

ORIGINAL ARTICLE

The IgE repertoire in children and adolescents resolved at component level: A cross-sectional study

Giovanni Melioli¹, Laura Marcomini¹, Alessia Agazzi¹, Gyada Bazurro¹, Mariangela Tosca², Giovanni A. Rossi², Paola Minale³, Renato Rossi⁴, Giorgio Reggiardo⁵, Giorgio W. Canonica⁶ & Giovanni Passalacqua⁶

¹UOC Laboratorio Centrale di Analisi, Istituto Giannina Gaslini, Genoa; ²UOC di Pediatria ad indirizzo pneumologico ed allergologico, Istituto Giannina Gaslini, Genoa; ³UOC Allergologia, Azienda Ospedale San Martino, Genoa; ⁴Rete di Allergologia Regione Piemonte, Azienda Sanitaria Cuneo 1, Cuneo; ⁵Unità di Biometria, Mediservice Srl, Milano; ⁶Allergy and Respiratory Diseases, University of Genoa, Genoa, Italy

To cite this article: Melioli G, Marcomini L, Agazzi A, Bazurro G, Tosca M, Rossi GA, Minale P, Rossi R, Reggiardo G, Canonica GW, Passalacqua G. The IgE repertoire in children and adolescents resolved at component level: A cross-sectional study. *Pediatr Allergy Immunol* 2011; Doi: 10.1111/j.1399-3038.2011.01228.x

Keywords

IgE repertoire; microarray; molecular diagnosis; paediatric patients; sensitization.

Correspondence

Giovanni Passalacqua, Allergy and Respiratory Diseases, University of Genoa, Padiglione Maragliano, L.go R.Benzi 10, 16132 Genoa, Italy.
Tel.: +390103538933
Fax: +390103538904
E-mail: passalacqua@unige.it

Accepted for publication 24 September 2011

DOI:10.1111/j.1399-3038.2011.01228.x

Abstract

Background: It is well known that allergy evolves at clinical level from the birth to adulthood, and this has been clearly demonstrated also at a level of sensitization. However, little information is available on the evolution of the IgE repertoire directed to single allergenic components. In this cross-sectional, observational study, the evolution of the IgE repertoire was analysed at component level.

Methods: Serum samples from 901 allergic patients, stratified in 6 groups according to age, were analysed by ImmunoCAP ISAC, a microarray chip that allows to identify the presence of specific IgE towards 103 different allergen components. Total IgE were also evaluated.

Results: The behaviour of total IgE according to age strictly paralleled that of the sum of specific IgE directed to molecular components. As expected, food-related components (in particular those of milk and egg) were the most frequently recognized in the earliest ages, whereas specific IgE to plant allergens appeared invariably later. Nonetheless, IgE specific to mite components was the most represented in all age classes. Of note, specific IgE against cross-reacting allergens was virtually absent in the first years and tended to appear only after the age of 6.

Conclusion: Despite this was not a study performed on a cohort of patients followed up from birth to adolescence, the molecular patterns of allergen recognition resulted modified according to age. These findings may support, at molecular level, the clinical features of the allergic march.

It is a common notion that allergy modifies its characteristics during the life. Symptoms related to the skin as well as to the gastroenteric tract, usually associated with food allergy, are more frequent in the first infancy, while respiratory allergies (rhinitis/asthma) arising from inhalant allergens tend to appear later, in children older than five, up to adolescence (1–3). This phenomenon, called ‘allergic march’, has been partially explained by the evolution of the immune system during the first 3 yr in association with diet changes (4) and exposure to airborne allergens. In adolescents, also hormonal changes may play a role in the natural history of allergy (5).

Despite the numerous studies that followed up children during their first years of life, little is known on the modifica-

tions of the capacity for recognizing allergen components during childhood and puberty. In recent years, highly purified or recombinant allergens have become available in the routine clinical and laboratory practice, thus allowing a diagnostic approach of allergy at component level (6). In parallel, allergen microarrays have been developed. The most common microarray, commercially named ImmunoCAP Solid-phase Allergen Chip (ImmunoCAP ISAC; Phadia, Uppsala, Sweden), allows to detect specific IgE against a large variety (> 100) of molecular components belonging to inhalant, food allergens and hymenoptera (7, 8). These include both species-specific components and cross-reacting molecules (pan-allergens), such as profilins, lipid transfer proteins, calcium binding proteins, storage proteins, tropomyosin and serum

albumins (9). This technique has thus increased the capacity for accurately identifying the allergen components associated with clinical manifestation(s) and, more interestingly, to distinguish true multiple sensitizations from cross-reactions.

