISU-E	Undetectable
	nTri a aA_Tl	Alpha-amylase / Trypsin inhibitor	12 ISU-E	High	<0.3 ISU-E	Undetectable
	rTri a 14	Lipid transfer protein (nsLTP)	36 ISU-E	Very high	<0.3 ISU-E	Undetectable
Kiwi	rAct d 8	PR-10 protein	3.9 ISU-E	Moderate	0.7 ISU-E	Low
Apple	rMal d 1	PR-10 protein	31 ISU-E	Very high	22 ISU-E	Very high
Peach	rPru p 1	PR-10 protein	24 ISU-E	Very high	24 ISU-E	Very high
	rPru p 3	Lipid transfer protein (nsLTP)	14 ISU-E	Very high	<0.3 ISU-E	Undetectable
Celery	rApi g 1	PR-10 protein	2.9 ISU-E	Moderate	2.1 ISU-E	Moderate

Aeroallergens						
Grass pollen						
Bermuda grass	nCyn d 1	Grass group 1	6.1 ISU-E	Moderate	25 ISU-E	Very high
Timothy grass	rPhi p 1	Grass group 1	<0.3 ISU-E	Undetectable	59 ISU-E	Very high
	nPhi p 4	Berberine bridge enzyme	10 ISU-E	High	<0.3 ISU-E	Undetectable
Tree pollen						
Alder	rAln g 1	PR-10 protein	83 ISU-E	Very high	25 ISU-E	Very high
Birch	rBet v 1	PR-10 protein	>100 ISU-E	Very high	49 ISU-E	Very high
Hazel pollen	rCor a 1.0101	PR-10 protein	6.6 ISU-E	Moderate	4.3 ISU-E	Moderate
Japanese cedar	nCry j 1	Pectate lyase	4.2 ISU-E	Moderate	<0.3 ISU-E	Undetectable
Cypress	nCup a 1	Pectate lyase	5.9 ISU-E	Moderate	<0.3 ISU-E	Undetectable
Olive pollen	nOle e 7	Lipid transfer protein (nsLTP)	11 ISU-E	Very high	<0.3 ISU-E	Undetectable
	rOle e 9	Beta-1,3-glucanase	5.2 ISU-E	Moderate	<0.3 ISU-E	Undetectable
Plane tree	nPla a 2	Polygalacturonase	7 ISU-E	Moderate	<0.3 ISU-E	Undetectable
	rPla a 3	Lipid transfer protein (nsLTP)	29 ISU-E	Very high	<0.3 ISU-E	Undetectable
Weed pollen						
Mugwort	nArt v 3	Lipid transfer protein (nsLTP)	20 ISU-E	Very high	<0.3 ISU-E	Undetectable
Animals						
Dog	rCan f 1	Lipocalin	<0.3 ISU-E	Undetectable	33 ISU-E	Very high
	rCan f 2	Lipocalin	<0.3 ISU-E	Undetectable	30 ISU-E	Very high
	rCan f 5	Arginine Esterase	<0.3 ISU-E	Undetectable	>100 ISU-E	Very high
Horse	rEqu c 1	Lipocalin	3.3 ISU-E	Undetectable	>100 ISU-E	Very high
Cat	rFel d 1	Uteroglobin	<0.3 ISU-E	Undetectable	>100 ISU-E	Very high
	nFel d 2	Serum albumin	<0.3 ISU-E	Undetectable	0.6 ISU-E	Low
	rFel d 4	Lipocalin	<0.3 ISU-E	Undetectable	28 ISU-E	Very high
Mouse	nMus m 1	Lipocalin	<0.3 ISU-E	Undetectable	2.1 ISU-E	Moderate
Mold						
Aspergillus	rAsp f 6	Mn superoxide dismutase	3.4 ISU-E	Moderate	9.6 ISU-E	Moderate
Mite						
D. farinae (HDM)	nDer f 1	Cysteine protease	<0.3 ISU-E	Undetectable	46 ISU-E	Very high
	rDer f 2	NPC2 family	<0.3 ISU-E	Undetectable	>100 ISU-E	Very high
D. pteronyssinus (HDM)	nDer p 1	Cysteine protease	<0.3 ISU-E	Undetectable	9.8 ISU-E	Moderate
	rDer p 2	NPC2 family	<0.3 ISU-E	Undetectable	42 ISU-E	Very high
Other allergens						
Parasite						
Anisakis	rAni s 3	Tropomyosin	1.4 ISU-E	Moderate	<0.3 ISU-E	Undetectable
Latex						
Latex	rHev b 1	Rubber elongation factor	2.2 ISU-E	Moderate	<0.3 ISU-E	Undetectable
	rHev b 6.01	Prohevein	8.6 ISU-E	Moderate	<0.3 ISU-E	Undetectable
CCD	nMUXF3	CCD	14 ISU-E	High	<0.3 ISU-E	Undetectable

* < 0.3 Undetectable
0.3 - 0.9 Low
1.0- 14.9 Moderate / High
>15 Very High

Figure 1. Patient 1: (A) Exacerbation of AD and an eczema herpeticum during systemic cyclosporine treatment. (B) Marked improvement of skin-condition after adjusting the patient's diet and medication based on results from component-resolved diagnosis.



Figure 2. Patient 2, AD exacerbation.

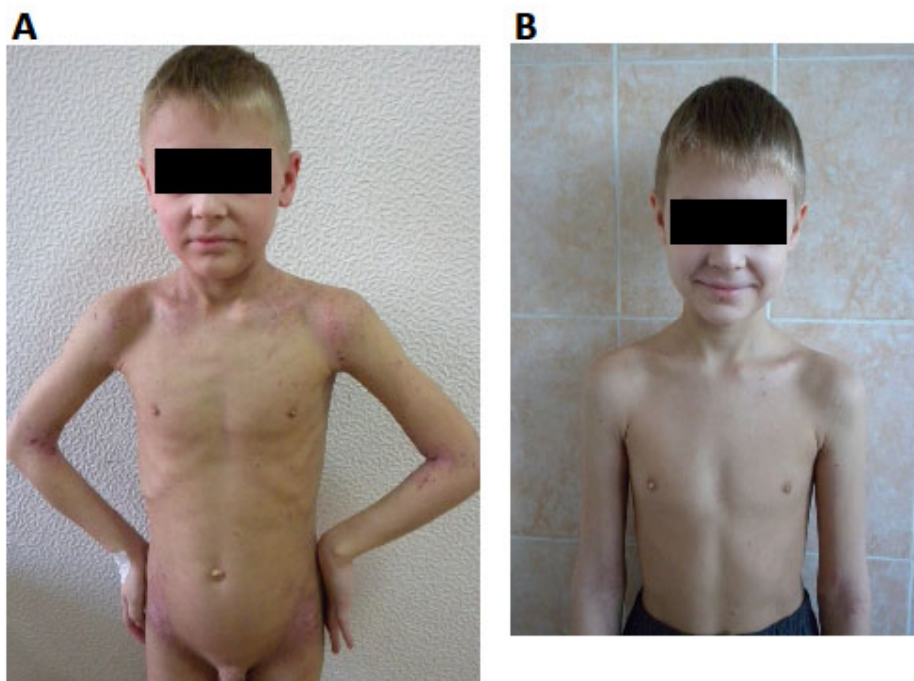


Figure S1. Patient 1: (A) Exacerbation of AD and eczema herpeticum during systemic cyclosporine treatment. (B) Swollen abdomen and enlarged lymph nodes.

A



B

