

ORIGINAL ARTICLE

Molecular microarray analysis reveals allergen- and exotoxin-specific IgE repertoires in children with atopic dermatitis

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Abstract

Background Children suffering from atopic dermatitis frequently show allergen-specific sensitization. However, the corresponding IgE-recognition patterns have not yet been extensively characterized using multiallergen microarrays.

Objective To provide comprehensive, molecular IgE repertoires in paediatric patients with atopic dermatitis using microarray technology.

Methods Sera of 140 affected children were screened with a protein microarray containing a panel of 95 inhalant, food and staphylococcal antigen components. In addition, total serum IgE levels and further clinical parameters were recorded.

Results At a mean total IgE level of 1528 kU/L, the number of sensitizations varied from 0 to 32 per patient, and regression analysis revealed a significant association between total IgE and the quantity of recognized antigens. A total of 78 single allergen and microbial components elicited at least one IgE response, while 11 plant and 13 non-plant molecules were recognized by more than 10% of patients. Specific IgE against *Staphylococcus aureus* could be detected in 14% of children. Sensitization rates against the studied allergen molecules differed significantly when stratified by age. Whereas reactivity against inhalant allergens and SEC was lowest in the youngest group (<24 months) reaching highest values in children ≥ 72 months, IgE responses against food allergen components peaked in younger age groups (0–48 months) and clearly declined in patients of higher age. The large amount of microarray data could be aggregated by centroid cluster analysis revealing valid allergen clusters possibly linked with higher disease severity as determined by multivariate analysis of covariance.

Conclusion Allergenic molecule microarray analysis can be regarded as a suitable research tool for large-scale IgE screening in infants and children with atopic dermatitis (AD). Still, further studies in well-defined populations are needed to exactly identify its tangible benefits in the diagnostic and therapeutic management of affected patients in daily clinical practice.

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Conflict of interest

Hagen Ott has received speaker's fees and research funding from Phadia/ThermoFisher.

Introduction

Affecting approximately 10% of the total population, atopic dermatitis (AD) represents the most common chronic inflammatory dermatosis in developed countries. It manifests primarily in infants and young children with an onset of eczematous skin lesions during the first year of life in 50%, and until age five in up to 80% of patients.¹

AD has a multifactorial pathogenesis involving genetically determined epidermal barrier dysfunction mainly due to filaggrin loss-of-function mutations, impaired antimicrobial protein expression and characteristic deviations of innate and adaptive immunity, which are reviewed in detail elsewhere.^{2–7} For example, these immunological characteristics include T-helper type 2-skewed cytokine patterns, increased numbers of skin-resident, immunoglobulin E (IgE)-bearing antigen-presenting cells and, most importantly, elevated serum levels of total and/or allergen-specific IgE (tIgE, sIgE). In particular, sIgE responses against

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inhalant and food allergens have been associated with distinct AD phenotypes in numerous clinical studies.^{6,7}

In addition, predominantly due to skin-barrier disruption, AD patients show a propensity to cutaneous colonization and infection with bacterial pathogens. In particular, *Staphylococcus aureus* (*S. aureus*) can be isolated in up to 90% of lesional skin and may secrete exotoxins that either operate as superantigens or as allergens eliciting specific humoral immune responses.⁷ Interestingly, exotoxin-directed sIgE antibody titres have been described to correlate with disease severity in children and adults with AD.^{8,9}

However, these data were mostly obtained in small-scale studies performing sIgE screening with limited allergen and enterotoxin panels only. Moreover, sIgE detection was almost exclusively based on allergenic extracts, which do not allow for the reliable identification of single culprit allergen components. Yet, molecular allergy diagnosis relying on single allergenic molecules is now widely considered as a valuable diagnostic method in clinical research, particularly with regard to AD patients.¹⁰ Thus, it was the aim of this study to establish comprehensive, molecular sIgE-recognition patterns in a large cohort of children affected by AD and to correlate the obtained sensitization profiles with clinical features, notably disease severity.

