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Evolution of the IgE and IgG repertoire to a comprehensive array of allergen molecules in the first decade of life.

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ABSTRACT

Background: In early childhood, the allergen-specific IgG repertoire is mainly directed to animal and vegetable food molecules and infrequently to airborne molecules. It is unknown whether this early pattern is maintained throughout childhood.

Objective: To investigate the evolution of IgG and IgE responses to a broad panel of allergenic molecules from birth to age 10yrs.

Methods: We examined the sera collected between birth and age 10yrs from participants in the German Multicentre Allergy Study, a birth cohort born in 1990. The IgE (cut-off ≥ 0.30 ISU) and IgG (cut-off ≥ 0.10 ISU) responses to 35 genuine allergenic molecules were measured with a multiplex microarray approach (ImmunoCAP ISAC™).

Results: IgE responses were mostly directed against a restricted group of airborne molecules, with a sequence and prevalence hierarchy (Phl p 1 > Bet v 1 > Fel d 1 > Phl p 5 > Der p 2 > Der p 1) largely maintained over time. Conversely, the IgG repertoire was much broader, starting with animal foodborne, then spreading to vegetable foodborne and finally to airborne molecules. A strong and persistent IgG response to a given airborne molecule almost invariably preceded or accompanied an IgE response to that molecule.

Conclusions: The evolution of IgG and IgE responses throughout childhood differs widely at population level. IgG responses are mostly directed to animal food allergens while IgE responses are dominated by airborne allergens. However, a strong IgG response almost invariably precedes or accompanies the appearance of IgE to the same molecule in specifically sensitized subjects.

Author Keywords

allergy, allergen molecule, birth cohort, children, Immunoglobulin G, Immunoglobulin E, micro-array

Abbreviations

IgG Immunoglobulin G

IgE Immunoglobulin E

Treg regulatory T cell

MAS Multicenter Allergy Study

PR-10 Pathogenesis-related group 10 protein

ISAC ImmunoCAP Solid-Phase Allergen Chip

ISU ISAC Standardized Units

kUA/L Kilounits/liter

CI Confidence interval

Introduction

IgE-mediated type-I-hypersensitivity is the major cause of allergic diseases (1, 2), such as allergic rhinitis, asthma, atopic dermatitis and food allergy. Especially in westernized countries like the USA, up to 30% of the adult population and 40% of the children (3) suffer from allergic symptoms when exposed to indoor, outdoor and/or food allergens. Although allergies have increased significantly over the last decades, it is since the 19th century, that researchers are investigating allergen sources in order to discover the unique properties, turning an innocuous biological substance into the cause of a severe health threat for parts of the population (4). While the contact with pathogenic organisms, namely antigens, usually results in the production of specific IgG antibodies, the immune system of allergic patients produces not only IgG, but also IgE antibodies towards the eliciting allergen. This difference in the immune response draws the attention towards the differential characteristics between antigens and allergens.

Platts-Mills et al. reported that almost all the defined inhalant allergens are small (10-50kDa), stable, and freely soluble proteins, which are rapidly released from airborne particles following uptake into the lung (4). When investigating the effect of airborne particles vs. their soluble allergen fraction on the adaptive immune system, Bacher et al. recently discovered that the response of memory T cells against the soluble fraction, but not the whole particle was strongly increased in allergic patients when compared to healthy subjects (5). This discovery proves the role of solubility for the distinction between antigens and allergens. But physic-chemical properties are not the only factor of importance for the allergenicity of proteins. Also the route and dose of exposure (4, 6), the characteristics of the anatomic contact site (5), as well as the individual's immune status (7) need to be taken into consideration when evaluating the allergenic potential of a source.

One option to obtain information on the immunogenic features of allergens is the analysis of humeral immune responses activated within an allergic reaction. Apart from the previously well-studied IgE-responses (1, 8-15), the evolution of allergen-specific IgG antibodies is here of particular interest as its characteristics may defer from other pathogen-related dynamics. During the past years, allergen-related IgG has been considered a marker of natural exposure (16) and was attributed to be protective (17), rather than part of a pathologic phenomenon (18). Because of their occurrence within the course of and after immunotherapy (19), IgG antibodies are considered to "block" the pathologic function of IgE (20, 21), although there is evidence showing that allergen-specific IgG might also be involved in anaphylactic events in mice (22), the existence of IgG-mediated anaphylaxis in humans is a highly debated topic(23). Various studies have shown that IgG responses towards allergens are more frequent and/or stronger in IgE-sensitized than in non-sensitized subjects (24-28). A characterization of the natural evolution of IgG to PR.10 proteins throughout childhood showed a clear difference between a "default" response (i.e. early, weak, transient and primarily food-related) among healthy children versus a pre-atopic response (i.e. later, strong, persistent and directed primarily towards airborne allergens) in birch-allergic patients (29). Additionally, the corresponding IgG antibodies among all children appeared almost invariably before or simultaneously with the IgE response. When expanding the set of analysed allergens, the IgG

prevalence could be clearly grouped into different categories. While foodborne allergens triggered IgG responses in almost all subjects, airborne molecules did so much less frequently, but evoked rather often an IgE-sensitization (30). This shows that the frequency, route and dose of exposure lead to a distinctly diverging repertoire of allergen-specific IgG antibodies, representing a specific B-cell response. The aim of the present study is to deepen the understanding of this phenomenon through a longitudinal analysis of IgG and IgE profiles to a broad panel of allergenic molecules in childhood.

Methods

Study design and population

The Multicenter Allergy Study (MAS), a prospective observational birth cohort, recruited 1314 of 7609 infants born in 1990, in 6 delivery wards in 5 German cities (Berlin, Düsseldorf, Mainz, Freiburg, and Munich). A detailed description of the stratified sampling scheme and study subjects is given elsewhere (31). The study was approved by the local ethics committees. Each parent gave written informed consent at the time of enrolment. All children were followed-up at ages 1, 3, 6, 12, 18 and 24 months and yearly, within 4 weeks of the child's birthday, from age 3y to age 13y and lastly at age 20y. For the present study, the data of the first decade of life of children with >1 serum sample at age 1y-3y; >2 serum samples at age 5y-7y; a serum sample at age 10y were examined [Figure 1].

Assessment of IgE and IgG antibodies against allergenic molecules

IgE and IgG antibodies to native and recombinant allergenic molecules were tested with a multiplex microarray approach (ImmunoCAP ISAC™-103 and ISAC™-112, TFS, Sweden). The sera were tested undiluted for IgE detection, and diluted 1:50 for IgG detection according to the manufacturer's instructions. Results were expressed in ISAC Standardized Units (ISU) with a cut-off for positivity of 0.3 ISU for IgE and of 0.1 ISU for IgG as previously reported (30). Thirty-five molecules from 16 allergen sources, among the 91 molecules available and comparable in both ISAC™-103 and ISAC™-112, were selected and examined in the present study (see Fig E1 in this article's Online Repository at www.jacionline.org). The exclusion criteria, already validated in a previous study (30), were the following: A) the molecules belonging to sources with irrelevant exposure in Germany (eg, Brazil nut, pellitory, and olive pollen); B) when 2 highly cross-reacting molecules belonging to the same source were tested (eg, Phl p 5 and Phl p 6), one of them was excluded (eg, Phl p 6); C) highly cross-reacting molecules belonging to different categories with different routes of exposure (eg, PR-10 molecules, profilin, and serum albumin) have also been excluded, with the only exception being Bet v 1, which has been used as a marker of sensitization to birch pollen. Overall, analyses at the source level included 35 molecules and 16 sources. A participant was defined as "sensitized" if sIgE level to at least one molecule was ≥ 0.30 ISU for at least one available serum and as "non-sensitized" otherwise.

