Evolution and predictive value of IgE responses toward a comprehensive panel of house dust mite allergens during the first 2 decades of life

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Background: The evolution of the IgE response to the numerous allergen molecules of *Dermatophagoides pteronyssinus* is still unknown.

Objectives: We sought to characterize the evolutionary patterns of the IgE response to 12 molecules of *D pteronyssinus* from birth to adulthood and to investigate their determinants and clinical relevance.

Methods: We investigated the clinical data and sera of 722 participants in the German Multicenter Allergy Study, a birth cohort started in 1990. Diagnoses of current allergic rhinitis (AR) related to mite allergy and asthma were based on yearly interviews at the ages of 1 to 13 years and 20 years. IgE to the extract and 12 molecules of *D pteronyssinus* were tested by means of ImmunoCAP and microarray technology, respectively, in sera collected at ages 1, 2, 3, 5, 6, 7, 10, 13, and 20 years. Exposure to mites at age 6 and 18 months was assessed by measuring Der p 1 weight/weight concentration in house dust.

Results: One hundred ninety-one (26.5%) of 722 participants ever had IgE to *D pteronyssinus* extract (≥0.35 kU_A/L). At age 20 years, their IgE recognized most frequently Der p 2, Der p 1, and Der p 23 (group A molecules; prevalence, >40%), followed by Der p 5, Der p 7, Der p 4, and Der p 21 (group B molecules; prevalence, 15% to 30%) and Der p 11, Der p 18, clone 16, Der p 14, and Der p 15 (group C molecules; prevalence, <10%). IgE sensitization started almost invariably with group A molecules and expanded sequentially first to group B and finally to group C molecules. Early IgE sensitization onset, parental hay fever, and higher exposure to mites were associated with a broader polymolecular IgE sensitization pattern. Participants reaching the broadest IgE sensitization stage (ie, ABC) had significantly higher risk of mite-related AR and asthma than unsensitized participants. IgE to Der p 1 or Der p 23 at age 5 years or less predicted asthma at school age.

Conclusions: Parental hay fever and early exposure to *D pteronyssinus* allergens promote IgE polysensitization to several *D pteronyssinus* molecules, which in turn predicts current mite-related AR and current/future asthma. These results might inspire predictive algorithms and prevention strategies against the progression of IgE sensitization to mites toward AR and asthma. (J Allergy Clin Immunol 2016;■■■:■■■-■■■.)

Key words: *House dust mite allergy, recombinant allergens, microarray, allergic rhinitis, asthma, birth cohort, children, component-resolved diagnostics,* Dermatophagoides pteronyssinus*, IgE, prediction*

House dust mites (HDMs) are one of the most prevalent sources of indoor allergens.[1] The assumption that high exposure to HDM allergens during early life facilitates the development of allergic sensitization[2] has been challenged by several studies suggesting that early allergen exposure to other allergen sources (eg, cat[3] and peanut[4]) can induce tolerance. IgE sensitization to HDM contributes to allergic rhinitis (AR) and asthma, as well as atopic dermatitis, and therefore it is an enormous worldwide health and economic burden.[5] *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* are the 2 most important HDM species, with up to 85% of allergic asthmatic children sensitized to either or both of them.[6]

In vitro diagnosis of HDM allergy has long been based on the detection of serum specific IgE antibodies to the whole extract,

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Abbreviations used
AR: Allergic rhinitis
CRD: Component-resolved diagnostics
HDM: House dust mite
ISAAC: International Study of Allergy and Asthma in Childhood
MAR: Mite-related allergic rhinitis
MAS: Multicenter Allergy Study
OR: Odds ratio

which is a "crude, unfractionated mixture of allergenic and non-allergenic proteins, polysaccharides, and lipids obtained by extraction from an allergen source."[7] With the introduction of allergen microarrays, it has become possible to test IgE responses against a large number of natural or recombinant molecules,[8-10] compelling *in vitro* diagnosis of allergy in the era of so-called precision medicine.[11] The detection of IgE against allergenic molecules was named component-resolved diagnostics (CRD)[12] and allows for determining the molecular profile of the patient's IgE sensitization.[7,13,14] Studies in 2 different birth cohorts[15,16] have also shown that in many patients this IgE response increases with time both in serum concentration and in molecular complexity. The sequential development of IgE responses to distinct non–cross-reacting molecules from the same antigenic (allergenic) source may start with an initial first molecule that is recognized and followed by other allergen molecules until stable sensitization profiles are established.[15,17]

More than 20 allergenic molecules of *D pteronyssinus* have been identified and sequenced.[18-20] The "major" allergenic molecules (ie, those causing IgE responses in >50% of the allergic patients) are Der p 1, Der p 2, and Der p 23.[21-23] The IgE responses to 7 *D pteronyssinus* molecules have been investigated in a cross-sectional study of mite-sensitized asthmatic and nonasthmatic children, showing that the former recognize more molecules.[24] Thus far, only the IgE responses to Der p 1 and Der p 2 molecules have been investigated in a birth cohort study, which showed that the current and future risk of asthma is stronger in children with dual sensitization to Der p 1 and Der p 2 compared with those sensitized to only one of them.[16]

The aim of this study is to examine the evolution of the IgE response to a wide array of *D pteronyssinus* molecules, the factors contributing to this evolution, and the implications of this process on the development of allergic symptoms. To this end, we examined the IgE responses to 12 *D pteronyssinus* molecules, factors involved in the molecular sensitization process, and the implications of molecular sensitization profiles on the symptoms of AR and asthma in the participants of the German Multicenter Allergy Study (MAS), a cohort of children born in 1990 and monitored from birth until age 20 years.[25]

METHODS

Study design and population

The MAS, a prospective birth cohort study, recruited a selection of 1314 of 7609 infants born in 1990 on 6 delivery wards in 5 German cities (Berlin, Dusseldorf, Mainz, Freiburg, and Munich).[25] The study was approved by local ethics committees. Each parent provided written informed consent at the time of enrollment. All children were followed up at ages 1, 3, 6, 12, 18, and 24 months and then yearly from the age of 3 to 13 years and then at 20 years of age. In this analysis we included subjects with not more than 3 missing clinical follow-up points between 3 and 20 years of age who had at least 1 serum sample examined for total IgE and specific IgE to *D pteronyssinus*.

Clinical end points

A questionnaire/interview including International Study of Allergy and Asthma in Childhood (ISAAC) questions was used to assess allergic symptoms at each follow-up.[26] Asthma was defined as 2 or more of the following criteria during a single follow-up visit: physician's diagnosis of asthma ever, use of asthma medication in the last 12 months, and any indicative symptom (wheezing, shortness of breath, or dry cough at night) in the last 12 months.[27] AR was defined as reporting, in at least 1 clinical follow-up, sneeze attacks or a runny, blocked, or itchy nose in the absence of the common cold in the last 12 months. The occurrence of AR during October, November, December, or January was used to define mite-related allergic rhinitis (MAR).