Materials and methods

Patient population

In this prospective study, 140 consecutive children were recruited presenting to our dermatological referral centre due to chronic eczematous skin reactions. In each patient, a definitive diagnosis was established in adherence to the Hanifin and Rajka diagnostic criteria for AD, while the grade of illness was classified according to the SCORAD index devised by the European Task Force on AD.¹¹ Besides demographic data, detailed information concerning atopic family history, associated comorbidities and prior bacterial skin infections were recorded. After obtaining written informed consent, blood samples were collected upon the first patient visit and the corresponding serum probes were stored at -80 °C until microarray analysis and tIgE measurement. Data processing was performed in compliance with ethical standards on human experimentation and with the Helsinki Declaration of 1975, as revised in 1983. The study was approved by the local ethical committee of the University Hospital Kiel.

Determination of total serum IgE levels

Determinations of tIgE levels were performed using a commercially available fluorescence enzyme immunoassay (FEIA) as proposed by the manufacturer (UniCAP™; Phadia, Uppsala, Sweden).

Protein microarray assays

In this investigation, we employed customized, commercially available microarrays (ImmunoCAP ISAC™; Phadia) containing

95 components of inhalant, food and microbial antigen sources (Table 1). This allergenic molecule-based test system has been validated as a useful *in vitro* instrument in prior clinical studies, particularly with regard to atopic diseases in children and adults.^{12–16}

Microarray immunoassays were performed according to the manufacturer's recommendations as previously published.¹⁷ Briefly, each microarray reaction site was incubated with 20 µL of undiluted patient serum to capture sIgE antibodies by their corresponding allergen molecules. In a second step, the microarray slides were washed and finally dried under nitrogen flow. Hereafter, microarray-bound IgE was marked with a secondary, fluorescence-tagged antihuman IgE. The corresponding fluorescence signals were scanned using a biochip reader (Scan Array Express™; Perkin Elmer Life Sciences, Boston, MA, USA). Based on calibration sera of known specific IgE content, raw data analysis of the corresponding digitized microarray images was performed using the QuantArray™ 3.1 software (Perkin Elmer Life Sciences). Image information was transformed into numerical data yielding four semiquantitative classes of sIgE levels, which were then categorized as either negative or positive for further statistical calculations.

Statistics

Continuous variables are expressed as mean values $\pm$ standard deviation. Data obtained by microarray analysis included 78 antigens recognized by at least one study patient and described by dichotomous variables. In a first step, these 78 variables were clustered using the centroid method.

A multivariate analysis of covariance (ANCOVA) was carried out to investigate the effects of the clusters, superantigens and clinical parameters on disease severity depicted by the dimensionless SCORAD score. The sum of sensitizations was formed for each cluster and the staphylococcal superantigens respectively. Conducting a preceding variable selection, only those clinical parameters were included in the multivariate ANCOVA, which showed a *P*-value below 0.25 in the univariate analysis. Homoscedasticity and normal distribution of residuals were investigated by both residual and normal probability plots.

Furthermore, a linear regression analysis investigated the influence of all recognized allergens (sum) on tIgE. A log-transformation on tIgE yielded the required homoscedasticity and normal distribution of residuals. The significance level was $\alpha = 0.05$. All statistical analyses were conducted using the Statistical Analysis System (SAS Version 9.2; SAS Institute, Cary, NC, USA).

Results

Demographics and clinical baseline data

A total of 140 children (55 girls, 85 boys) with a mean age of 74 ± 53 months (range 5–206 months) entered this study. All patients suffered from AD of variable severity as depicted by a

Table 1 Prevalence of IgE-mediated sensitization against single microarrayed allergen and microbial antigen components in the total population (*n* = 140)