It is conceivable that the knowledge of the evolution of IgE sensitizations with time could allow to identify unique characteristics and therefore could imply novel strategies for diagnosis, therapy and follow-up protocols in paediatric and adolescent patients. Aim of our study was to describe the evolution of the IgE repertoire at a molecular level, by means of the ISAC microarray. For this reason, the results obtained during routine detection of specific IgE using the ISAC microarray in a population of children and adolescents were analysed. Of note, ImmunoCAP ISAC was introduced, in the present 103 allergen form, in late 2008; thus, a prospective study in a cohort of patients to be followed up for years was not feasible. Therefore, serum samples from a population of patients with allergic symptoms at different ages were used.

Methods

Patients

Serum samples were always obtained from patients with clinical and laboratory (skin prick test and/or specific IgE assay) evidence of allergy, including rhinitis, asthma, conjunctivitis, urticaria and food allergy. Samples were collected by venipuncture from September 2008 to December 2010 and centralized in a reference laboratory to perform the assays. Blood was clotted and serum was obtained by centrifugation. Aliquots were stored frozen at -30°C . Although the results of skin prick tests and specific IgE assays with the whole extractive allergens were available, these were not considered in this study, because the aim of the work was the analysis at component level. For this study, only samples with at least a single positive component were included. Of note, these 'positive' samples represented 45% of the whole population assayed using ISAC. Finally, total serum IgE was considered as an internal control.

Patients were grouped according to usual convention by age as follows:

Group 1: from birth to the end of the 2nd yr (early infancy);

Group 2: from the beginning of the 3rd yr to the end of the 5th yr (preschool age);

Group 3: from the beginning of the 6th yr to the end of the 9th yr (primary school);

Group 4: from the beginning of the 10th yr to the end of the 13th yr (first adolescence and secondary school);

Group 5: from the beginning of the 14th yr to the end of the 17th yr (end of adolescence);

Group 6: 18 yr and older (adults).

Laboratory tests

ISAC tests were performed as previously described (10), following the producer's recommendations (Phadia, Milan, Italy, now ImmunoDiagnostics Division; Thermo-Fisher Sci-

entific, Milan Italy). Briefly, 0.02 ml of serum was incubated on the microchip containing 103 allergen spots. After a 60-min incubation, slides were washed and a monoclonal anti-IgE antiserum labelled with a fluorochrome was added and incubated for 60 min. Slides were washed again and the chips were analysed using a Laser Scan Confocal microarray reader (LuxScan 10K/A; CapitalBio, Beijing, China). Analysis of the results was automatically performed using a Microarray Image Analyser. All samples were identified using a single barcode, and the results were calculated by the software without any intervention of the technicians. All slides were visually scored to detect the presence of defects (e.g. smears of fluorescence or drops interfering with the reading). Only in these cases, a manual correction (or a refusal of the result) was decided. The ISAC score was reported as ISAC Standardized Units (ISU), which ranges from 0 to 100. Data were obtained and grouped by specific software. Calibration and quality control of the assays were performed as described (10). To evaluate the results of the 103 allergen microchip, the following parameters were used: the Total ISAC Score (TIS), calculated as the sum of all 103 scores of the single patient. In addition, the Average ISAC Score (AIS) was calculated dividing TIS by 103. Finally, the Allergen Family ISAC Score (AFIS) was obtained by summing the ISU of the different components within a single family such as mites, trees, grasses, milk, egg, peanut, shrimp, etc. For instance, mite AFIS was the sum of the ISU of Der p1, Der p2, Der f1 and Der f2. To reduce the effects of pan-allergens and an overestimation of AFIS arising out of cross-reactions, the following components were excluded from calculation: Act d 8, Aln g 1, Ara h 8, Cor a 1.0101, Mal d 1; (for PR-10), Cor a 8 (for lipid transfer proteins); Hev b 8, Mer a 1, Ole e 2, Phl p12 (for profilins); Ani s 3, Bla g 7, Pen a 1, Pen i 1, Pen m 1 (for tropomyosins); Can f 3, Equ c 3; (for serum albumins) Gad c 1 (for parvalbumins); Cyn d 1 (for grass group 1); Cry j 1 (for pectate lyases); Phl p 7 (for polcalcins) and Eur m 2.

Total IgE assay was performed on an Immulite 2000 instrument (Siemens Diagnostics, Milano, Italy), using a random access chemiluminescence method. The test was performed according to the producer's recommendations. Results were expressed as IU/ml.