IgE assays

Serum samples were obtained from the children at 1, 2, 3, 5, 6, 7, 10, 13, and 20 years of age (see Table E1 in this article's Online Repository at www.jacionline.org). All of the available serum samples were tested for IgE antibodies against the extract of *D pteronyssinus* (ImmunoCAP FEIA: Thermo Fisher Scientific, Uppsala, Sweden), with a detection range 0.35 to 100 kU_A/L. Sera with a specific IgE concentration of greater than 85 kU_A/L were diluted 1:10. A result of 0.35 kU_A/L or greater was considered positive, and the corresponding serum was further tested with a customized microarray (Thermo Fisher Scientific) to detect IgE to Der p 1, Der p 2, Der p 4, Der p 5, Der p 7, Der p 11, Der p 14, Der p 15, Der p 18, Der p 21, Der p 23, and clone 16.[24,28] Details of the major characteristics and distribution in HDM of these molecules are presented in this article in Table E2 in this article's Online Repository at www.jacionline.org. A result of 0.3 ISAC standardized units or greater was considered positive.

Dust sample analysis

At the ages of 6 and 18 months, parents were asked to collect dust samples from carpets in the living room, as previously reported.[29] The relative (weight/weight) content in Der p 1 was then measured with a sandwich ELISA (ALK-Abelló, Copenhagen, Denmark), and the average values ([Value at 6 months + Value at 18 months]/2) expressed in nanograms of Der p 1 per gram of extracted dust were calculated[29,30] and used after $\log_{10}$ transformation for analysis.

Statistical analysis

Study population. Differences between groups were compared by using a χ^2 test for categorical variables, and the Mann-Whitney *U* test was applied for quantitative variables that were not normally distributed. Poisson regression was used to calculate the incidence rate ratio of MAR or asthma between MAS participants included and those excluded from our study population.

Prevalence by age of IgE to *D pteronyssinus* molecules. The prevalence of IgE sensitization (≥0.3 ISAC standardized units) to the 12 *D pteronyssinus* molecules during increasing age and the frequencies of all the different sensitization profiles identified at age 20 years were calculated by using combinatorial analysis.

Sequential IgE sensitization and ABC categorization. The sequence of sensitization to *D pteronyssinus* molecules was assessed with the following 5 procedures: (1) average age at first detection of IgE sensitization; (2) average yearly incidence rate; (3) sensitization-free time calculated with Kaplan-Meier survival curves (participants were censored if they did not have the event of interest); (4) average order of appearance (calculated at individual level) of IgE to each *D pteronyssinus* molecule; and (5) frequency in which a molecule, alone or in combination, induced IgE sensitization first.

Risk factors of molecular spreading. The effects of age and age at IgE sensitization onset on the number of *D pteronyssinus* molecules recognized by IgE were evaluated with Poisson regression by using multilevel mixed effects to take into account repeated measures of the same patients. The effect of allergen exposure and parental hay fever on the number of *D pteronyssinus* molecules recognized by IgE was estimated with a multivariable Poisson regression.

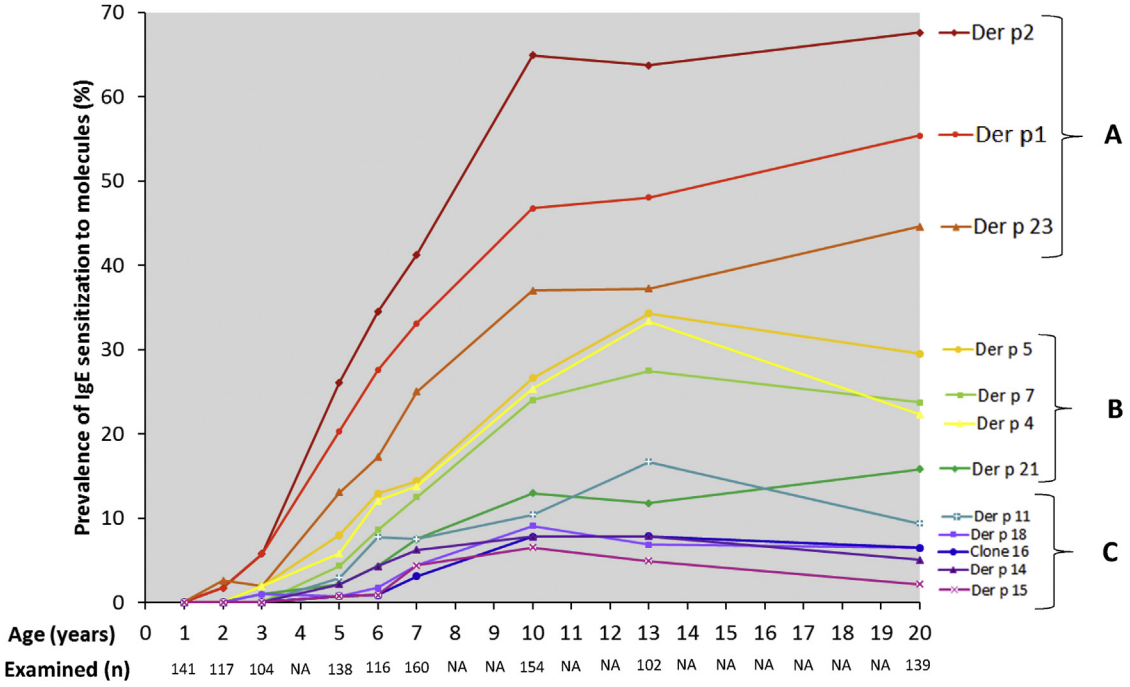

FIG 1. Evolution of IgE responses to 12 *D pteronyssinus* molecules from birth to age 20 years (n = 191). Prevalence of IgE sensitization to the 12 *D pteronyssinus* allergen molecules among the ever mite-sensitized subjects by age at follow-up is shown. A, B, and C indicate groups of molecules according to the prevalence of IgE response at age 20 years. The number of participants examined at each time point is indicated under the *x-axis*.

Clinical relevance of molecular IgE reactivity profiles. Association between trajectories of IgE response to *D pteronyssinus* molecules with MAR and asthma from birth to age 20 years was evaluated by using univariable logistic regression. The effect of IgE sensitization at age 5 years or less on asthma after age 6 years was evaluated with univariable and stepwise multivariable logistic regression analysis after adjusting for relevant confounders. Adjusted odds ratios (ORs) and their 95% CIs were calculated.

Missing values were not considered for statistical analyses. A *P* value of less than .05 was considered statistically significant. Statistical analyses were performed with R software (R Core Team, 2014; version 3.2.3).

RESULTS

Study population

Overall, 722 of the 1314 subjects recruited in the MAS cohort met the inclusion criteria (see Fig E1 in this article's Online Repository at www.jacionline.org) for this study. We found no difference in parental history of hay fever, number of older siblings, and asthma person-year rates between subjects included and excluded from the study. By contrast, German nationality, higher parental education, longer breast-feeding, mother's nonsmoking status during pregnancy, and MAR were more common in the group of participants. Among participants, 35.3% had MAR ever, and 19.7% had asthma, with a person-year rate constantly increasing with age for both MAR (2.3% per year; 95% CI, 2.0-2.6) and asthma (1.1% per year; 95% CI, 1.1-1.3; see Table E3 in this article's Online Repository at www.jacionline.org).