Rank	Antigen	Source	Code†	n	%	Rank	Antigen	Source	Code†	n	%
1	Lol p 1	Rye grass	450	56	40	23	Bos d 7	cow's milk	166	8	6
	Der f 2	House dust mite	302	56	40		Bos d 8	cow's milk	167	8	6
2	Der p 1	House dust mite	310	54	39		Bet v 2	birch	127	8	6
3	Der p 2	House dust mite	316	52	37		Ole e 2	olive tree	490	8	6
4	Der f 1	House dust mite	295	51	36	24	Ana c 2	ananas	694	7	5
5	Fel d 1	Cat	345	50	36		Act d 11	kiwi	5903	7	5
6	Phi p 1	Timothy grass	549	48	34		Hor v 17	barley	420	7	5
7	Der s 1	House dust mite	1804	45	32		Par j 2	pellitory	507	7	5
8	Bet v 1	Birch	90	44	31		Pho d 2	date palm	571	7	5
9	Phi p 5	Timothy grass	558	38	27		Can f 3	dog	176	7	5
10	Mal d 1	Apple	464	32	23	25	Act d 1	kiwi	1	6	4
11	Aln g 1	Alder	7	31	22		Phi p 12	timothy grass	553	6	4
12	Gal d 1	Hen's egg	359	29	21		Par j 3	pellitory	510	6	4
13	Gal d 2	Hen's egg	360	25	18	26	Bos d 4	cow's milk	163	5	4
	Gal d 4	Hen's egg	362	25	18		Bos d 6	cow	165	5	4
	Phi p 6	Timothy grass	569	25	18		Bos d 8 alpha S1	cow's milk	2734	5	4
	Can f 1	Dog	174	25	18		Hel a 2	sunflower	377	5	4
14	Phi p 2	Timothy grass	555	24	17	27	Bos d 8 Beta	cow's milk	2736	4	2
15	Cor a 1	Hazelnut	232	23	16		Gal d 3	hen's egg	361	4	2
16	Alt a 1	Alternaria	722	22	16		Pru p 3	peach	603	4	2
17	Pla a 2	Plane tree	573	17	12	28	Act d 2	kiwi	747	3	2
	Sta a SEC	Staphylococcus	2141	17	12		Der p 10	house dust mite	311	3	2
18	Art v 1	Mugwort	753	16	11		Blag 2	cockroach	141	3	2
19	Cor a 8	Hazelnut	245	13	9		Pen i 1	shrimp	527	3	2
20	Ole e 1	Olive tree	482	11	8		Pen m 1	shrimp	972	3	2
21	Act d 7	Kiwi	3976	10	7		Ani s 3	anisakis simplex	37	3	2
	Cyn d 12	Bermuda grass	279	10	7	29	Bos d Lactoferrin	cow's milk	1065	2	1
	Mer a 1	Annual mercury	476	10	7		Bos d 5.0101	cow's milk	2739	2	1
22	Api g 1	Celery	40	9	6		Bos d 8 Kappa	cow's milk	2737	2	1
	Cup a 1	Cypress	256	9	6		Dau c 1	carrot	287	2	1
	Hev b 8	Latex	397	9	6		Alt a 6	alternaria	14	2	1
	Sta a SEA	Staphylococcus	2139	9	6		Per a 7	cockroach	542	2	1

†Allergome allergen code (<http://www.allergome.org>).

mean SCORAD score of 36 ± 20 points (range 5–99 points). Atopic comorbidities consisted of reported food allergy in 62 (44%) and bronchial asthma in 39 (28%) children, whereas allergic rhinitis or conjunctivitis was observed in 50 (36%) and 52 (37%) patients respectively. A total of 57 individuals (41%) had suffered from additional bacterial superinfection (impetigo) at least once since AD onset. Finally, parental history for any atopic disease was positive in both parents in 35% of cases, whereas the father or the mother was exclusively affected in 26% and 24% respectively.

Allergen-specific IgE-recognition patterns and tIgE serum levels

At a mean total serum IgE of 1528 ± 3824 kU/L, tIgE levels revealed marked interindividual variability ranging from a minimum of 3 kU/L to a maximum of 26567 kU/L. The total number of sensitizations to any of the studied antigens varied from 0 to maximally 32 per patient (mean: 7.6 ± 7.7). Of included patients, 14% ($n = 20$) did not show any allergen-specific sensitization, as determined by microarray analysis.

Analysed separately, mean numbers of sensitizations against allergen components and *Staphylococcus aureus* superantigens were 7.4 ± 7.5 (range 0–32) and 0.2 ± 0.6 (range 0–3) respectively. Linear regression analysis disclosed a statistically significant association between tIgE and the sum of all recognized antigens (Fig. 1).