Statistical Methods

The differences of frequency of general characteristics between participants in the MAS birth cohort included and excluded from the present study were compared by χ^2 test. The prevalence by age of atopic sensitization to the 35 allergen molecules (sIgE ≥ 0.3 ISU) was calculated. The prevalence by age of IgG response (cut-off: ≥ 0.1 ISU), as well as the geometric mean levels of IgG antibodies to each of the 35 allergen molecules, when detectable, were calculated in the whole population, among the IgE sensitized and among the non-sensitized children. The sequence of IgE and IgG responses to the 35 allergen molecules was assessed with the following four procedures: A) frequency of allergen response; B) average age at first detection of allergen response; C) average order of appearance (calculated at individual level) according to temporal sequence in which each molecule-specific is detected; D) average yearly incidence rate. The persistence of IgG response to each molecule was computed as follows: 1) the percentage of positive IgG responses to the molecule, within the sera collected and examined after the onset of the IgG response, has been calculated for each subject; 2) the average percentage has been then calculated within the whole population of subjects. Spearman's rank correlation coefficient was used to evaluate the relationship between prevalence and persistence of the IgG response to each of the 35 molecules. Mann Whitney U test and Fisher exact test were applied to compare the difference in persistence and prevalence, respectively, of the IgG response to each of the airborne molecules between subjects never and ever IgE sensitized to that molecule. Missing values were not considered for statistical analyses. A p-value <0.05 was considered statistically significant. Statistical analyses were performed with R Core Team (2014), version 3.2.3.

Results

Participants

Overall, 104 children (55 males) of the 1314 children recruited in the MAS birth cohort were included in our study [Figure 1]. The included children did not differ significantly from those not included regarding sex, parental education, parental history of allergy, elder siblings, breast-feeding, whereas they had more frequently German nationality, allergic rhinitis and IgE sensitization [Table 1]. Of the 104 included children, 59 were never sensitized (IgE <0.35 kU/L) to all the nine allergen extracts examined and were therefore considered as “never atopic” (see methods for details). [Figure e2]

Evolution of IgE responses to 35 molecules in the first decade of life.

As expected, IgE responses to different molecules ranged from: **A)** frequently detected, of which 13 airborne (rAlt a 1, rBet v 1, nPla a 2, rPhl p 1, rPhl p 2, rPhl p 5, rPhl p 11, rCan f 1, rCan f 2, rFel d 1, rFel d 4, nDer p 1, nDer p 2) and 3 foodborne (nGal d 1, nGal d 2, nAct d 2) [Figure 2]; **B)** sporadically detectable (nBos d 4, nBos d 5, nBos d 8, nBos d lactoferrin, nGal d 3, nAct d 1, nAct d 5, rAna o 2, nAra h 1, nAra h 2, nCor a 9, nSes i 1, rAlt a 6) and **C)** always undetectable (nAra h 3, nGly m 5, nGly m 6, nTri a aA_TI, rTri a 19.0101, rPla a 1) [not shown]. . The prevalence of IgE sensitization to most of the airborne molecules and to nAct d 2 rapidly increased throughout the first decade of life; that to rFel d 4, rCan f 1, rCan f 2 slowly increased until age 7y and plateaued thereafter, while the prevalence of IgE responses to the animal foodborne molecules nGal d 1 and nGal d 2 was higher at

pre-school age and slightly declined at school age. At age 10y, IgE to Phl p 1 and Bet v 1 were the most prevalent responses ($\geq 20\%$), followed by IgE responses to Fel d 1, Phl p 5, nDer p 2, nDer p 1, nPla a 2 (10%-20%), and to rPhl p 2, rAlt a 1, rPhl p 11 and nAct d 2 ($<10\%$) [Figure 2]. Interestingly, a hierarchy was maintained throughout the observation period within the subgroup of *Phleum pratense* molecules, with Phl p 1 being always the most frequently recognized by IgE, followed by Phl p 5, then Phl p 2 and Phl p 11 [Figure 2, Table 2a].

Sequence of IgE and IgG response to molecules

Among the 45 atopic children, only 3 (7%) had an IgE response starting in the first 2 years of life and directed to milk or egg proteins. In most atopic children, the first IgE responses were usually detected around age 5y and were directed to airborne allergens. Twelve molecules (Phl p 1, Bet v 1, Fel d 1, Phl p 5, Alt a 1, Der p 2, Der p 1, Pla a 2, Phl p 2, Phl p 11, Fel d 4, Act d 2) induced an IgE response in more than five IgE-sensitized participants; of these, only one (Act d 2) was vegetable foodborne, while all the others were airborne allergens [Table 2a]. The sequence of IgE sensitization – at population level - to the 12 molecules was similar to their prevalence ranking [Figure 2]. Phl p 1 (17/45, 38%) and Bet v 1 (17/45, 38%) were by far the most frequent molecules observed in a child's first IgE positive serum, the most prevalent ones and the earliest [Table 2a]. At 10 years of age, the prevalence of asthma and/or allergic rhinitis among participants with IgE to airborne molecules (rPhl p 1, rBet v 1, rFel d 1, nDer p 2, nDer p 1) was significantly higher than among non-atopic participants [Figure E3].

By contrast, the first IgG responses were directed to animal foodborne molecules (e.g. Bos d 5, Bos d 8, Bos d 4, Gal d 2), followed by those to vegetable foodborne molecules and last by those to airborne molecules [Table 2b]. This sequence was in general confirmed by different statistical methods and ranking criteria, including age at first appearance, score of the appearance order, average yearly incidence and the frequency of first appearing response [Table 2b].

Prevalence and levels of the IgG antibodies to 35 molecules from 1 to 10 years of age

The prevalence and the levels of IgG antibodies to most animal foodborne molecules were stable and, with the exception of Gal d 3, extremely high during the whole first decade of life [Figure 3a-b]. The prevalence and the levels of the IgG response to vegetable foodborne molecules were more heterogeneous, less stable and lower than those of IgG to animal foodborne [Figure 3c-d]. Some of them remained almost stable (nAct d 2, nAra h 3, nCor a 9, nTri a aA_Tl, rAna o 2), some slightly decreased over the time (nAct d 5, nGly m 5, nGly m 6, rTri a 19.0101) and others (nAct d 1, nAra h 1, nAra h 2, nSes i 1) gradually grew until 10 years of age [Figure 3c]. Finally, the IgG prevalence to all airborne molecules but Pla a 1 showed upward trends [Figure 3e]. Interestingly, the ranking of the prevalence of IgG responses was identical to that of IgE responses when *Phleum pratense* genuine molecules were considered: Phl p 1>Phl p 5>Phl p 2>Phl p 11 [Figure 3e].

At all the follow-up time points, highest, intermediate and lowest IgG levels were observed to animal foodborne [Figure 3b], vegetable foodborne [Figure 3d] and to airborne [Figure 3f] molecules, respectively. However, the levels of IgG to most (5/7) animal foodborne molecules and to 6/13 vegetable food molecules declined with age [Figure 3b,d]. By contrast, the levels of IgG response to most airborne molecules (14/15) significantly increased with age. The ranking of the levels of IgG to the four grass-specific molecules was the following: Phl p 1>Phl p 5>Phl p 2>Phl p 11. The prevalence

and the levels of IgG responses among the 45 IgE-sensitized children [Figure E1,a-f] and among the 59 non-sensitized children [Figure E2,a-f] followed the same trends as in the global population sample.