Evolution of the IgE response to *D pteronyssinus* allergen molecules during the first 2 decades of life

There were 191 participants with IgE sensitization to *D pteronyssinus* extract at least once in life (see Fig E1). Among them, the prevalence of IgE sensitization to each of the 12 *D pteronyssinus* allergenic molecules progressively increased among the mite-sensitized children throughout the first decade of life and plateaued thereafter (Fig 1). The ranking of prevalence of the IgE responses to most of the 12 molecules did not change in a relevant manner between 5 and 20 years of age. Three groups of molecules could be defined according to IgE sensitization frequency at 20 years of age (Fig 1):

- group A (Der p 2, Der p 1, and Der p 23): prevalence greater than 40%;
- group B (Der p 5, Der p 7, Der p 4, and Der p 21): prevalence, 15% to 30%; and
- group C (Der p 11, Der p 18, clone 16, Der p 14, and Der p 15): prevalence, less than 10%.

The repertoire of molecules recognized by IgE at 20 years of age was extremely heterogeneous. Among the 119 sera tested with microarray at age 20 years, 48 profiles were identified. Of these, 27 were monomolecular: Der p 2 (n = 15), Der p 23 (n = 7), Der p 1 (n = 4), and Der p 5 (n = 1, see Fig E2 in this article's Online Repository at www.jacionline.org).

Sequential IgE sensitization and ABC categorization

The sequence of IgE sensitization to the 12 *D pteronyssinus* molecules was similar to the prevalence ranking described above (Table I). This sequence was also relatively consistent independent of the 5 methods used for its determination. The molecules first recognized by IgE were Der p 2, Der p 1, and Der p 23 (group A), followed by Der p 5, Der p 4, Der p 7, and Der p 21 (group B) and finally Der p 11, Der p 14, Der p 15, Der p 18, and clone 16 (group C). In the evolution of *D*

TABLE I. Sequence of sensitization to *D pteronyssinus* molecules in the chip-tested population

Sequence	Molecule	IgE sensitization ever*		Age of first detection		Scoring system†			Average yearly incidence (%)	20% survival time (y)‡	Molecules recognized in the first mite-positive serum		
											Alone or in combination§		Alone
		No.	Percent‖	Mean	SD	Mean	SD	95% CI			No.	Percent¶	Percent#
A													
1	Der p 2	147	77.0	8.7	4.3	1.2	0.5	1.1-1.3	7.3	5	119	70.8	40.3
2	Der p 1	117	61.3	8.9	4.7	1.4	0.9	1.3-1.6	5.0	6	86	51.2	26.7
3	Der p 23	97	50.8	9.6	4.9	1.9	1.6	1.5-2.2	3.8	7	63	37.5	27.0
B													
4	Der p 5	71	37.2	10.3	4.9	2.3	1.3	2.0-2.6	2.6	10	28	16.7	3.6
5	Der p 4	70	36.6	10.3	4.7	2.7	1.6	2.3-3.1	2.5	10	24	14.3	4.2
6	Der p 7	59	30.9	10.1	4.6	2.9	1.9	2.4-3.4	2.1	10	21	12.5	4.8
7	Der p 21	43	22.5	11.2	5.2	3.8	2.7	3.0-4.6	1.4	20	13	7.7	0.0
C													
8	Der p 11	42	22.0	10.2	4.9	4.0	2.2	3.3-4.7	1.3	13	10	6.0	0.0
9	Der p 14	34	17.8	9.9	4.5	4.9	2.7	3.9-5.8	1.1	—	7	4.2	0.0
10/11	Der p 15	22	11.5	10.0	4.0	4.9	2.8	3.8-6.1	0.7	—	4	2.4	0.0
10/11	Der p 18	23	12.0	10.1	4.5	5.2	3.1	3.9-6.5	0.7	—	4	2.4	0.0
12	Clone 16	21	11.0	9.6	3.6	4.9	3.3	3.4-6.3	0.7	—	4	2.4	0.0

Data were summarized as numbers and frequencies (percentages) if they were categorical and as means and SDs if they were quantitative.
*Cutoff for positivity ≥ 0.30 ISAC standardized units.
†Ranking system based on the average score calculated according to the temporal sequence in which each *D pteronyssinus* molecule–specific IgE was detected.
‡Ranking system based on Kaplan-Meier estimates: for each molecule, time (in years) of 20% survival probability to remain free from IgE sensitization was evaluated.
§Number and frequency by which a molecule, alone or in combination with other molecules, induces IgE sensitization first.
‖Percentages calculated for 191 subjects tested to allergenic molecules.
¶Percentages calculated for 168 subjects with IgE positivity to at least 1 of the 12 tested molecules.
#Percentages calculated for the number of subjects whose IgE response starts with that specific molecule.

pteronyssinus–specific IgE responses, the most frequently and early recognized molecules were Der p 2 (70.8%, of which 40.3% was alone and 30.5% was in combination with other molecules), followed by Der p 1 (51.2%, of which 26.7% was alone and 24.5% was in combination) and Der p 23 (37.5%, of which 27.0% was alone and 10.5% was in combination), all belonging to group A (Table I).

Molecules of group A > B > C determine the longitudinal sensitization trajectories

Taking advantage of the above-described ABC grouping, we classified all the sera (n = 486) with IgE to 1 or more *D pteronyssinus* molecules according to the 7 possible combinations (A, B, C, AB, AC, BC, and ABC) based on the presence of IgE to 1 or more molecules of the respective groups (see Fig E3 in this article's Online Repository at www.jacionline.org). Interestingly, almost all the sera displayed the patterns A (258 [53.1%]), AB (125 [25.7%]), and ABC (90 [18.5%]). In contrast, those with other combinations were rare exceptions: B (3 [0.6%]), C (1 [0.2%]), BC (0; [0%]), and AC (9 [1.8%]). Thus we rarely found IgE to B molecules in the absence of IgE to A molecules and IgE to C molecules in the absence of IgE to both A and B molecules (see Table E4 in this article's Online Repository at www.jacionline.org).

Then we analyzed IgE sensitization trajectories between their onset and the point of their maximal spreading of each of the 129 participants with at least 2 serum samples with IgE to *D pteronyssinus* molecules (Fig 2). Ninety-four (72.9%) of 129 of the children started their sensitization with IgE to group A only; 48 (51.1%) of them remained in this condition, whereas 35 proceeded to an AB and 11 to an ABC pattern, respectively. Those proceeding to an AB pattern (n = 35) could either stay at that stage (n = 26) or further proceed to an ABC pattern (n = 9). Similarly, children starting with an AB pattern (n = 25) either stayed at that stage (n = 13) or proceeded further to the ABC pattern (n = 12, Fig 2).

Risk factors associated with increases in molecular sensitization profiles

The number of molecules recognized by IgE increased significantly with age in the early years, followed by a plateau. The IgE of children with an earlier onset (age ≤3 years) of sensitization recognized significantly more molecules than that of children with delayed (age 5 years or age >5 years) onset (Fig 3).