A total of 17 microarrayed antigens did not yield any positive sIgE result in the studied population (Gal d 5, Act d 5, Act d 6, Phl p 7, Pla a 1, Asp f 1, Bla g 5, Hev b 3, Hev b 5, Hev b 7, Hev

b 10, Hev b 11, Ani s 1, Sta a SED, Sta a SEH, Sta a SEJ, Sta a SEK), whereas 78 single allergen and microbial components elicited an sIgE response in at least one child. The corresponding prevalence of sensitizations is fully displayed in Table 1, revealing a set of 11 plant (Lol p 1, Phl p 1, Bet v 1, Phl p 5, Mal d 1, Aln g 1, Phl p 6, Phl p 2, Cor a 1, Pla a 2, Art v 1) and 13 non-plant (Der f 2, Der p 1, Der p 2, Der f 1, Fel d 1, Der s 1, Aln g 1, Gal d 1, Gal d 2, Gal d 4, Can f 1, Alt a 1, Sta a SEC) allergenic molecules recognized by more than 10% of patients.

Considering primary allergen sources, inhalant sensitization was most often caused by house dust mites, tree and grass pollens, animal epithelia and the mould *Alternaria* (*A. alternata*). Likewise, sIgE responses to food allergen sources were most frequently due to hen's egg, hazelnut, cow's milk and kiwi (Table 2). Specific IgE against *Staphylococcus aureus* could be detected in 14% of all patients and was shown to be directed predominantly against one molecule, namely Sta a SEC (Table 1).

Plotted separately in five groups of rising age, sIgE reactivity against inhalant allergens and Sta a SEC was lowest in patients younger than 24 months of age, reaching highest values in children ≥ 72 months (Fig. 2a). In contrast, sensitization rates against food allergen components (Gal d 1, Bos d 8, Cor a 8) peaked in younger age groups and clearly declined in patients of higher age (Fig. 2b). In infants and children younger than 48 months, food proteins most frequently yielding positive test results comprised Gal d 1 (29% of patients), Gal d 2 (25%) and Gal d 4 (17%) followed by Cor a 8 (25%) and Bos d 8 (5%). Moreover, cross-reactivity rates against plant and non-plant panallergens were either generally low in all groups (e.g. dust mite [Der p 10] and anisakis [Ani s 3] tropomyosins) or slightly

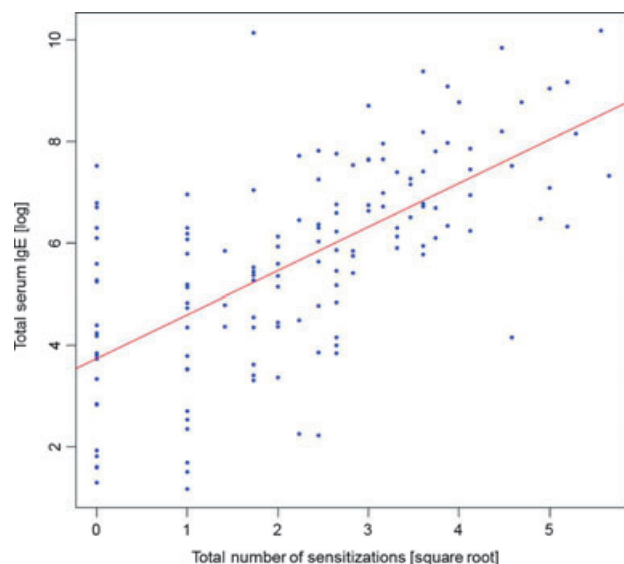


Figure 1 Linear regression analysis of the association between total serum IgE (tIgE) and the sum of all recognized allergen and microbial antigen components.

Table 2 Prevalence of sensitization against allergen sources eliciting specific IgE responses in at least 10% of the total population ($n = 140$)

Rank	Allergen source	<i>n</i>	%
1	House dust mite	64	46
2	Timothy grass	58	41
3	Rye grass	56	40
4	Cat	50	36
5	Hen's egg	49	35
	Birch	49	35
6	Apple	32	23
7	Hazelnut	31	22
	Alder	31	22
8	Dog	28	20
9	Alternaria	22	16
10	Kiwi	21	15
11	Cow's milk	20	14
	Staph. aureus	20	14
12	Plane tree	17	12
13	Mugwort	16	11