Statistical analysis

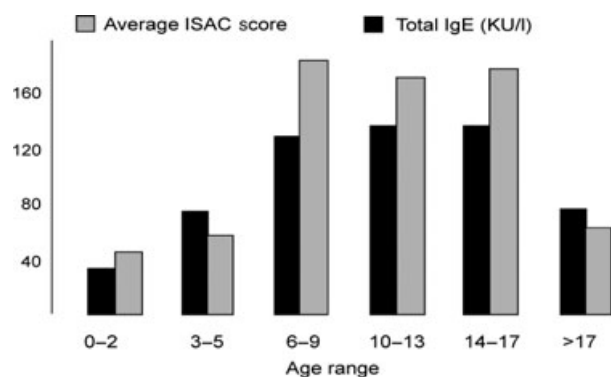
All results were elaborated using a FileMaker Pro 10 (FileMaker, Inc., Santa Clara, CA, USA) relational database and simple statistics (MedCalc Software, Mariakerke, Belgium) and the free statistical software PAST. The correlation between TIS and total IgE per age group was analysed by Spearman's test. The percentage of patients positive to genuine components and pan-allergens among age groups was compared by chi-square test. Age and AFIS were analysed by Mann-Whitney test.

Results

Sera from 901 patients (425 males, age ranging from 1 month to 65 yr) with clinical and laboratory evidence of allergic dis-

Table 1 Distribution of patients by age

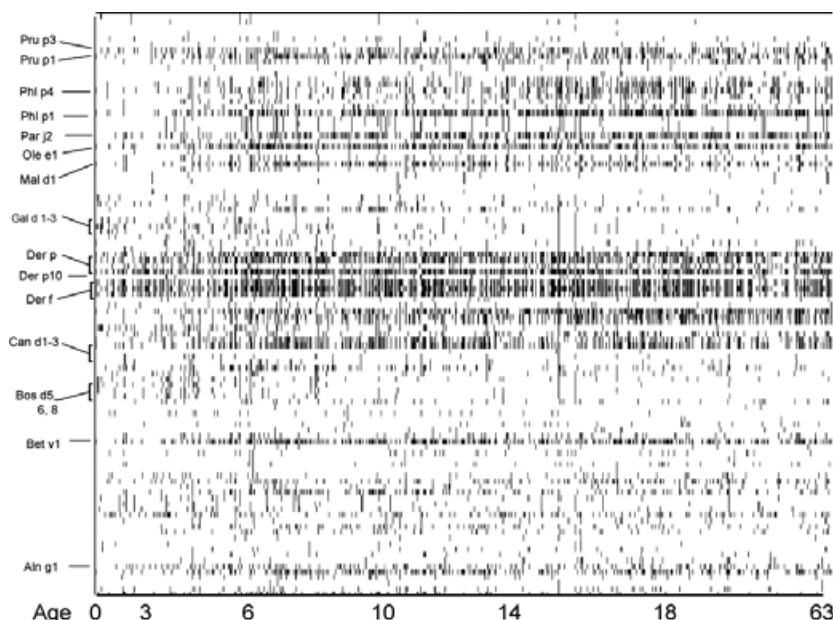
Groups	Age interval	Total patients	Male	Female
Group 1	0–3	48	23	25
Group 2	3–4	122	60	62
Group 3	6–9	164	75	89
Group 4	10–14	165	82	83
Group 5	14–18	112	54	58
Group 6	>18	290	131	159
Total		901	425	476

**Figure 1** Evolution of total IgE during the different the period of life. On the vertical axis, total serum IgE median values in IU/ml (black) and median average ISAC Score (grey) are given.

eases were studied by the ISAC microarray. This technique has been extensively validated (10) and can be considered as highly reliable. The distribution of patients according to their sex and age is reported in Table 1. To evaluate the consistency of the sample, total IgE concentration was also analysed. As expected, low concentrations were detected in early infancy and then a continuous increase was observed, followed by a decrease starting from the age of 18. To support this finding, the time-related evolution of AIS was also calculated. As shown in Fig. 1, the total IgE significantly paralleled the AIS during life (Spearman $r = 0.92$ $p = 0.003$). This represents a suitable quality control of the patients' cohort.

The actual evolution of the IgE against the different allergen components during life is plotted in Fig. 2, where horizontal axis represents the 901 patients sorted by age and the vertical axis represents the 103 allergens assayed. It is apparent that during the first years of life, the number and intensity of the IgE-allergen reactions, as measured by the ISAC score, are lower than in the subsequent analysed periods. This was confirmed by calculating the number of negative allergens per patient during the different periods. The number of negative allergens (i.e. components not recognized by specific IgE) decreased from the first period (70% negative allergens) to the subsequent periods, being 25% in group 2, 13% in group 3, and 8% in adolescents and adults.