The number of molecules recognized by IgE was significantly higher in children with parental hay fever and significantly increased with the level of exposure to Der p 1 at 6 and 18 months of age. After adjusting for exposure to Der p 1, the maximum number of *D pteronyssinus* molecules ever recognized by IgE was significantly higher among children with at least 1 parent with hay fever than in children without parental hay fever (Fig 4).

Clinical relevance of molecular IgE reactivity profiles

The average number of *D pteronyssinus* molecules recognized by IgE antibodies was the lowest in disease-free participants, intermediate in those with MAR only or asthma only, and highest in those with both MAR and asthma (Fig 5, *A*). Throughout the first 2 decades of life, participants reaching

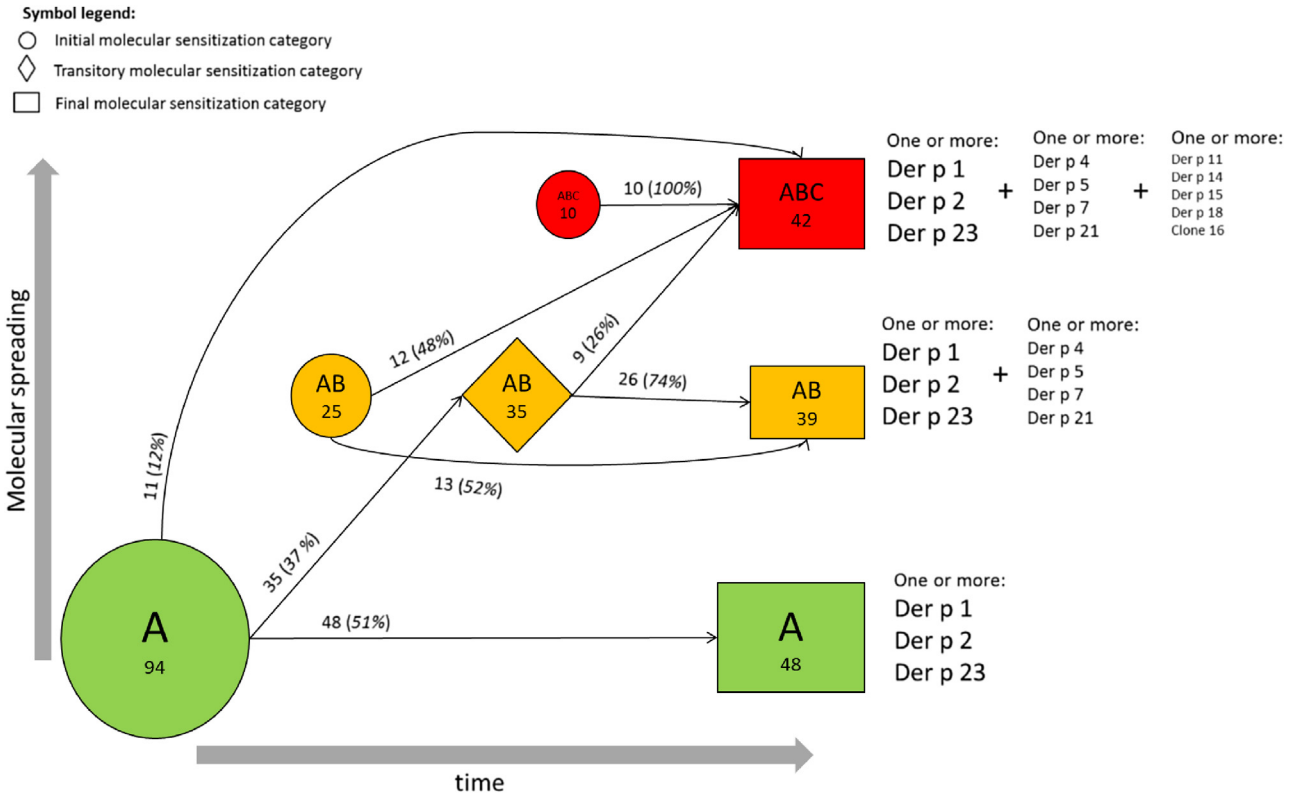

FIG 2. Trajectories of IgE sensitization in mite-sensitized subjects (n = 129). Evolution of the IgE responses to 12 *D pteronyssinus* allergen molecules according to the A, AB, or ABC classification in participants sensitized at 2 or more follow-up points is shown. The *round*, *rhombus*, and *rectangular boxes* represent the initial, intermediate, and final sensitization stages, respectively. Numbers (percentages) refer to participants, and areas are proportional to their frequency.

an ABC stage of IgE sensitization to *D pteronyssinus* had the highest cumulative incidence of MAR and asthma compared with those in groups A and AB and nonsensitized subjects (Fig 5, *B* and *C*). All 3 IgE sensitization patterns were associated with MAR, asthma, or both, but the ABC group had the highest ORs (5.5 [95% CI, 2.9-10.8; $P < .001$] for MAR, 6.1 [95% CI, 3.2-11.5; $P < .001$] for asthma, and 6.9 [95% CI, 3.4-13.6; $P < .001$ for MAR and asthma]; Table II). The association between the ABC group and MAR, asthma, or both MAR and asthma persisted after adjusting for IgE sensitization to cat and/or dog as confounders in the multivariable analysis, and no interaction was observed between IgE sensitization to mite and IgE sensitization to cat and/or dog (data not shown).

A stepwise multivariable logistic regression analysis accounting for relevant confounders showed that early IgE sensitization to any A molecule in healthy preschool children (age, ≤5 years) predicted asthma at school age (≥6 years; OR, 3.2; 95% CI, 1.4-7.0; $P < .001$). In particular, early IgE sensitization to Der p 1 (OR, 3.0; 95% CI, 1.1-8.2; $P = .031$) and Der p 23 (OR, 5.3; 95% CI, 1.5-19.5; $P = .009$), but not Der p 2 (OR, 1.9; 95% CI, 0.7-4.8; $P = .175$), predicted asthma at school age (Table III).

DISCUSSION

Our study is the first to analyze the evolution of the IgE response to a comprehensive panel of 12 HDM allergens in a longitudinal manner during the first 2 decades of life. In more than 700 German participants followed from birth to age 20 years (the MAS birth cohort study), we found that IgE sensitization to individual HDM allergens increases in prevalence and breadth regarding the number of recognized allergen molecules during the first decade of life. During the first decade of life, the IgE response seems to show plasticity, whereas thereafter, stable IgE recognition profiles are established. Children differed regarding the times of onset of IgE sensitizations (≤3 years, 5 years, and >5 years), with a few subjects persistently sensitized to 1 molecule only (mostly Der p 2, infrequently Der p 23, and rarely Der p 1) and others recognizing several of the 12 molecules. Interestingly, the evolution of IgE sensitization followed certain rules and most frequently started with those HDM allergens that are known as major allergens for being recognized by more than 50% of adults with HDM allergy (ie, Der p 1, Der p 2, or Der p 23) and that were included in molecule group A. The second most frequently recognized allergens during the evolution of the IgE response were those we defined as group B of molecules (ie, Der p 4, Der p 5, Der p 7, or Der p 21). These molecules represent quite frequently (approximately 30% of patients with HDM allergy) recognized allergens in adult patients. The allergens of group A and B, which are the most frequently and early recognized allergens in the course of the evolution of the HDM-specific IgE response in childhood share a common feature: they occur in the mite feces, which contain adjuvants or costimulants of T_H2 responses, including endotoxin, chitin, enzymatic activity, and