Relationship between prevalence and persistence of IgG response to individual molecules

The prevalence and the persistence of the IgG responses to individual molecules were positively correlated ($r_s=0.89$) [Figure 4a]. Interestingly, the IgG responses associated to an IgE response to the same molecule had a significantly higher prevalence and persistence than the IgG responses to the same molecules but not associated to an IgE response [Figure 4b].

Discussion

In a birth-cohort study of German children we found a differential evolution of the IgG and IgE responses to a broad set of allergen molecules: 1) IgE responses were mostly directed against a restricted group of airborne molecules, with a sequence and prevalence hierarchy that - at population level - is largely maintained throughout the whole first decade of life: Phl p 1 > Bet v 1 > Feld d 1 > Phl p 5 > Der p 2 > Der p 1; 2) In contrast, the IgG repertoire was much broader, spreading in most children first to animal foodborne molecules, then to vegetable foodborne and finally to airborne molecules; 3) An abnormally strong and persistent IgG response precedes or accompanies an IgE response to the same airborne molecule.

IgE repertoire - The earliest and most frequent IgE responses all recognize airborne sources (grass, birch, cat and mite) and are in this birth cohort strongly related to allergic rhinitis and asthma (29, 32-34). In contrast, the IgE responses induced by animal foodborne molecules were sporadic, declining and mostly transient. This detailed, molecular analysis confirms well-known trends of IgE responses previously examined at source level, i.e. with allergen extracts (35, 36). Our results are consistent with the notion that the lymphoid tissue associated with the gastrointestinal tract (GALT) induces better immune tolerance than the one associated with the nasal and bronchial mucosae (NALT, BALT) (37, 38). In addition, we show a list of “initiators” of the allergic response (39), i.e. those molecules that are able to start an IgE response. A detailed analysis of their characteristics may be useful to ascertain what makes an allergen an “initiator”. Interestingly, their molecular weight are all comprised between 14 and 38 kDa [www.allergome.org], which is consistent with the hypothesis that “strong” allergen molecules are relatively small macromolecules (4). An important, recent study, has experimentally demonstrated that not only a relatively low molecular weight, but also a high molecular solubility is essential to escape the allergy preventing control from T regulatory cells (5). Interestingly, it has been shown that the strong allergenic power of Phl p 1/Phl p 5, Der p 1/Der p 2, Bet v 1 (5) as well as Fel d 1 (40) is linked to their high solubility, a relevant factor for the induction of Th2 responses.

IgG repertoire – Our study is the first reporting a systematic, longitudinal analysis of IgG responses to a broad panel of allergenic molecules throughout the first decade of life. Studies in the eighties had already shown that IgG responses to animal foods are very common in childhood (41). More recent studies separately focused on IgG responses to a few food (27, 42), pet (6) or pollen (25) allergen molecules. Recently, we cross-sectionally examined the IgG response to 91 allergen molecules at 2y of age in 148 participants of the MAS cohort (30). We found in every single child a

physiologic, early and strong IgG response to cow's milk and hen's egg allergen molecules (30). The present longitudinal study confirms and expands our previous cross-sectional study, showing that the different trends of IgG responses to animal food, vegetable food, and airborne molecules are maintained in non-atopic children over the whole first decade of life. Cow's milk and hen's egg molecules act therefore as excellent "antigens" that induce abundant and persistent IgG responses but they can only sporadically function as "allergens" as the above mentioned six airborne molecules. Most of these molecules are made totally free and soluble after ingestion, suggesting that not only intrinsic molecular characteristics (5) but also the route and amount of exposure determines the antigenicity versus allergenicity of the examined molecules. Different allergen exposure and persistence of allergen are causally associated to different prevalence of IgE responses in the population. The exposure to seasonal allergens (e.g. pollen) is minimal, but the prevalence of IgE is the highest [Figure 2]. The exposure to many indoor allergens is perennial (e.g. dust mites) and the respective prevalence of IgE responses is slightly lower than in the case of pollens [Figure 2]. The exposure to animal and vegetable foods is maximal and related to the lowest prevalence of IgE [Figure 2]. Thus, the quantity and the frequency of exposure to allergens seems to be inversely related to allergy and IgE production and directly related to tolerance and IgG production [Figure 3].

"(Pre-)Atopic" IgG response to airborne molecules – We had recently shown that normal children can develop a "default" physiological, weak, transient IgG response to ingested PR-10 molecules (e.g. Mal d 1, Pru p 1, etc.) (29). In the same study, we have also shown that birch allergic children develop a strong and persistent "(Pre-)Atopic" IgG response against inhaled Bet v 1, which precedes or accompanies the synchronous or subsequent IgE response against Bet v 1 itself (29). A similar phenomenon had been also shown in 1979 by Platts-Mills, observing a strong and persistent IgG response to Der p 1 in the nasal secretions of patient with mite-related allergic rhinitis developing IgE responses to Der p 1 itself (25). In the present study, we confirm and expand that observation, showing that a persistent IgG response to an airborne molecule (e.g. Der p 1, Fel d 1, etc.) is invariably associated to an IgE response to that molecule [Figure 4b]. In other words, the present study suggests that the "(Pre-)Atopic" IgG response to allergen airborne molecules is a general phenomenon, not limited to PR-10 molecules. All these studies lead to the hypothesis that the generation of high affinity specific-allergen IgE needs in most cases an intermediary IgG1 phase and sequential class switching ($\mu \rightarrow \gamma_1 \rightarrow \epsilon$) (43, 44). Accordingly, we speculate that B memory cells expressing an IgG1 BCR at high affinity for the allergen molecule is the precursor of the B cell switching to IgE production. It is not to be excluded that the observation of persistent, strong and high affinity IgG responses to an allergy "initiator" molecule in early childhood may be used to predict the incipient onset of an IgE response to the same molecule.

Limitations

We have to acknowledge some limitations of our study. First, the included participants do not represent the whole MAS cohort or the German general population. Therefore, although general conclusions are robust enough, associations concerning individual molecules may be context dependent and less reproducible in different settings. Second, high concentrations of IgG antibodies partially inhibit the binding of IgE antibodies in the ISAC test (45), so that the IgE-Ab levels we measured are probably underestimated. Third, we did not test IgG1 antibodies, as the ISAC kit is not considering this possibility (IgG1 antisera are questioned). Nevertheless, IgG2 and IgG3

responses to allergenic molecules, to our knowledge, are regarded as being irrelevant and the IgG4 represent only less than 5% of the overall signal of these responses (30). Thus, in our opinion, the IgG response is an IgG1 response with a negligible, if any IgG4 component.

Conclusion

In the first decade of life IgE responses start with a restricted number of “initiators” including Phl p 1, Bet v 1, Fel d 1, Der p 2 and Der p 1. In contrast, the IgG repertoire is much broader, spreading in most children first to animal foodborne molecules, then to vegetable foodborne and finally to airborne molecules. IgE responses to airborne molecules are preceded or accompanied by strong and persistent IgG responses to the same molecules.

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- Accepted Article
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Legend of figures

Figure 1

Flow diagram showing the selection of the population subsets used for analysis.

Figure 2

Evolution of the prevalence of IgE responses to 16 allergen molecules from 1 to 10 years of age (n=104). The prevalence of IgE sensitization (≥ 0.3 ISU) to the 16 allergen molecules by age at follow-up is shown. The number of participants examined at each follow-up is reported under the x-axis. NA = not applicable (no blood drawn at this follow-up).