IgE specific to foods (in particular milk and egg) was more frequently detected in the first 2 yr, while other allergens, especially inhalants, were more represented in the subsequent periods of life (Table 2). No significant difference existed between the first two age periods (group 1 and group 2),

**Figure 2** The ISAC results in the 901 patients. Patients are sorted by age on the horizontal axis. On the vertical axis, the positivity of specific IgE for the 103 allergen components is given. White spots

represent IgE-specific negative results. Light grey = 0.3–0.9 ISAC Standardized Units (ISU). Grey = 1–14.9 ISU. Black ≥ 15 ISU. The most relevant allergens are indicated on the left.

Table 2 Sensitization to allergen families in the different age groups (absolute number and percentages within each group). Only genuine components were included (see text for the list of excluded pan-allergens)

	Number of positive patients per group						Percentage of positive patients per group					
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Total (N)	48	122	164	165	112	290	48	122	164	165	112	290
Egg	14	22	24	11	6	4	29.2	18.0	14.6	6.7	5.3	1.5
Milk	8	25	21	13	10	13	16.7	20.5	12.8	7.9	8.8	4.4
Mites	19	60	101	104	68	128	39.6	49.2	61.6	63.0	60.2	44.2
Grass	4	27	72	86	72	148	8.3	22.1	43.9	52.1	63.7	50.9
Birch	6	29	70	76	36	102	12.5	23.8	42.7	46.1	31.9	35.1
Parietaria	5	14	52	60	41	111	10.4	11.5	31.7	36.4	36.3	38.3
Plat	1	10	21	20	20	38	2.1	8.2	12.8	12.1	17.7	13.2
Olea	7	29	77	71	47	84	14.6	23.8	47.0	43.0	41.6	29.0
Cypress	1	9	44	51	40	105	2.1	7.4	26.8	30.9	35.4	36.3
Cat	11	30	72	66	41	97	22.9	24.6	43.9	40.0	36.3	33.3
Group 1 vs. 2, 3, 4, 5, 6							16.3 ns	56.8***	84.2***	94.4***	107***	
Group 2 vs. 3, 4, 5, 6								21.6**	40.8***	48.5***	64.2***	
Group 3 vs. 4, 5, 6									5.93 ns	13.6 ns	22.6**	
Group 4 vs. 5 and 6										5.58 ns	8.69 ns	
Group 5 vs. 6											6.5 ns	

Chi-squared comparisons are evidenced in grey. In the first grey row, group 1 is compared to other groups, in the second row group 2 to the other groups, until the 5th grey row comparing group 5 vs. group 6.

ns, not significant.

p < 0.01; *p < 0.001.

while the IgE repertoire of these two groups was significantly different from groups 3, 4, 5 and 6. In addition, concerning the IgE repertoire towards genuine components, group 3, 4, 5 and 6 were homogenous, being not significant in the statistical differences. IgE specific to food was more frequent in children under 6 yr, but mite components represented an exception as they were invariably recognized as the most frequent allergens in all age groups: this is also evident at component level (Table 3). Of note, specific IgE to foods was characterized by low AFIS (with an ISU ranging from 1 to 10), while inhalant allergens involved a stronger immunological reaction, with an AFIS up to 100 ISU for grasses and up to 1000 ISU for mites (Fig. 3). The difference age-AFIS for milk and egg resulted not significant (Mann–Whitney $U = 3836$ and $U = 3980$, respectively; $p > 0.05$), while all the other comparisons resulted highly different, indicating that not only the components of different allergen families sensitize the patients in different period of life, but also that the amount of specific IgE is different.

An interesting characteristic of the evolution of IgE repertoire during the first years of life was the almost complete absence of IgE specific to cross-reacting antigens (profilins, lipid transfer proteins, tropomyosin, etc.) during the first 2 yr of life. These cross-reacting allergens appeared in the IgE repertoire only in the subsequent years, as shown in Table 4. Also for pan-allergens, the first two periods of life were homogenous, and the differences appeared only in the subsequent periods, as observed for genuine allergens. During the

first period of life, food-related cross-reacting components (i.e. tropomyosins and seed allergens) did not belong to the IgE repertoire, despite food allergens are significantly represented in this period. Starting from the second period, novel IgE sensitizations to food-related pan-allergens appeared and then remained unchanged later on.

The number of ISAC positivities (expressed as average AFIS) in the different age clusters increased with age only for inhalant allergens, while for food-related allergens remained stable. Also, the average AFIS and average TIS for inhalants strongly increased with ageing, whereas for food allergens only a modest increase was observed (Fig. 4). The significant differences of the two distributions (Kolmogorov–Smirnov $D = 1$; $p = 0.0013$ and $D = 0.83$; $p = 0.012$, respectively) confirm that this is another specific characteristic of the IgE repertoire changes during ageing. To better define the component specificities of IgE, the most frequent positive ‘genuine’ allergen molecules were studied. As shown in Table 3, components related to mites (Der f 1, Der p 1, Der f 2, Der p 2) were the most frequently recognized molecules. Allergens related to food (Gal d 2 and Bos d 6) were the most represented in the first years. From the second period to adolescence, IgE specific to food-related components were less frequent and tended to disappear, while mite-specific IgE remained. Of note, Der p2 was identified as an IgE-specific genuine component from 44%, 55%, 58% and 71% of patients during the second, third, fourth and fifth periods of life, respectively. After 18 yr, mite-related components