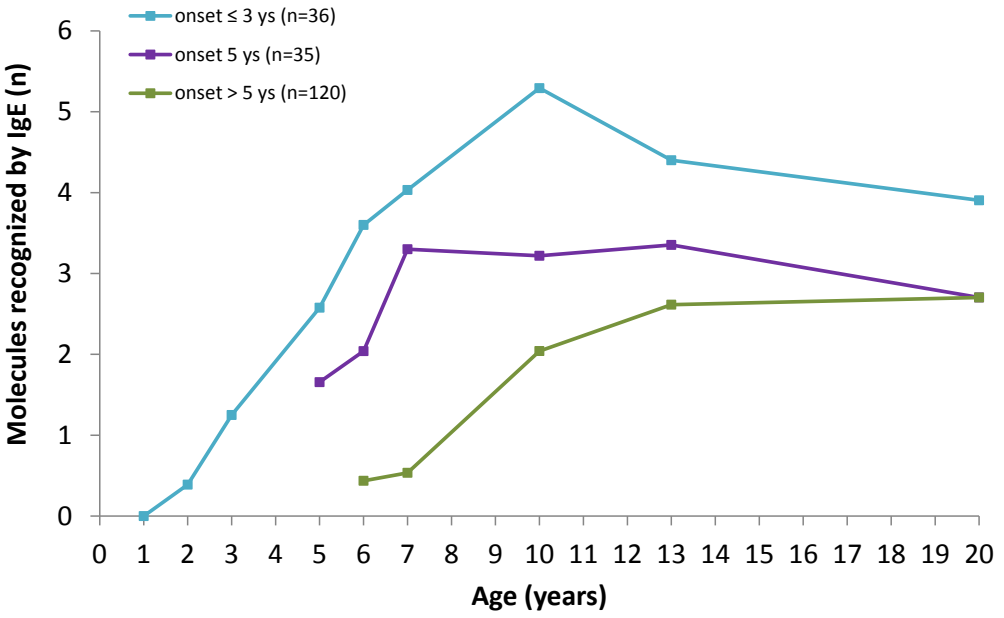

FIG 3. Influence of age and age at sensitization onset on the mite-specific IgE response (n = 191). Average number of all allergen molecules of *D pteronyssinus* recognized by IgE at each follow-up by age at first detection of IgE to *D pteronyssinus* extract is shown. A multilevel mixed-effects Poisson regression was applied. The dependent variable was the number of *D pteronyssinus* molecules recognized by IgE. The independent variables (fixed-effects) were as follows: age (β_{age} 0.55 [95% CI, 0.54-0.55; $P < .001$]) and, assuming a nonlinear relationship, the quadratic term of age (β_{age^2} −0.02 [95% CI, −0.02 to −0.02; $P < .001$]) and the categorical variable of age at first detection of IgE sensitization ($\beta_{onset\ 5\ ys}$ −0.31 [95% CI, −0.31 to −0.31; $P = .001$]) and ($\beta_{onset\ >\ 5\ ys}$ −1.12 [95% CI, −1.12 to −1.12; $P < .001$]). Three years or less at first detection is the reference category.

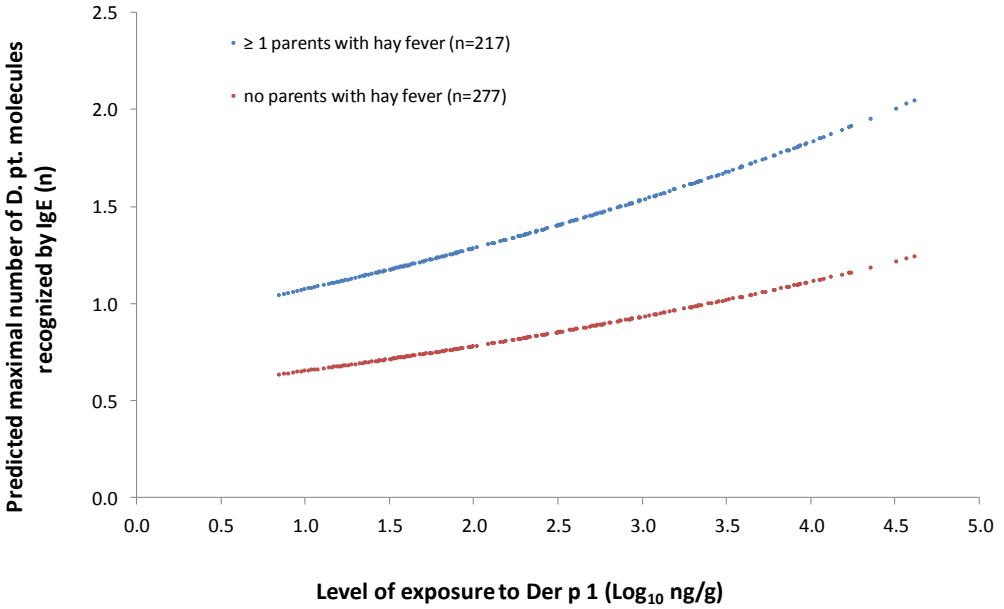

FIG 4. Influence of parental hay fever and early exposure to mite allergens on the mite-specific IgE response (n = 494). Marginal mean of the maximal number of *D pteronyssinus* molecules ever recognized by IgE by level of exposure to Der p 1 and by parental hay fever calculated after a multivariable Poisson regression is shown. The dependent variable was the maximal number of *D pteronyssinus* molecules, and the independent variables were parental hay fever (β_1 regression coefficient = 0.50 [95% CI, 0.32-0.68; $P < .001$]) and exposure to Der p 1 (see the Methods section for definition: $\beta_2 = 0.18$ [95% CI, 0.12-0.24; $P < .001$]). Only subjects with complete information on parental hay fever, mite exposure, and IgE sensitization are included.