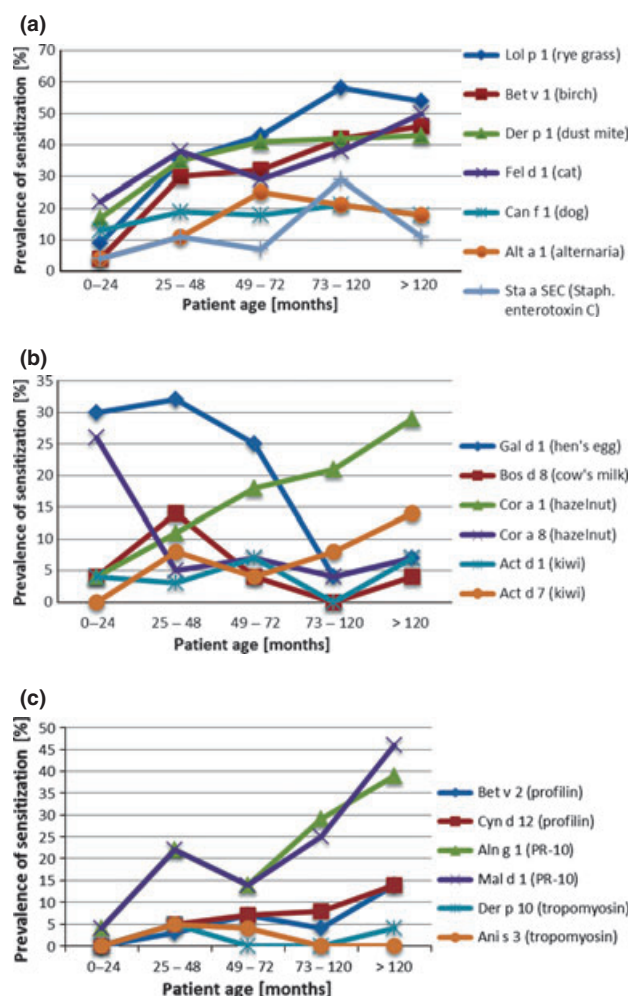


Figure 2 Prevalence of sensitization against the most frequently recognized inhalant (a), food (b) and cross-reactive (c) allergen components plotted separately according to age.

rising with increasing age (e. g. birch [Bet v 2] and bermuda grass [Cyn d 12] profilins). Although low in infants and young children, sensitization prevalence against birch pollen-associ-

ated, pathogenesis-related (PR-10) proteins from other plant allergen sources (e.g. alder [Aln g 1], apple [Mal d 1]) was elevated in all patient groups of higher ages (Fig. 2c).

Cluster analysis and association of clinical parameters with disease severity

Using the centroid method, 10 allergen clusters of varying size were established explaining 57.82% of total variation. Interestingly, statistical analysis yielded clusters (C) of cross-sensitization comprising homologous allergenic proteins (e.g. C1, C4, C7, C9) and clusters of co-sensitization containing non-homologous components, which frequently elicit simultaneous IgE responses in clinical practice (e.g. C3, C5, C7) (Table 3). Investigating the effects of these allergen clusters as well as of Staphylococcal superantigens and clinical parameters on disease severity (SCORAD), four clusters (C2, C6, C8, C10) and one clinical parameter (previous bacterial superinfection) were found to be significantly correlated with grade of illness in multivariate ANCOVA ($P < 0.05$). This association could neither be established for the other clusters nor for those clinical parameters that had passed previous univariate variable selection: patient age, concomitant asthma, allergic rhinitis, allergic conjunctivitis, parental atopy or sensitization to staphylococcal superantigens.

Discussion

To the best of our knowledge, this is the first study to establish sIgE sensitization profiles by allergenic molecule-based microarray analysis in a well-defined group of children suffering from AD. To avoid the restrictions of conventional test systems with pre-selected, numerically limited allergen panels, we relied on multiplex microarrays for the simultaneous detection of sIgE against an extensive range of allergenic proteins. Also designated as allergen chips, these devices have only recently been validated by us and other groups as promising diagnostic tools in atopic disease.^{13–15,18–20}

First, we were able to confirm previous studies in that the total number of sIgE responses is significantly associated with tIgE serum levels in paediatric AD patients ($P < 0.0001$) (Fig. 1). Applied to clinical research, this strongly implies that

Table 3 Grouping of sIgE-eliciting allergen components into 10 clusters calculated by the centroid method