Figure 3

Prevalence and concentration of IgG responses to 35 molecules from 1 to 10 years of age. Prevalence (a, c, e) and level (b, d, f) of IgG responses to 35 allergenic molecules, grouped by their reference category (a-b: animal foodborne; c-d: vegetable foodborne; e-f: airborne) in 104 children of the MAS cohort from 1 up to 10 years of age. For prevalence calculation at each follow-up only participants with available sera were examined (n examined = {50, 81, 84, NA, 96, 91, 97, NA, NA, 104} for the respective follow-up (years)= {1, 2, 3, 4, 5, 6, 7, 8, 9, 10}). IgG levels were evaluated as the geometric mean of detectable IgG responses (cut-off: ≥ 0.1 ISU)

Figure 4

Relationship between prevalence and persistence of IgG response to individual molecules considering (a) all children (n=104); (b) sensitized versus never sensitized children for each specific molecule. The prevalence of IgG response (y-axis) is defined as the proportion of children with at least one IgG response (cut-off for positivity ≥ 0.10 ISU) to that given molecule in the first decade of life. The persistence of IgG response to each molecule (x-axis) was computed as follows: 1) the

percentage of positive IgG responses to the molecule, within the sera collected and examined after the onset of the IgG response, has been calculated for each subject; 2) the average percentage has been then calculated within the whole population of subjects. **a)** Prevalence and persistence of IgG responses were calculated considering 104 children examined with IgG and 35 specific molecules selected; r_s and p represent respectively Spearman correlation and the relative p-value. **b)** Prevalence and persistence of IgG responses were evaluated between sensitized and never sensitized groups, taking into account the sensitization for each specific molecule. In this case, only airborne molecules with at least 5 subjects ever sensitized were considered. P values refer to comparisons of the prevalence and persistence between sensitized vs never sensitized groups for each considered molecule and were calculated with a Fisher exact test and a Mann Whitney U test, respectively. Statistically significant differences were highlighted (* $p < 0.05$ for prevalence, * $p < 0.05$ for persistence).

Table 1

Table 1 - Characteristics of the population sample.

	Included (n=104)*		not Included (n=1210)*		p*
	n	%	n	%	
Males	55	52.9	629	52.0	0.941
Nationality					
German	102	100.0	1097	93.5	0.015
Other origin	0	0.0	76	6.5	
Parental education ($\geq 12y$)	53	52.0	610	52.1	1.000
Older siblings	46	44.2	494	40.8	0.567
Breast-feeding (>1 month)	79	76.0	814	68.5	0.143
Parental history of atopy (at least one parent)	61	58.7	619	51.6	0.203
Clinical outcome					
Wheezing (≥ 1 among 1-10 ys)	46	44.2	411	37.2	0.193
Atopic dermatitis (≥ 1 among 1-10 ys)	38	36.5	351	30.2	0.217
Allergic rhinitis (≥ 1 among 1-10 ys)	56	53.8	388	44.0	0.006
IgE sensitization†	67	64.4	474	39.2	<0.001

* sporadic missing values for a few parameters

*Chi square test is used for comparing frequencies between groups

†at least one serum with sIgE ≥ 0.35 kJ/l to at least one of 9 extracts of *Dermatophagoides pteronyssinus*, cat, dog, grass and birch pollens, milk, egg, soy, wheat.

Table 2

Table 2 - Sequence of IgE (a) and IgG (b) response to molecules in 104 subjects of MAS cohort.

	Sequence ¶	Molecule~	Source	Category*	Total prevalence *		Age of the first detection		Scoring system †		Average yearly incidence (%)	Molecules recognized by IgE (a) or IgG (b) for the first time		
					n	% ₁	mean	SD	mean	95% CI		Alone or in combination‡		Alone
												n	%₂	
a)	1/2	rPhl p 1	Timothy	AIR	29	27.9	5.4	2.1	2.2	1.4 - 3.1	3.2	17	37.8	23.5
	1/2	rBet v 1	Birch	AIR	25	24.0	5.3	2.2	2.0	1.2 - 2.8	2.7	17	37.8	35.3
	3	rFel d 1	Cat	AIR	21	20.2	5.6	2.6	2.8	1.6 - 4.0	2.2	12	26.7	16.7
	4	rPhl p 5	Timothy	AIR	18	17.3	5.7	2.6	2.9	2.1 - 3.8	1.9	6	13.3	16.7
	5	rAlt a 1	Alternaria	AIR	8	7.7	4.9	1.2	1.8	0.7 - 2.8	0.8	6	13.3	16.7
	6	nDer p 2	D. pteron.	AIR	16	15.4	6.0	2.3	2.9	1.8 - 4.0	1.6	7	15.6	42.9
	7	nDer p 1	D. pteron.	AIR	12	11.5	6.0	2.7	3.3	2.0 - 4.6	1.2	4	8.9	0.0
	8	nPla a 2	Plane tree	AIR	13	12.5	8.2	2.2	3.4	1.2 - 5.6	1.3	6	13.3	83.3
	9	rPhl p 2	Timothy	AIR	10	9.6	6.9	2.6	3.7	2.2 - 5.2	1.0	2	4.4	0.0
	10	nAct d 2	Kiwi	VEG	8	7.7	6.0	2.1	3.9	1.5 - 6.5	0.8	2	4.4	0.0
	11	rPhl p 11	Timothy	AIR	8	7.7	6.5	2.4	4.6	3.5 - 5.8	0.8	11	24.4	0.0
	12	rFel d 4	Cat	AIR	5	4.8	5.6	3.2	7.2	2.1 - 12.3	0.5	1	2.2	0.0
b)	1/2	nBos d 5	Milk	ANI	102	98.1	1.6	0.8	1.1	0.9 - 1.4	54.3	101	97.1	0.0
	1/2	nBos d 8	Milk	ANI	104	100.0	1.7	0.7	1.3	0.9 - 1.7	59.8	101	97.1	0.0
	3	nBos d 4	Milk	ANI	101	97.1	1.7	0.8	1.5	1.0 - 2.1	50.0	97	93.3	0.0
	4	nGal d 2	Egg	ANI	102	98.1	1.7	0.9	1.5	1.0 - 2.0	52.3	98	94.2	0.0
	5	nCor a 9	Hazelnut	VEG	100	96.2	1.9	1.3	2.5	1.6 - 3.5	43.7	90	86.5	0.0
	6/7	nBos d lactoferrin	Milk	ANI	104	100.0	2.0	1.2	3.3	2.1 - 4.4	51.0	89	85.6	0.0
	6/7	nGal d 1	Egg	ANI	102	98.1	1.9	1.1	2.8	1.8 - 3.9	47.4	89	85.6	0.0
	8	nTri a aA_TI	Wheat	VEG	100	96.2	2.1	1.7	2.8	1.9 - 3.7	40.2	86	82.7	0.0
	9	nPla a 2	Plane tree	AIR	102	98.1	2.2	1.2	4.8	3.5 - 6.1	42.0	74	71.2	0.0
	10/11/12	nAct d 2	Kiwi	VEG	89	85.6	2.3	1.7	4.5	3.1 - 5.9	25.3	68	65.4	0.0
	10/11/12	nGly m 6	Soy	VEG	96	92.3	2.3	1.9	4.4	2.9 - 5.9	31.9	77	74.0	0.0
	10/11/12	rAna o 2	Cashewnut	VEG	100	96.2	2.4	1.8	5.3	3.8 - 6.7	35.8	71	68.3	0.0
	13	nGal d 3	Egg	ANI	87	83.7	2.8	2.0	8.4	6.5 - 10.4	21.1	49	47.1	0.0
	14	nGly m 5	Soy	VEG	37	35.6	2.9	2.2	8.6	5.3 - 12.0	4.7	23	22.1	0.0
	15	nAct d 5	Kiwi	VEG	42	40.4	3.1	2.0	10.8	7.8 - 13.8	5.6	19	18.3	0.0
	16	nSes i 1	Sesame	VEG	93	89.4	3.1	1.9	9.5	7.7 - 11.4	23.2	45	43.3	0.0
	17	rPhl p 1	Timothy	AIR	101	97.1	3.1	2.3	9.4	7.5 - 11.2	29.1	52	50.0	0.0
	18	nAct d 1	Kiwi	VEG	98	94.2	3.2	2.0	10.9	9.1 - 12.7	26.2	39	37.5	0.0
	19	rCan f 1	Dog	AIR	60	57.7	3.3	2.2	9.9	7.2 - 12.5	9.4	31	29.8	0.0
	20	nAra h 3	Peanut	VEG	65	62.5	3.3	2.2	9.8	7.4 - 12.2	10.8	35	33.7	0.0
	21	rAlt a 1	Alternaria	AIR	66	63.5	3.4	2.3	11.1	8.8 - 13.4	10.9	28	26.9	0.0
	22	rTri a 19.0101	Wheat	VEG	66	63.5	3.5	2.4	11.1	8.6 - 13.6	10.9	31	29.8	0.0
	23	rBet v 1	Birch	AIR	63	60.6	3.5	2.0	12.8	10.4 - 15.1	10.0	22	21.2	0.0
	24	rAlt a 6	Alternaria	AIR	94	90.4	3.6	2.2	12.5	10.6 - 14.4	21.6	33	31.7	0.0
	25	rPla a 1	Plane tree	AIR	56	53.8	3.7	2.7	11.2	8.6 - 13.8	8.1	25	24.0	0.0
	26	nAra h 1	Peanut	VEG	86	82.7	3.9	2.6	12.5	10.3 - 14.7	16.8	35	33.7	0.0
	27	nDer p 1	D.pteron.	AIR	82	78.8	3.9	2.3	12.4	10.3 - 14.4	15.2	29	27.9	0.0
	28	nAra h 2	Peanut	VEG	43	41.3	4.1	2.3	15.5	12.8 - 18.3	5.5	10	9.6	0.0
	29	rPhl p 2	Timothy	AIR	32	30.8	4.3	2.6	15.6	12.1 - 19.1	3.7	9	8.7	0.0
	30	nDer p 2	D.pteron.	AIR	46	44.2	4.3	2.4	15.0	12.0 - 17.9	5.9	13	12.5	0.0
	31	rFel d 1	Cat	AIR	65	62.5	4.6	3.1	13.3	10.7 - 15.8	9.5	22	21.2	0.0
	32	rCan f 2	Dog	AIR	61	58.7	4.6	2.5	14.9	12.4 - 17.5	8.6	16	15.4	0.0
	33	rPhl p 11	Timothy	AIR	22	21.2	4.6	2.7	15.8	11.2 - 20.3	2.4	6	5.8	0.0
	34	rPhl p 5	Timothy	AIR	67	64.4	4.7	2.4	16.5	14.4 - 18.6	9.8	12	11.5	0.0
	35	rFel d 4	Cat	AIR	56	53.8	4.7	2.8	16.6	13.9 - 19.3	7.5	14	13.5	0.0