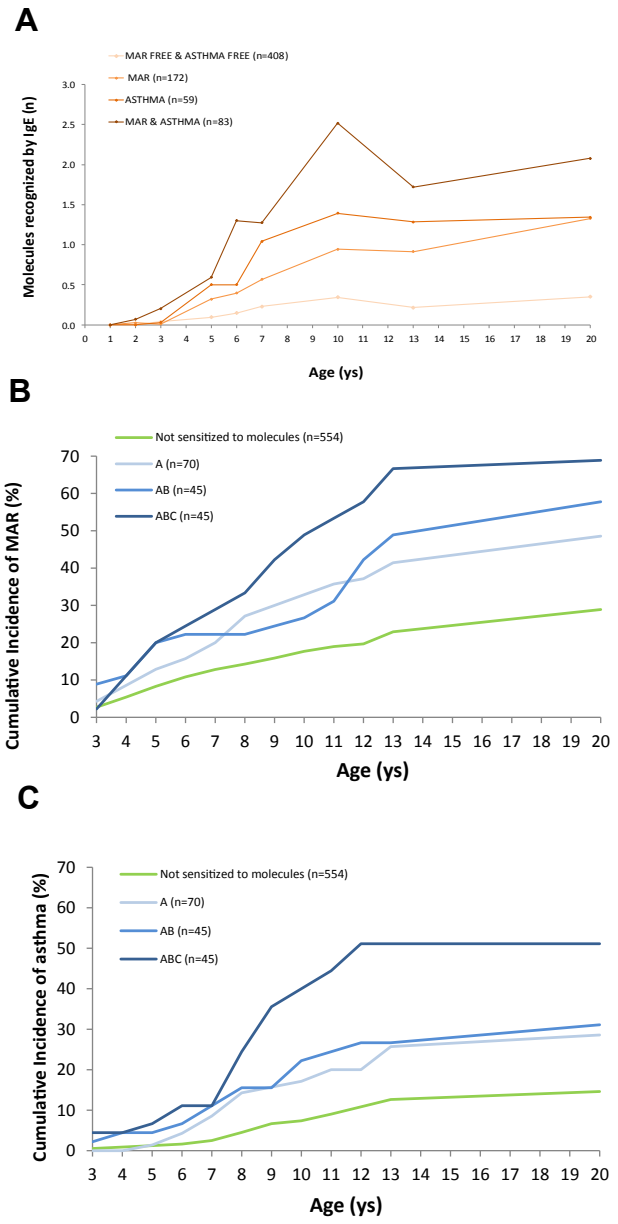

FIG 5. Relationship of spreading of the IgE response to *D pteronyssinus* molecules with MAR and asthma (n = 722). **A,** Average number of *D pteronyssinus* molecules recognized by IgE by age in sensitized but asymptomatic participants and in those with MAR, asthma, or both. **B** and **C,** Cumulative incidence of MAR (Fig 5, *B*) or asthma (Fig 5, *C*) in participants reaching IgE sensitization stages (A, AB, or ABC) between birth and age 20 years. Sporadic cases (n = 8) with other sensitization patterns are excluded from this analysis.

unmethylated DNA, whereas the allergens of group C, which appeared as the last allergens in the evolution of the IgE response (ie, Der p 11, Der p 14, Der p 15, Der p 18, and clone 16) are mainly located in the mite bodies.[31,32]

At present, we can only speculate why Der p 2, Der p 1, and Der p 23 are among the earliest recognized allergens. Der p 2, the earliest recognized allergen, was reported to be similar to MD-2, an LPS-binding protein of the Toll-like receptor signaling complex,[33] and might induce sensitization through a possible adjuvant effect as one of the first allergens. Der p 1 or Der p 2 have been described already as initial molecules involved in HDM sensitization, which is not surprising because these proteins are abundant in HDM.[16] The interesting finding is that Der p 23, a recently cloned major allergenic protein that occurs in HDM in only small amounts,[23,34-36] was among the first recognized allergens in 63 children, and in 17 of them appeared as the only first recognized allergen. This might be because Der p 23 is closely associated with the feces of HDMs, which are major allergen carriers.

It is well known that in addition to genetic factors exposure in early childhood to HDM allergens (Der p 1) is an important determinant of the subsequent development of asthma.[37] Our study expands this knowledge and shows that both atopic predisposition (parental hay fever) and higher exposure to *D pteronyssinus* in infancy strongly facilitate the molecular evolution of the IgE response. Moreover, our data show that the IgE response to *D pteronyssinus* molecules evolves faster and is more diversified in children with early sensitization and in those with both MAR and asthma. Interestingly, early IgE sensitization to Der p 1 and Der p 23, but not Der p 2, was significantly associated with asthma appearing at school age. Moreover, children with asthma recognized more *D pteronyssinus* molecules in the first 2 decades of life than those with MAR only or who were disease free. A recent cross-sectional study has shown that *D pteronyssinus*–specific IgE of asthmatic children recognizes more molecules than that of nonasthmatic children.[24] Similarly, elegant statistical analyses of the Manchester Asthma and Allergy Study (MAAS) birth cohort data have shown that asthmatic children are characterized by more complex molecular patterns of IgE sensitization to grass and mite molecules (Der p 1 and Der p 2).[16] Our results further confirm and expand those observations, showing at a higher level of complexity that asthmatic children usually reach stage ABC of the IgE sensitization to *D pteronyssinus* molecules and that some molecules (eg, Der p 1 and Der p 23) are more relevant then others as predictors of asthma.

As has been observed for grass pollen allergy, children seem to start with an early and often clinically silent sensitization to 1 or a few allergens and then acquire oligomolecular profiles that can progressively increase in complexity[15] until a stable sensitization profile is reached that is linked to symptoms. The fact that IgE responses can be detected often in serum at a preclinical stage before they progress to symptomatic allergy (rhinitis and asthma) is important in helping to define a window for early allergen-specific intervention, such as allergen-specific immunotherapy and allergen avoidance aimed to prevent the progression of IgE sensitization to mite allergen to AR and/or asthma.[17]

In addition to the many time points of serologic assessment, a worldwide unique characteristic of the MAS birth cohort, and in addition to the many interesting findings of potential clinical application, our study has some limitations. We did not test IgE responses to Der p 10, because it is a highly cross-reacting tropomyosin, and to Der p 3, Der p 6, Der p 9, and Der p 30, the clinical relevance of which is low or has not been demonstrated. Moreover, the prevalence rates of IgE sensitization to individual *D pteronyssinus* allergen molecules observed in the MAS cohort might be slightly underestimated because the effect of competing IgG antibodies can be stronger in the microarray (low binding capacity) than in ImmunoCAP (high binding capacity).

Another possible limitation is that other indoor allergens (cat and dog) might have occasionally contributed to AR and asthma

TABLE II. Relationship of trajectories of IgE response to *D pteronyssinus* molecules with MAR and asthma from birth to age 20 years (n = 722)

	Group A (n = 70)			Group AB (n = 45)			Group ABC (n = 45)		
	OR	95% CI	*P* value	OR	95% CI	*P* value	OR	95% CI	*P* value
MAR	2.3	1.4-3.9	.001	3.4	1.8-6.3	<.001	5.5	2.9-10.8	<.001
Asthma	2.3	1.3-4.1	.004	2.6	1.3-5.1	.005	6.1	3.2-11.5	<.001
MAR and asthma	3.1	1.6-6.0	.001	3.1	1.3-6.7	.001	6.9	3.4-13.6	<.001

P values are from univariable logistic regression. The reference category is participants with no IgE sensitization (n = 554). Sporadic cases (n = 8) with other sensitization patterns are excluded from this analysis.