Table 3 The twelve most expressed components in different ranges of age (percentage positive subjects within each group)

Group 1 (%)	Der f 1 (43.75)	Der p 1 (37.5)	Gal d 2 (37.5)	Bos d 6 (31.25)	Der f 2 (31.25)	Der p 2 (31.25)	Equ c 3 (25)	Eur m 2 (25)	Fel d 1 (25)	Bos d 8 (18.75)	Lactof. (18.75)	Gal d 3 (15.2)
Group 2 (%)	Der p 2 (43.9)	Der f 2 (40.9)	Eur m 2 (33.3)	Bet v 1 (30.3)	Der f 1 (30.3)	Der p 1 (30.3)	Ole e 1 (27.3)	Cor a 1.01 (24.2)	Cor a 1.04 (24.2)	Phl p 1 (22.7)	Api m 4 (21.2)	Cor a 9 (21.2)
Group 3 (%)	Der f 2 (55.3)	Der p 2 (54.3)	Ole e 1 (46.8)	Bet v 1 (45.7)	Fel d 1 (45.7)	Eur m 2 (44.7)	Der p 1 (43.6)	Cor a 1.01 (42.6)	Der f 1 (41.5)	Mal d 1 (37.2)	Cup a 1 (36.2)	Cor a 1.04 (34.0)
Group 4 (%)	Der f 2 (57.9)	Der p 2 (56.6)	Der p 1 (48.7)	Eur m 2 (48.7)	Der f 1 (46.1)	Ole e 1 (43.4)	Phl p 1 (43.4)	Bet v 1 (42.1)	Par j 2 (40.8)	Cup a 1 (39.5)	Cor a 1.01 (38.2)	Fel d 1 (38.2)
Group 5 (%)	Der f 2 (71.4)	Der p 2 (71.4)	Der p 1 (62.9)	Eur m 2 (62.9)	Der f 1 (54.3)	Fel d 1 (51.4)	Phl p 1 (48.6)	Ole e 1 (45.7)	Cyn d 1 (42.9)	Par j 2 (31.4)	Bet v 1 (25.7)	Cor a 1.01 (25.7)
Group 6 (%)	Der p 2 (41.0)	Der f 2 (40.6)	Par j 2 (39.0)	Phl p 1 (36.2)	Bet v 1 (35.9)	Fel d 1 (34.0)	Cor a 1.01 (32.4)	Der p 1 (31.1)	Der f 1 (30.2)	Eur m 2 (30.2)	Cup a 1 (28.6)	Aln g 1 (28.3)

become less represented, and specific IgE to Par j 2, Phl p 1 and Bet v 1 increased. The 'genuine' components of Grass (Phl p 1), Parietaria (Par j 2) and Birch (such as Bet v1) were rare before 3 yr.

Discussion

Allergic disorders display a certain clinical evolution during childhood, adolescence and adulthood (1). The progression from atopic dermatitis and food allergy to rhinoconjunctivitis and, finally, asthma, with a partial remission of food-related symptoms is usual, although not mandatory (11), and this phenomenon is known as 'allergic march'. From an immunological viewpoint, this is related to the appearance of sensitizations to food allergens during the first years of life, followed by the onset of sensitization to inhalant allergens. In addition, the number of allergen sensitizations increases from childhood, when mono- or oligo-sensitizations are common, to the adolescence and adulthood, when poly-sensitization is more frequent (12).

The use of specific allergen components, represented by highly purified or recombinant allergens, has deeply improved the accuracy of analysis of sensitization patterns in allergic patients (6, 13). This approach has been further refined with the recent introduction of the ISAC technology, which detects the presence of IgE specific to 103 different components in a single test. In this context, this approach seems to be of relevance in paediatric age, where a so wide analysis would be virtually impossible, especially in newborns and small children, using standard techniques (such as ImmunoCAP with recombinant allergens), because of the large amount of blood required.