Data were summarized as numbers (n) and frequencies (%) if they were categorical and as mean and standard deviation (SD) or confidence interval (95 % CI) if quantitative.

¶ The order of sequence is calculated for each molecule as the average of ranks obtained considering all statistical methods separately.

~ Only molecules with at least 5 subjects who had a molecule-specific IgE (panel a) or IgG (panel b) response were considered for this analysis.

* Category label of molecules: ANI, animal food; VEG, vegetable food; AIR, airborne.

*Cut-off for positivity: ≥ 0.30 ISU for IgE (a); ≥ 0.10 ISU for IgG (b).

† Ranking system based on the order of appearance average score calculated according to temporal sequence in which each molecule-specific is detected with IgE (a) or IgG (b).

‡ Percentages calculated on 104 subjects tested to allergenic molecules.

¶ Number and frequency by which a molecule, alone or in combination with other molecules, is recognized by IgE (a) and IgG (b) for the first time.

₂ Percentages calculated on subjects with (a) IgE response (n=45) and (b) IgG response (n=104) to at least one of the 35 tested molecules.

₃ Percentages calculated on the number of subjects whose (a) IgE response and (b) IgG response starts with that specific molecule.

Figure 1

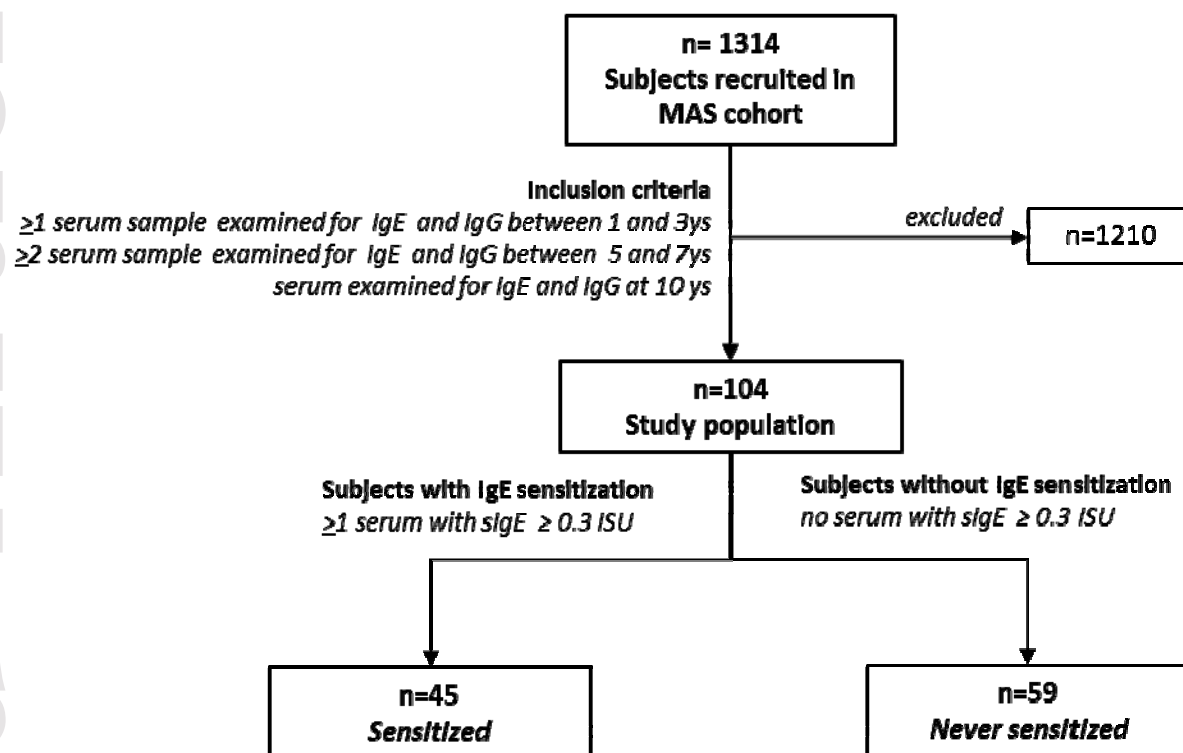


Figure 2

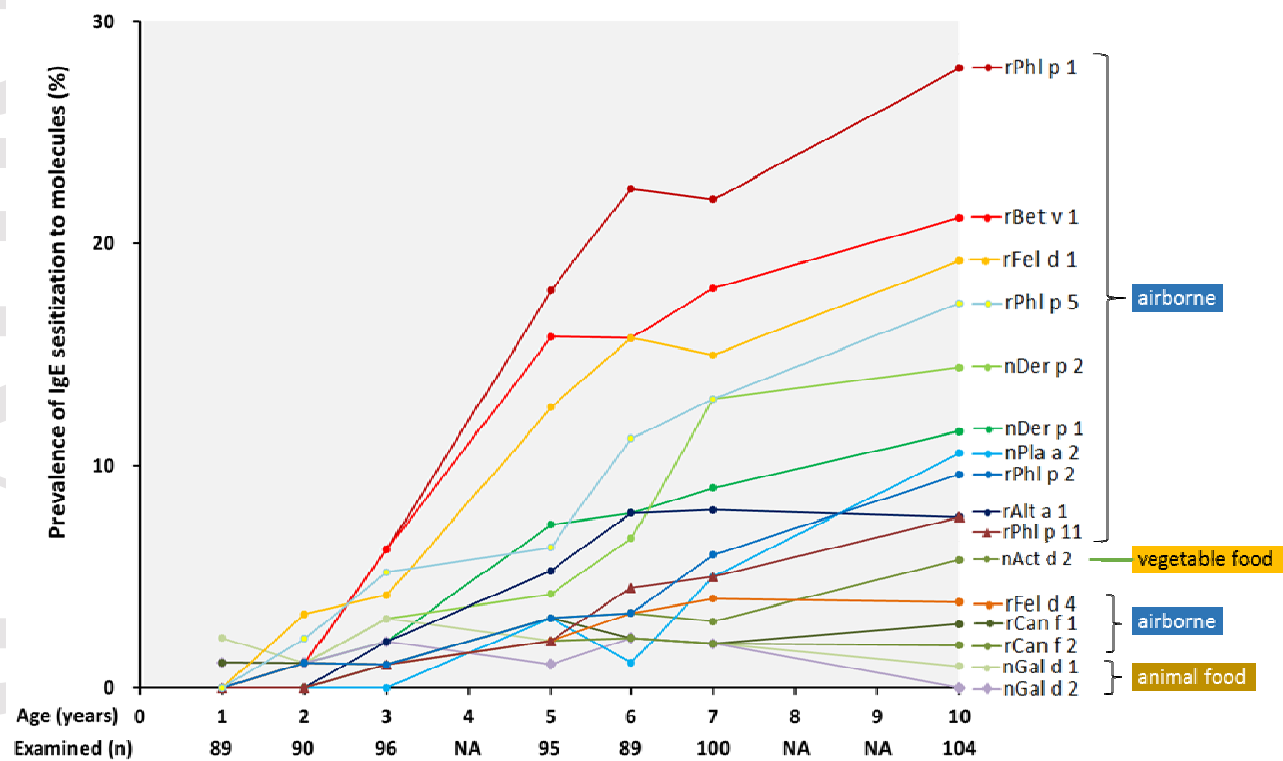


Figure 3

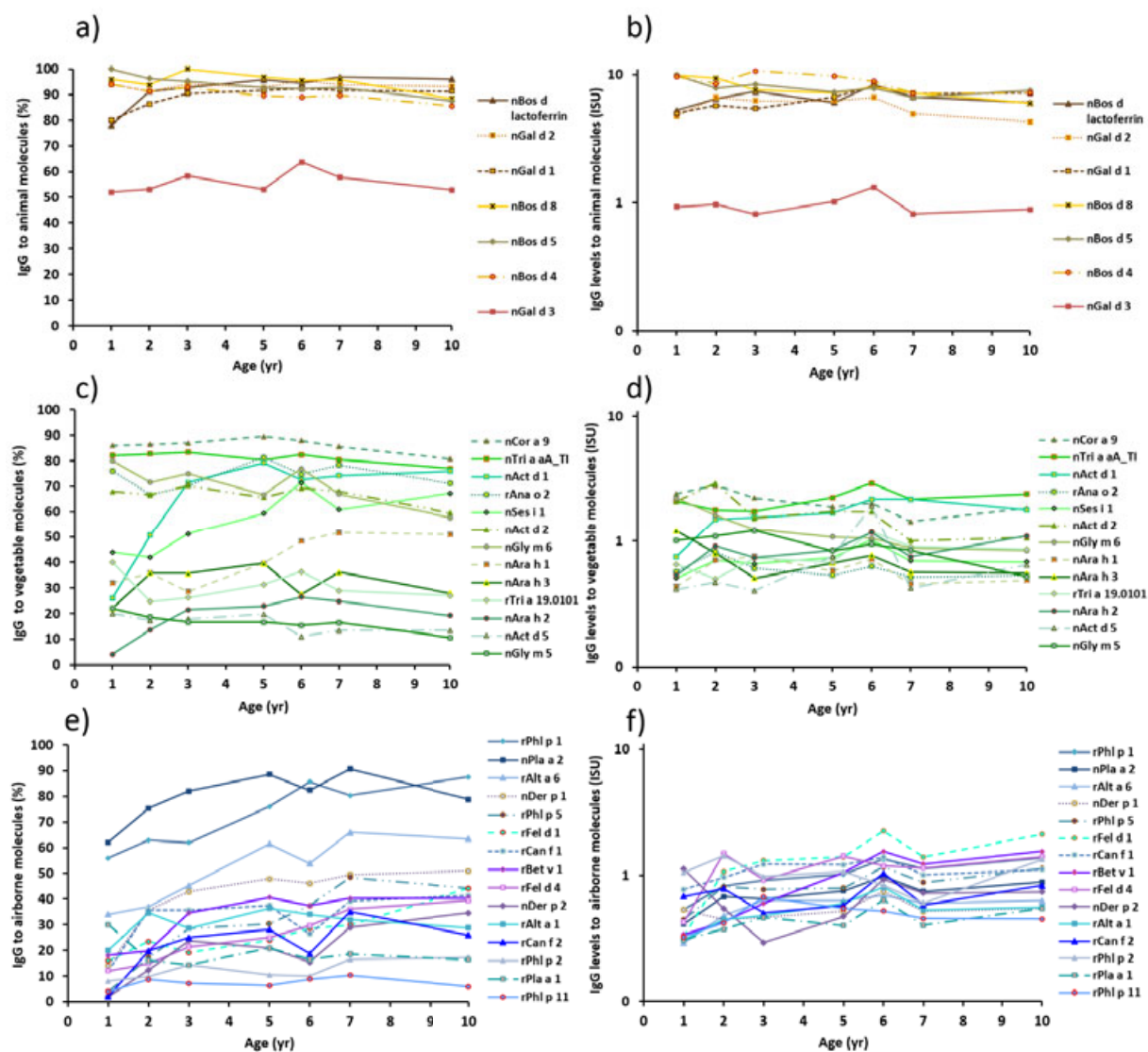
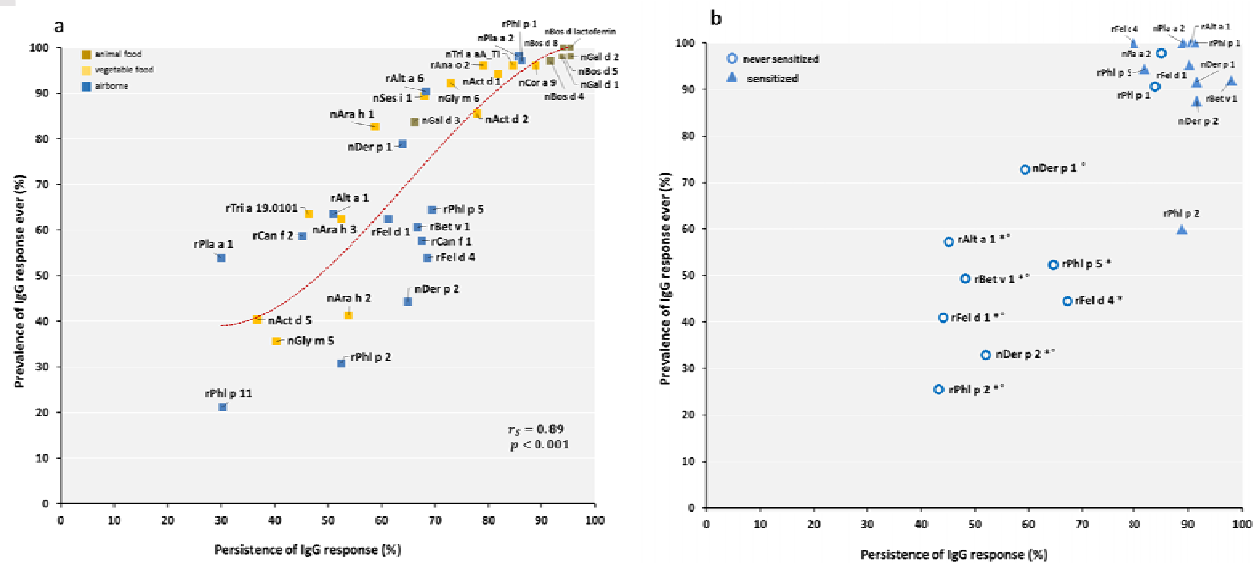