Cluster number	Included allergen components
C1	Bet v 2, Phl p 12, Cyn d 12, Hel a 2, Art v 1, Mer a 1, Par j 3, Pho d 2, Ole e 2, Hev b 8
C2	Bos d 6, Bos d 8 alpha, Ana o 2, Can f 3, Hev b 9
C3	Phl p 1, Phl p 5, Phl p 2, Phl p 6, Lol p 1, Alt a 6, Cla h 8
C4	Der p 10, Per a 7, Pen i 1, Pen m 1, Hel a 1, Ani s 3
C5	Bos d 4, Bos d 5, Bos d 8, Bos d 8 beta, Bos d kappa, Gal d 1, Gal d 2, Gal d 3
C6	Tri a 18, Pru p 3, Act d 1, Act d 2, Hev b 1, Hev b 6
C7	Der f 1, Der f 2, Der p 1, Der p 2, Der s 1
C8	Ana c 2, Act d 7, Hor v 17, Pla a 2, Cup a 1, Ole e 1
C9	Mal d 1, Api g 1, Act d 11, Bet v 1, Cor a 1, Aln g 1
C10	Bos d lactoferrin, Bos d 7, Gal d 4, Tri a 19, Dau c 1, Cor a 8, Hor v 16, Par j 2, Alt a 1, Fel d 1, Can f 1, Bla g 2

allergen-specific sensitizations might pass undetected in atopic individuals with high tIgE titres if sIgE testing is not performed with a broad spectrum of allergen components. This hypothesis is further corroborated by the fact that our patients were frequently sensitized against *S. aureus* exotoxins (14%), *A. alternata* (16%) and/or hazelnut (22%) allergens, which are not routinely represented in currently established sIgE screening panels.²¹ Hence, microarray-based molecular allergy diagnosis could significantly enhance future epidemiological investigations permitting a more accurate distinction of AD phenotypes, particularly the sIgE-associated extrinsic and the 'non-atopic' intrinsic disease variants.¹⁴ In addition, multiplex analysis required only very small volumes of patient serum (20 µl) which can also be collected by capillary blood sampling.²² This strongly suggests that allergen microarrays are particularly suitable for minimal-invasive sIgE screening in AD, a disorder characterized by its early onset during infancy and toddlerhood in a majority of affected patients.

Resolved at allergen-source level, air-borne allergens most frequently recognized in our patient population included house dust mites, tree and grass pollens, animal epithelia (cat, dog) and the mould *A. alternata* (Table 2). Analysed cross-sectionally in five different age groups, sIgE positivity against these allergens was lowest in patients younger than 24 months and reached highest values in children > 72 months of age (Fig. 2a). In direct contrast, sensitization rates against the major animal food components (hen's egg, cow's milk) peaked in the younger groups (0–48 months) and clearly declined in patients of higher age (Fig. 2b). This set of data is in accordance with many previous publications reporting highly similar inhalant- and animal food-specific sIgE repertoires and their age dependency in children affected by AD or related atopic diseases.^{23,24}

Intriguingly, we were also able to demonstrate that sensitization against cross-reactive panallergens of plant or animal origin was practically inexistent in AD patients younger than 24 months. Correspondingly, low levels of sensitization ($\leq 5\%$) against the panallergenic tropomyosins of dust mite and anisakis persisted in all age groups. In contrast, grass and birch profilin reactivity slowly increased reaching rates >10% in the highest age group only. Even more strikingly, sIgE reactivity directed at birch pollen-associated PR-10 proteins was very low in children younger than 24 months, but then steadily rose in older patients (Fig. 2c). Interestingly, Melioli and co-workers reported similar panallergen recognition patterns in a very large group of patients with different allergic diseases excluding AD. However, after age stratification of sensitization profiles, the youngest patients (<2 years) mounted sIgE responses to PR-10 proteins in a sizeable number of cases (20%). Besides geographical factors, this discrepancy to our data is most probably due to differences in patient selection favouring inclusion of individuals with pollen-related, inhalant allergies (rhinitis, conjunctivitis, asthma) in the cited study.²⁴