By means of this test, we studied the IgE sensitization pattern to the 103 allergens of the ISAC microarray in a large population of allergic subjects, clustered according to their age, to identify the molecular evolution of allergy. The panel of 103 allergens is wide, but does not include all known allergens, and some clinically relevant ones are still missing. In fact, while mites (represented by six both genuine and cross-reacting components) and grasses (represented by nine genuine components and pan-allergens) are suitably covered, Ambrosia and Parietaria (two of the most frequent allergens in our regions) are represented only by a single component (Amb a 1 and Par j 2, respectively). Even if these characteristics could affect in part the quality of the results, some controls were introduced to support the data reliability. In this context, the preliminary analysis of the evolution of total IgE and AIS (a parameter related expressing the average amount of IgE in the ISAC) demonstrated that the group was representative of the already described evolution of IgE in allergic children and adolescents (14–17). Thus, any evidence collected at molecular level should also be representative of this evolution. The main finding was a clear time-related modification of the IgE repertoire at component level. Along this line, the percentage of positive components (representative of the IgE repertoire) during the first period of life was relatively small (20–30% of the 103 ISAC molecules) and this percentage increased during the subsequent periods. It may

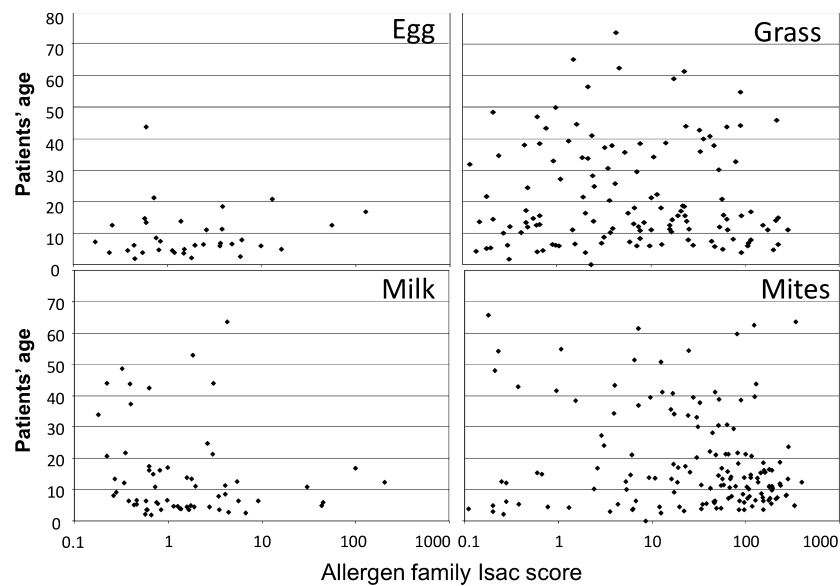


Figure 3 Age-related distribution of Allergen Family ISAC score (AFIS) for different allergenic families: Egg (Gal d 1, Gal d 2, Gal d 3, Gal d 5), Milk (Bos d 4, Bos d 5, Bos d 6, Bos d 8, lactoferrin), Grasses (Phl p 1, Phl p 2, Phl p 4, Phl p 5, Phl p 6, Phl p 7, Phl p 11, Phl p 12, Cyn d 1) and Mites (Der p 1, Der p 2, Der p 10, Der p 1, Der f 2, Eur m 2). On the horizontal axis, the AFIS for each posi-

Table 4 Sensitization to pan-allergens in the age groups (absolute number and percentage within each group)

	Number of positive patients per group						Percentage of positive patients per group					
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Total (N)	48	122	164	165	112	290	48	122	164	165	112	290
Tropomyosin	0	9	22	17	11	13	0.0	7.4	13.4	10.3	9.7	4.5
Profilins	2	9	17	15	16	27	4.2	7.4	10.4	9.1	14.2	9.2
LTP	8	25	36	38	32	63	16.7	20.5	22.0	23.0	28.3	21.6
Pectate-lyase	1	9	47	56	40	111	2.1	7.4	28.7	33.9	35.4	38.3
PR-10	10	37	79	84	46	119	20.8	30.3	48.2	50.9	40.7	41.0
Polcalcins	0	0	5	5	0	9	0.0	0.0	3.0	3.0	0.0	3.1
Parvalbumins	2	9	10	8	3	5	4.2	7.4	6.1	4.8	2.7	1.9
Albumins	11	25	18	14	16	35	22.9	20.5	11.0	8.5	14.2	12.1
Cupins	13	28	34	25	14	12	27.1	23.0	20.7	15.2	12.4	4.3
Group 1 vs. 2, 3, 4, 5, 6							11.8 ns	45.4***	55.6***	50.1***	65.4***	
Group 2 vs. 3, 4, 5, 6							20.1**	29.2***	26.3***	44.3***		
Group 3 vs. 4, 5, 6							2.26 ns	9.91 ns	17.1*			
Group 4 vs. 5 and 6							8.1 ns	9.95 ns				
Group 5 vs. 6							9.79 ns					

Chi-squared comparisons are evidenced in grey. In the first grey row, group 1 is compared to other groups, in the second row group 2 to the other groups, until the 5th grey row comparing group 5 vs. group 6.

ns, not significant.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

be argued that these little patients have IgE directed to components specific to that period of life (such as milk proteins), and the possibility that they never become sensitized to other (inhalant) components during growth cannot be ruled out.