Figure 4



ELECTRONIC REPOSITORY

Evolution of the IgE and IgG repertoire to a comprehensive array of allergen molecules in the first decade of life.

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Figures: 3

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40 **Legend to figures**

41 **Figure S1**

42 Evolution of the prevalence and levels of IgG responses to 35 molecules from 1 to age 10 years in sensitized
43 children, n=45, subjects with IgE positivity (≥ 0.3 ISU) to at least one of the 35 tested molecules. Prevalence of
44 IgG to 35 molecules grouped by their reference category (a-b: animal foodborne; c-d: vegetable foodborne;
45 e-f: airborne). Panels a), c), e) represent the IgG prevalence; panels b), d), f) represent the IgG levels. IgG
46 levels were evaluated as the geometric mean of detectable IgG responses (cut-off: ≥ 0.1 ISU).

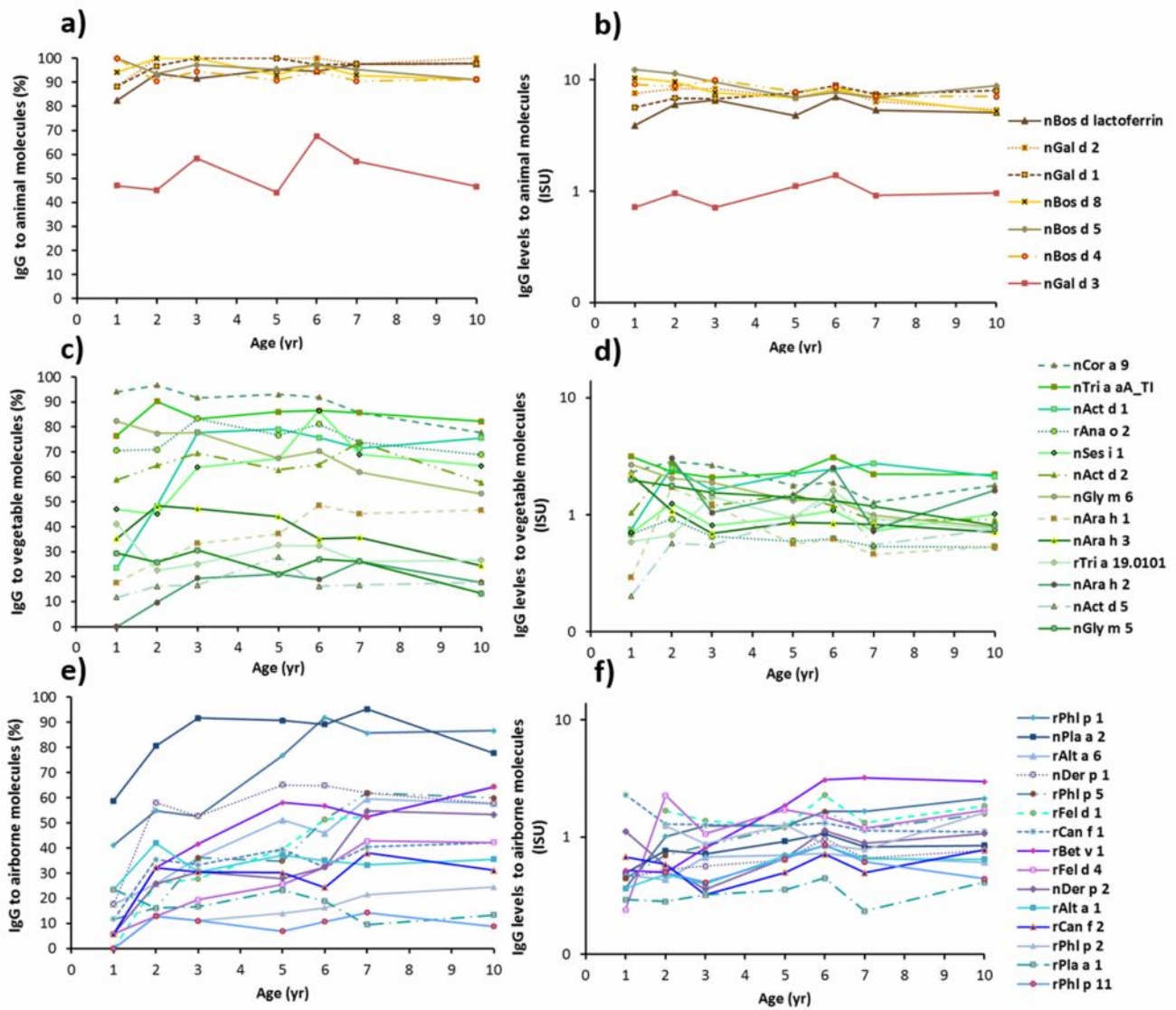
47 **Figure S2**

48 Evolution of the prevalence and levels of IgG responses to 35 molecules from 1 to age 10 years in non-
49 sensitized children, n=59, subjects without IgE positivity (≥ 0.3 ISU) to any of the 35 tested molecules.
50 Prevalence of IgG to 35 molecules grouped by their reference category (a-b: animal foodborne; c-d:
51 vegetable foodborne; e-f: airborne). Panels a), c), e) represent the IgG prevalence; panels b), d), f) represent
52 the IgG levels. IgG levels were evaluated as the geometric mean of detectable IgG responses (cut-off: ≥ 0.1
53 ISU).