TABLE III. Association of an IgE response to group A *D pteronyssinus* molecules (Der p 1, Der p 2, and Der p 23) at preschool age (≤5 years) with the onset of asthma at school age (6-20 years) in 709 participants of the MAS birth cohort

		Univariable analysis			Multivariable analysis*		
IgE response ≤ 5 y of age	Percent	OR	95% CI	*P* value	OR	95% CI	*P* value
Der p 1 sensitization	4.5	4.4	2.0-9.2	<.001	3.0	1.1-8.2	.031
Der p 2 sensitization	5.4	2.8	1.3-5.5	.005	1.9	0.7-4.8	.175
Der p 23 sensitization	2.8	4.3	1.7-11.0	.002	5.3	1.5-19.5	.009
Group A	6.9	4.0	2.1-7.5	<.001	3.2	1.4-7.0	.004

The dependent variable was at least 1 year with asthma between 6 and 20 years of age in 709 children (binary variable), in particular 580 observations for level 1 and 129 observations for level 2.

*Each line refers to an independent model adjusted for parents with hay fever, exposure, breast-feeding after 1 month, mother smoking during pregnancy, older siblings, and German nationality. The reference category is participants with no IgE sensitization.

symptoms.[38] However, sensitization to HDM was by far the most common type of sensitization in this study population, and the association between an ABC pattern of sensitization and clinical outcomes persisted after adjusting for sensitization to cat, dog, or both.

Furthermore, we acknowledge that all participants were not followed at all given time points and that the MAS biodata banks (partially spoiled by many studies) are limited by a quite consistent amount of missing samples and data, especially in the early follow-up points. However, in most cases IgE sensitization to mites started after 3 years of age (ie, in age groups with enough sera available for our study).

Finally, allergen exposure was considered only at 6 and 18 months of age. However, to our knowledge, no other large birth cohort study on allergies has prospectively collected clinical and above all serologic information at so many points in time (ie, from birth to adulthood) as the MAS birth cohort.

In conclusion, our study is the first to provide information on the evolution of the development of IgE sensitization and symptoms toward a comprehensive set of HDM allergen molecules in the first 2 decades of life. The results might be useful to develop predictive medicine–like algorithms for preventing the progression of sensitization to HDM to childhood allergy.[11,17]

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Clinical implications: Parental hay fever, higher mite allergen exposure in infancy, and early IgE sensitization predict a broader IgE response to *D pteronyssinus* molecules, which in turn predicts current MAR and current and future asthma.

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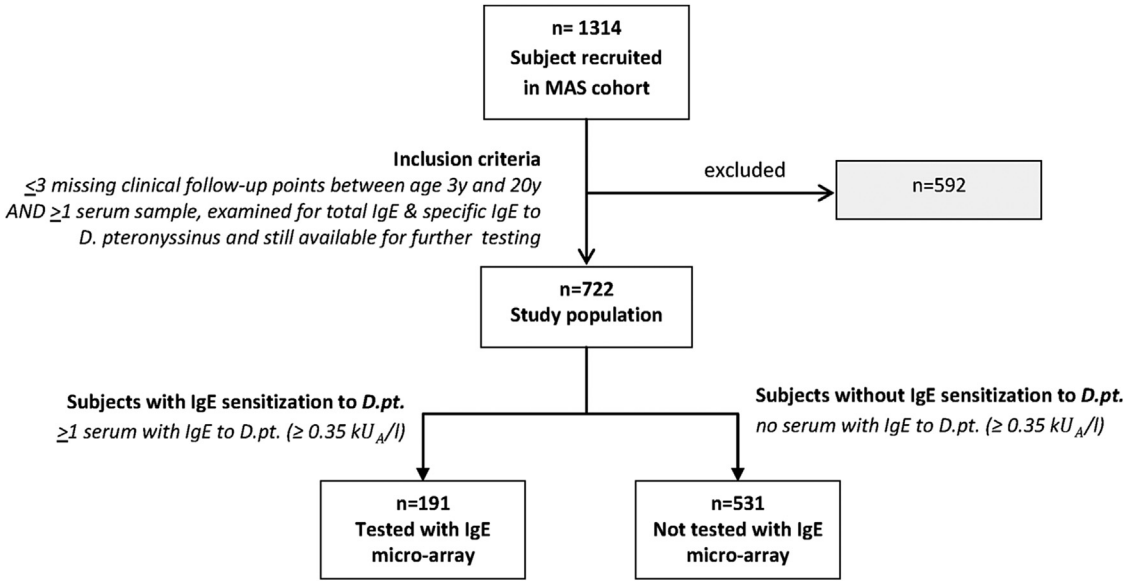

FIG E1. Flow diagram showing selection of the population subsets used for analysis.

Number of molecules	Der p 1	Der p 2	Der p 4	Der p 5	Der p 7	Der p 11	Der p 14	Der p 15	Clone 16	Der p 18	Der p 21	Der p 23	n	%
1		•											15	12.6
2	•	•											10	8.4
0													10	8.4
3	•	•										•	7	5.9
1												•	7	5.9
3	•	•		•									5	4.2
3	•	•	•										4	3.4
2		•										•	4	3.4
1	•												4	3.4
7	•	•	•	•	•						•	•	3	2.5
4	•	•	•									•	3	2.5
4	•	•		•							•		3	2.5
2	•											•	3	2.5
10	•	•	•	•	•	•	•		•		•	•	2	1.7
6	•	•	•	•	•							•	2	1.7
6	•	•	•		•						•	•	2	1.7
5	•	•	•		•							•	2	1.7
5	•	•		•							•	•	2	1.7
4	•	•			•							•	2	1.7
11	•	•	•	•	•	•	•	•	•	•		•	1	0.8
10	•	•	•	•	•	•	•			•	•	•	1	0.8
10	•	•	•	•	•	•	•	•		•		•	1	0.8
9	•	•	•	•	•	•				•	•	•	1	0.8
9	•	•	•	•	•	•	•		•			•	1	0.8
9	•	•	•	•	•	•	•	•				•	1	0.8
8	•	•	•	•	•	•					•	•	1	0.8
8	•	•	•	•	•					•	•	•	1	0.8
8	•	•	•	•	•				•		•	•	1	0.8
8	•	•	•	•	•	•				•		•	1	0.8
7	•	•	•	•	•					•		•	1	0.8
6	•	•	•	•		•						•	1	0.8
6	•	•		•	•						•	•	1	0.8
6	•	•		•	•					•		•	1	0.8
6	•	•		•	•				•			•	1	0.8
6	•	•	•	•	•						•		1	0.8
5	•	•							•	•		•	1	0.8
5	•	•		•	•				•				1	0.8
5	•	•		•					•		•		1	0.8
4	•	•		•								•	1	0.8
4	•	•				•						•	1	0.8
4	•	•		•	•								1	0.8
4	•	•			•	•							1	0.8
4		•		•	•							•	1	0.8
4		•		•							•	•	1	0.8
3		•		•								•	1	0.8
3		•			•							•	1	0.8
3		•									•	•	1	0.8
1				•									1	0.8
													119	100

FIG E2. IgE profiles to 12 *D pteronyssinus* molecules in 119 subjects with an IgE reaction to *D pteronyssinus* at 20 years of age tested with microarray and the complete data set. Absolute frequencies are shown. Profiles are ordered by decreasing frequency.