Nevertheless, it should be noted that about 75% of ISAC components are recognized by children aged 3–6, then 88% by children aged 6–10. After 10 yr of age, the percentage of components not recognized remains stable. In these groups

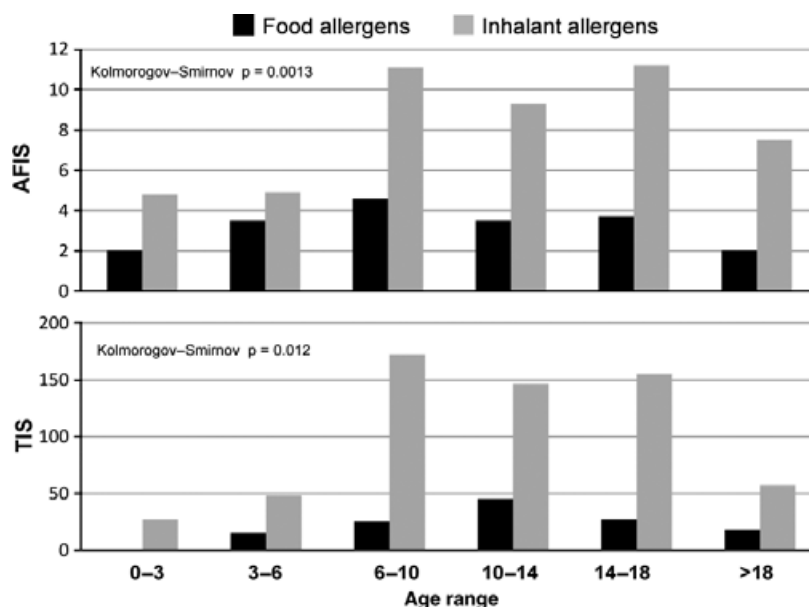


Figure 4 Changes in AFIS and Total ISAC Score (TIS) in the different age intervals. Upper panel: AFIS values for inhalants (black columns) and foods (grey columns). Lower panel: TIS values for inhalants (black columns) and foods (grey columns). The statistical comparison was made using the Kolmogorov-Smirnov test.

of patients, inhalant allergens (such as mite components) are positive in the presence of sensitization (at single patient level) to food allergens. As stated before, this was a retrospective study that did not provide a follow-up of a selected cohort of patients during childhood and adolescence. Thus, no molecular proof of a previous sensitization to food components followed by sensitization to inhalant allergens could be provided. Nevertheless, the presence of IgE specific to either milk or egg components in patients with IgE specific to Der f 2 indicates that a simultaneous food and inhalant sensitization is possible during the second and third period of life. Along this line, it is also likely that the increased possibility to encounter different allergens during ageing and the contemporary maturation of the immune system are responsible for the modification of the IgE repertoire.

Indeed, mites belonging to the environment of newborns and children become the source of the most frequent recognized allergens. In the same early period of life, egg and milk belong to the typical food, while components such as the shrimp (Pen) and the peanut (Ara) families, which in our country are not considered common food in the first year of life, did not represent frequent sensitizations. On the contrary, components associated with inhalant allergy, in particular from plants, tended to appear later (in patients belonging to group 2 and 3) even if airborne allergens belonging to these families are common in the environment since birth, and Parietaria is an almost perennial allergen. These differences could be explained, at least in part, by the large molecular structure of animal-derived components (ranging from 60 to 100 kDa for allergens included in ISAC) in comparison with the small molecular weight (ranging from

15 to 32 kDa) of plant-inhalant components which, frequently, are glycosylated (18). The well-known incapacity of newborn and small children to raise an immune response against polysaccharides could be involved in this phenomenon (19).

In conclusion, the modification of IgE repertoire at component level, from the birth to adolescence, seems to reflect well the clinical characteristics of the allergic march. This fact is sustained by the initial mono- or oligo-sensitization, followed by poly-sensitization during adolescence, the increase in IgE positive components during time, the early sensitization to milk and egg components characterized by a poor number of specific IgE (as shown by the low scores observed), followed, after the third year of life, by sensitization to inhalant allergens belonging to the mite family (in particular Der f 2) and characterized by a high number of specific IgE (as shown by the high scores observed). In addition, cross-reacting components and pan-allergens are negative up to 6–10 yr, becoming positive later on, in terms of number and ISU score of components identified by specific IgE. Furthermore, sensitization towards certain food allergens, represented by component of the Cupin (Ara a1, Ara a3, Ara...) and Tropomyosin (Pen a1, Der p10, etc.) families, appear only after the 6th yr of life. This is the period where sensitization to Grasses (in particular Phl p 1) and birch (Bet v 1) tends to appear. All these features not only support the clinical evidence of the allergic march, but shed some light on the molecular basis of this phenomenon so strictly related to the evolution of the IgE repertoire towards allergen components during childhood and adolescence.