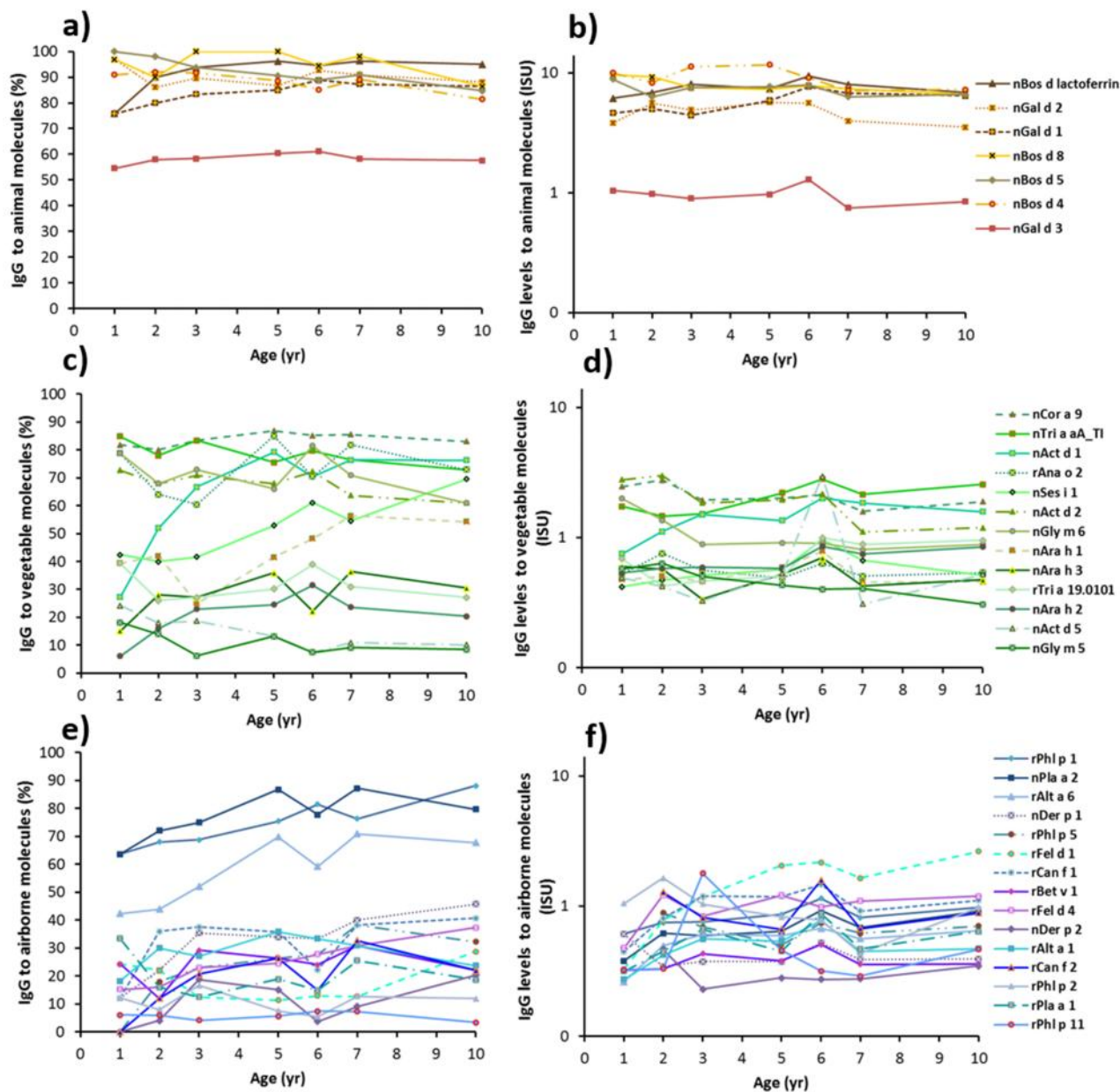
54 **Figure S3**

55 The prevalence of asthma (**a**) and allergic rhinitis (**b**) in participants with (black column) or without (hatched
56 column) serum IgE antibodies to five airborne molecules at 10 years old. Fisher exact test was used to
57 compare frequency between groups. Statistically significant differences were highlighted (* $p < 0.05$,
58 ** $p < 0.01$).

59 Figure S1 Sensitized subjects (n=45)

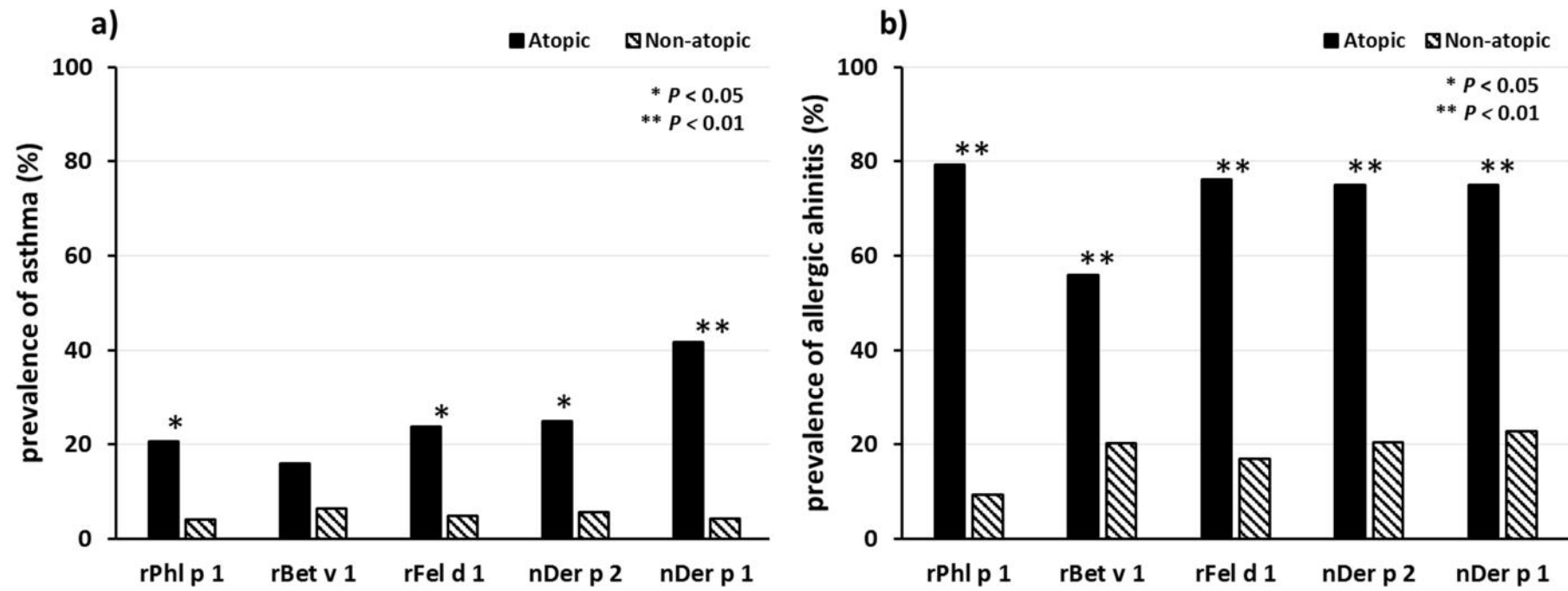


62 **Figure S2 Non-sensitized subjects (n=59)**



63

64 Figure S3
65



66