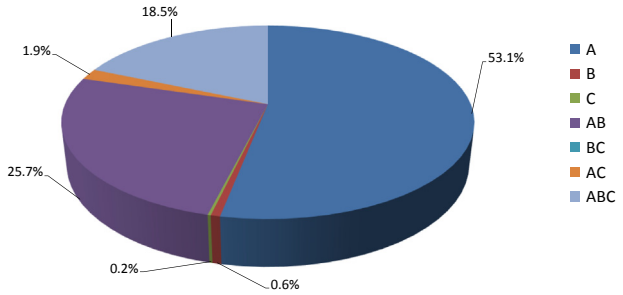

FIG E3. Molecular sensitization profiles (according to the ABC categorization) in sera with IgE to *D pteronyssinus* extract and IgE to 1 or more *D pteronyssinus* allergen molecules (n = 486).

TABLE E1. Sera availability

Variable	Age (y)													
	1	2	3	4	5	6	7	8	9	10	11	12	13	20
Study population	722	722	722	722	722	722	722	722	722	722	722	722	722	722
Questionnaire data	722	722	721	721	722	722	721	722	720	718	721	715	719	633
Sera tested with *D pteronyssinus* extract*	516	464	433	NA	493	407	552	NA	NA	513	NA	NA	550	457
Sera with no IgE to *D pteronyssinus* extract†	515	446	408	NA	437	346	455	NA	NA	392	NA	NA	402	337
Sera with IgE level ≥ 0.35 kU_A/L against *D pteronyssinus* extract	1	18	25	NA	56	61	97	NA	NA	121	NA	NA	148	120
Sera tested with microarray‡	1	8	13	NA	51	60	94	NA	NA	119	NA	NA	81	119

NA, Not applicable (no blood drawn at this follow-up).
*Participants who accepted blood draw at the follow-up.
†IgE to *D pteronyssinus* extract <0.35 kU_A/L on UniCAP.
‡Sera still available at the time of microarray testing.

TABLE E2. List of HDM allergens located on the chip, indicating biological function, molecular weight, localization, and structure

Allergen	Biological function	Molecular weight (kDa)	Localization in feces	PDB ID*	Secondary structures
Der p 1[E1-E3]	Cysteine protease	25	Yes	2AS8	10% α-Helix; 50% β-sheets; 40% random coil
Der p 2[E4-E6]	ML domain lipid-binding protein	14	Yes	1KTJ	10% α-Helix; 50% β-sheets; 15% β-turn; 25% random coil
Der p 4[E7]	α-Amylase	57	Yes	NA	27% α-Helix; 21% β-sheets; 22% β-turn; 31% random coil†
Der p 5[E8,E9]	Unknown	14	Yes	3MQ1	86% α-Helix; 0% β-sheets; 5% β-turn; 8% random coil
Der p 7[E10-E12]	Lipid-binding protein	22	Yes	3H4Z	56.6% α-Helix; 38.3% random coil
Der p 11[E13,E14]	Paramyosin	103	No	NA	Predominantly α-helix
Der p 14[E15]	Apolipophorin	190	No	NA	18% α-Helix; 26% β-sheets; 25% β-turn; 31% random coil‡
Der p 15[E16,E17]	Chitinase	62	No	NA	16% α-Helix; 26% β-sheets; 27% β-turn; 31% random coil†
Der p 18[E16-E18]	Chitinase-like protein	51	No	NA	18% α-Helix; 22% β-sheets; 18% β-turn; 42% random coil†
Der p 21[E19]	Unknown	15	Yes	NA	88% α-Helix; 5% β-sheets; 2% β-turn; 4% random coil
Der p 23[E20-E23]	Peritrophin-like protein	8	Yes	4ZCE	Predominantly β-sheets
Clone 16	Peritrophin-like protein	26	Yes	NA	NA

NA, Data not available.
*http://www.rcsb.org/pdb/home/home.do.
†Resch Y and Vrtala S, unpublished data.
‡Banerjee S and Vrtala S, unpublished data.

TABLE E3. Characteristics of the study population

	Study population (n = 722)*	Excluded (n = 592)*	*P* value†
Male sex (%)	375 (51.9)	309 (52.2)	.926
Parental history of allergy (%)	379 (52.8)	301 (51.5)	.632
German nationality (%)	677 (95.5)	522 (92.2)	.015
Older siblings (%)	297 (41.1)	243 (41.0)	.974
Parental education (>12 y [%])	388 (55.3)	275 (48.2)	<.001
Breast-feeding (≥1 mo [%])	548 (76.6)	345 (59.8)	.011
Mother smoking in pregnancy (%)	149 (20.7)	195 (33.2)	<.001
Clinical outcome			
MAR (%)	255 (35.3)	114 (25.7)	.001
Age at onset (y), median (IQR)	9 (5-13)	9 (4-20)	.568
Incidence (person-year), % (95% CI)	2.3 (2.0-2.6)	1.8 (1.5-2.2)	.045
Asthma (%)	142 (19.7)	67 (14.9)	.036
Age at onset, median (IQR)	9 (8-12)	9 (7-20)	.701
Incidence (person-year), % (95% CI)	1.1 (1.0-1.3)	1.0 (0.8-1.2)	.320

Data were summarized as numbers and frequencies (percentages) if they were categorical and/or medians and interquartile ranges *(IQR)* if they were quantitative.
*Sporadic missing values for each of the variable examined.
†The χ^2 test was used to evaluate the association of categorical data between groups. The Mann-Whitney *U* test was used to compare quantitative but not normally distributed variables between groups (Shapiro-Wilk test was used to assess normality of data). Poisson regression was applied to estimate person-year rates (percentages) and their 95% CIs of MAR and asthma between groups. The corresponding *P* values indicate the statistical significance of the incidence rate ratio.

TABLE E4. Sera tested with microarray classified according to ABC categorization by follow-up

	No. of tested samples	No. of positive samples*		A		B		C		AB		BC		AC		ABC	
Age	No.	No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent
1	1	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
2	8	4	50.0	4	50.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
3	13	8	61.5	5	38.5	0	0.0	0	0.0	2	15.4	0	0.0	1	7.7	0	0.0
5	51	46	90.2	32	62.7	0	0.0	0	0.0	9	17.6	0	0.0	1	2.0	4	7.8
6	60	48	80.0	30	50.0	1	1.7	1	1.7	8	13.3	0	0.0	0	0.0	8	13.3
7	94	85	90.4	52	55.3	1	1.1	0	0.0	18	19.1	0	0.0	1	1.1	13	13.8
10	119	113	95.0	56	47.1	0	0.0	0	0.0	31	26.1	0	0.0	2	1.7	24	20.2
13	81	73	90.1	29	35.8	0	0.0	0	0.0	20	24.7	0	0.0	2	2.5	22	27.2
20	119	109	91.6	50	42.0	1	0.8	0	0.0	37	31.1	0	0.0	2	1.7	19	16.0
Total	546	486	89.0	258	47.3	3	0.5	1	0.2	125	22.9	0	0.0	9	1.6	90	16.5

*IgE ≥ 0.3 ISAC standardized units to at least 1 molecule.