Finally, the results of this work suggest that, at least at component level, a radical change in the IgE repertoire occurs starting from the age of 6, although a certain heterogeneity is present. This cut-off could be also important in the prognostic and therapeutic evaluation of the patient.

Disclosures

Giovanni Melioli, Giovanni Passalacqua, Giorgio W. Canonica and Renato Rossi are members of the Italian Board for ISAC (IBI).

Author's contributions

GM, GWC and GP designed the study, collected and analysed the results and wrote the manuscript. MT, RR, PM, GAR did the clinical work; AA, ER, LM, GB performed the laboratory analyses. GR was the biostatistician. All Authors equally contributed to data collection and to the revision of the manuscript.

References

1. Hahn EL, Bacharier LB. The atopic march: the pattern of allergic disease development in childhood. *Immunol Allergy Clin North Am* 2005; **25**: 231–46.
2. Ker J, Hartert TV. The atopic march: what's the evidence? *Ann Allergy Asthma Immunol* 2009; **103**: 282–9.
3. Tse K, Horner AA. Allergen tolerance versus the allergic march: the hygiene hypothesis revisited. *Curr Allergy Asthma Rep* 2008; **8**: 475–83.
4. Wahn U. What drives the allergic march? *Allergy* 2000; **55**: 591–9.
5. Westergaard T, Begtrup K, Rostgaard K, Krause TG, Benn CS, Melbye M. Reproductive history and allergic rhinitis among 31145 Danish women. *Clin Exp Allergy* 2003; **33**: 301–5.
6. Mari A. When does a protein become an allergen? Searching for a dynamic definition based on most advanced technology tools *Clin Exp Allergy* 2008; **38**: 1089–94.
7. Ferrer M, Sanz ML, Sastre J, et al. Molecular diagnosis in allergology: application of the microarray technique. *J Investig Allergol Clin Immunol* 2009; **19** (Suppl 1): 19–24.
8. Scala E, Alessandri C, Bernardi ML, et al. Cross-sectional survey on immunoglobulin E reactivity in 23,077 subjects using an allergenic molecule-based microarray detection system. *Clin Exp Allergy* 2010; **40**: 911–21.
9. Bonds RS, Midoro-Horiuti T, Goldblum R. A structural basis for food allergy: the role of cross-reactivity. *Curr Opin Allergy Clin Immunol* 2008; **8**: 82–6.
10. Melioli G, Bonifazi F, Bonini S, et al. The ImmunoCAP ISAC molecular allergology approach in adult multi-sensitized Italian patients with respiratory symptoms. *Clin Biochem* 2011; **44**: 1005–11.
11. Barberio G, Pajno G, Vita D, Caminiti L, Passalacqua G. Does a reverse atopic march exist? *Allergy* 2008; **63**: 1630–2.
12. Marogna M, Massolo A, Berra D, et al. The type of sensitizing allergen can affect the evolution of respiratory allergy. *Allergy* 2006; **61**: 1209–15.
13. Sastre J. Molecular diagnosis in allergy. *Clin Exp Allergy* 2010; **30**: 1–15.
14. Gergen PJ, Arbes SJ Jr, Calatroni A, Mitchell HE, Zeldin DC. Total IgE levels and asthma prevalence in the US population: results from the National Health and Nutrition Examination Survey 2005–2006. *J Allergy Clin Immunol* 2009; **124**: 447–53.
15. Perkin MR, Strachan DP, He W, Lack G, Golding J; ALSPAC Study Team. The predictive value of early life total immunoglobulin E measurement in identifying atopic children in a population-based birth cohort study. *Pediatr Allergy Immunol* 2006; **17**: 118–24.
16. Bruno G, Cantani A, Ragno V, Milita O, Ziruolo G, Businco L. Natural history of IgE antibodies in children at risk for atopy. *Ann Allergy Asthma Immunol* 1995; **74**: 431–6.
17. Mediaty A, Neuber K. Total and specific serum IgE decreases with age in patients with allergic rhinitis, asthma and insect allergy but not in patients with atopic dermatitis. *Immun Ageing* 2005; **2**: 9.
18. Altmann F. The role of protein glycosylation in allergy. *Int Arch Allergy Immunol* 2007; **142**: 99–115.
19. Gil A, Rueda R. Interaction of early diet and the development of the immune system. *Nutr Res Rev* 2002; **15**: 263–92.