Furthermore, we determined hazelnut and kiwi as the leading triggers of plant food-specific IgE responses (Table 2). Interestingly, hazelnut-specific sensitization was bimodal, encompassing a first peak in the youngest age group, which was mainly due to sIgE against Cor a 8, a lipid-transfer protein from hazelnut. This was then followed by continuously rising hazelnut sIgE levels directed at a birch pollen-associated hazelnut PR-10 protein (Cor a 1). Similarly, specific IgE responses to kiwi detected in older children (>72 months) were predominantly due to cross-sensitization against the pollen-associated kiwi pectylmethylesterase (Act d 7) and not to the kiwi major allergen component Act d 1 (Fig. 2b). Thus, in our population, microarray-based molecular allergy diagnosis allowed for the distinction of genuine sensitization against plant food proteins from mere cross-sensitization against homologous proteins of other allergen sources. These recognition patterns are of great clinical interest, because sIgE positivity against components such as Cor a 8 has previously been shown to be associated with an elevated risk of actual clinical reactivity.²⁵ However, this could not be further explored in our study as placebo-controlled oral food challenges with the incriminated allergens were not systematically performed.

Colonization of both lesional and non-lesional skin with *Staphylococcus (S.) aureus* represents a key feature of AD, and up to 50% of *S. aureus* strains may secrete exotoxins.²⁶ While these bacterial products may act as superantigens directly activating T cells, they are also known to induce sIgE responses in some patients with AD. Thus, we analysed individual sIgE-recognition patterns with the broadest exotoxin panel in one of the largest paediatric AD populations to date.²⁷ Surprisingly, we detected IgE-mediated sensitization to any staphylococcal toxin in only 14% of our patients (Table 2). While Sta a SEC and Sta a SEA were the most frequently recognized single antigens (12% and 6% respectively), the other exotoxins elicited positive sIgE responses in either only 1% of the study population (Sta a SEB, Sta a SEE, Sta a SEI, Sta a TSST) or were not immunogenic at all (Sta a SED, Sta a SHE, Sta a SEJ, Sta a SEK) (Table 1). These results contrast with publications of other research groups indicating clearly higher sensitization rates against staphylococcal superantigens in children and adults suffering from AD.^{28,29} Although still unproven by large-scale and controlled trials, the disparity between these data and our findings may be related to differences in patient selection and the method employed for *in vitro* testing. For example, Lin and co-workers demonstrated Sta a SEA- and Sta a SEB-directed IgE antibodies in 70% of children with AD ($n = 60$) who displayed markedly higher mean tIgE levels than those found in our study population (1528 kU/L vs. 17191 kU/L). This might have significantly influenced sIgE positivity, because very high serum tIgE levels have been shown to raise the pretest probability of exotoxin-specific IgE detection in previous investigations.^{27,30} Furthermore, all cited research groups used conventional fluorescence-enzyme immunoassays

(FEIA) that might be more sensitive at detecting exotoxin-specific IgE, thus yielding higher positivity rates. In contrast, a significant influence of different microbial antigens used for microarray and FEIA analysis is improbable, because both devices rely on highly similar, purified, natural *Staphylococcus aureus* antigens. Unfortunately, this question could not be further clarified, because we did not perform parallel FEIA analyses that would have permitted direct comparison of both techniques.

In a last step, we aimed at analysing the influence of distinct sensitization patterns on disease severity, i.e. the SCORAD score. Prior to this, we applied centroid clustering to aggregate the large data set compiled by microarray analysis into 10 allergen clusters (Table 3). Of note, these newly identified clusters were particularly validated by their grouping of homologous, frequently cross-sensitizing proteins that are expected to form clusters *a priori* (e.g. profilins [C1], tropomyosins [C4] or PR-10 proteins [C8]). By further multivariate analyses of covariance, four allergen clusters (C2, C6, C8, C10) and a history of previous bacterial superinfections, but not sIgE positivity against any staphylococcal exotoxin, were found to be significantly associated with disease severity ($P < 0.05$). Yet, these data should be interpreted with caution, because some relevant allergens which were not present on the allergen chip at the time of our investigation (peanut, soy, fish components) might have influenced clustering analyses.³¹

In conclusion, this study validates allergen microarrays as powerful tools for clinical research in infants and children with AD. Facilitating simultaneous sIgE detection against multiple allergens in minute amounts of patient serum, this technique will empower future investigations of distinct disease phenotypes and their evolution during the atopic march. Still, further studies in well-defined populations are needed to clarify whether it can provide tangible benefits in the diagnostic and therapeutic management of affected patients in daily clinical practice.

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