Evolution pathways of IgE responses to grass and mite allergens throughout childhood

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Background: Little is known about longitudinal patterns of the development of IgE to distinct allergen components.
Objective: We sought to investigate the evolution of IgE responses to allergenic components of timothy grass and dust mite during childhood.
Methods: In a population-based birth cohort (n = 1184) we measured IgE responses to 15 components from timothy grass and dust mite in children with available samples at 3 time points (ages 5, 8, and 11 years; n = 235). We designed a nested, 2-stage latent class analysis to identify cross-sectional sensitization patterns at each follow-up and their longitudinal trajectories. We then ascertained the association of longitudinal trajectories with asthma, rhinitis, eczema, and lung function in children with component data for at least 2 time points (n = 534).
Results: Longitudinal latent class analysis revealed 3 grass sensitization trajectories: (1) no/low sensitization; (2) early onset; and (3) late onset. The early-onset trajectory was associated with asthma and diminished lung function, and the late-onset trajectory was associated with rhinitis. Four longitudinal trajectories emerged for mite: (1) no/low sensitization; (2) group 1 allergens; (3) group 2 allergens; and (3) complete mite sensitization. Children in the complete mite sensitization trajectory had the highest odds ratios (ORs) for asthma (OR, 7.15; 95% CI, 3.80-13.44) and were the only group significantly associated with comorbid asthma, rhinitis, and eczema (OR, 5.91; 95% CI, 2.01-17.37). Among children with wheezing, those in the complete mite sensitization trajectory (but not other longitudinal mite trajectories) had significantly higher risk of severe exacerbations (OR, 3.39; 95% CI, 1.62-6.67).
Conclusions: The nature of developmental longitudinal trajectories of IgE responses differed between grass and mite allergen components, with temporal differences (early vs late onset) dominant in grass and diverging patterns of IgE responses (group 1 allergens, group 2 allergens, or both) in mite. Different longitudinal patterns bear different associations with clinical outcomes, which varied by allergen. (J Allergy Clin Immunol 2015;136:1645-52.)

Key words: *IgE, childhood, component-resolved diagnostics, latent class analysis, allergens, dust mite, timothy grass, asthma, wheeze, rhinitis*

Atopic sensitization is a strong risk factor for asthma, rhinoconjunctivitis, and eczema.[1] Sensitization is traditionally defined as a positive allergen-specific serum IgE (sIgE) result or a positive skin prick test response using extracts from whole allergen sources.[2] These standard allergy tests have high sensitivity but relatively low specificity, and a positive test result does not confirm clinical reactivity on allergen exposure.[1] We have previously shown that quantification (ie, using the level of sIgE antibodies or the size of skin prick test–induced wheal) can increase the specificity (both in terms of diagnostic accuracy and the capacity to predict the persistence of symptoms),[3-6] but the problem of a significant number of false-positive test results remains.[7]

One potential problem related to the use of whole allergen extracts is that natural sources used for their preparation contain multiple allergenic proteins (components), and each component can contain multiple epitopes for binding IgE. Because of homology between proteins from the same family present in different allergen sources (eg, profilin and pathogenesis-related class 10 proteins in plants), a positive result might reflect reactivity to cross-reactive components rather than an independent sensitization event.[8] Consequently, interpretation of the tests routinely available in the clinic can be challenging.[7] Recent advances in biochemistry and molecular biology have led to the isolation and characterization of numerous allergen components. Their recombinant production facilitates the profiling of IgE reactivity to individual allergens at the molecular level, and this new approach to allergy diagnosis has been termed molecular diagnosis or component-resolved diagnostics (CRD).[8,9] One such emerging technology is the multiplex Immuno Solid-phase Allergen Chip (ImmunoCAP ISAC), an *in vitro* diagnostic tool that is based exclusively on allergen components. This array measures sIgE levels to more than 100 allergen components and has

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Abbreviations used
ATS: American Thoracic Society
CRD: Component-resolved diagnostics
eNO: Exhaled nitric oxide
FVC: Forced vital capacity
ISAC: Immuno Solid-phase Allergen Chip
ISU: ISAC standardized units
LCA: Latent class analysis
MAAS: Manchester Asthma and Allergy Study
OR: Odds ratio
sIgE: Serum IgE

been demonstrated to produce robust and reproducible results.[10] Recently, we have reported that machine learning modeling of ISAC data might facilitate a more accurate assessment of allergic diseases, showing that combinations of allergen components can predict concomitant asthma or rhinoconjunctivitis onset with fair sensitivity/specificity.[11] Hatzler et al[12] have used the CRD approach to demonstrate that IgE responses to timothy grass during childhood increase in molecular complexity over time through "molecular spreading," in which sensitization to a "lead allergen" (Phl p 1) precedes a characteristic sequence of further increase in the number of recognized molecules.

We hypothesize that there is an underlying complexity and heterogeneity in the longitudinal patterns of the development of component-specific IgE responses within individual patients and that different longitudinal patterns of IgE responses to distinct allergen components within the whole allergen source might be associated with different allergic diseases. To address our hypotheses, we conducted longitudinal analysis of ImmunoCAP ISAC CRD data, focusing on timothy grass and dust mite as examples of seasonal and perennial allergens linked to expression of rhinitis and asthma, respectively. We designed a nested latent class probabilistic modeling approach to allow the discovery of such a latent structure within the data that reflects the individual life course, with the aim of investigating the evolution of IgE profiles during childhood. These sensitization trajectories were then related to asthma, rhinitis, and eczema. We carried out the study among participants in a population-based birth cohort, in whom sIgE levels and detailed clinical assessment were ascertained at 5 follow-ups during the first 11 years of life.[13]

METHODS

Study population

The Manchester Asthma and Allergy Study (MAAS) is a population-based birth cohort (registration: ISRCTN72673620).[13,14] MAAS was approved by the local research ethics committee; parents provided written informed consent.

Data sources

Clinical follow-up. Participants attended follow-ups at ages 1, 3, 5, 8, and 11 years. Blood samples were collected from children who gave assent for venipuncture. Validated questionnaires were interviewer administered to collect information on parentally reported symptoms, physician-diagnosed diseases, and treatment received. At age 11 years, we measured lung function using spirometry and airway reactivity in a 5-step protocol using quadrupling doses of methacholine.[15] The fraction of exhaled nitric oxide (eNO) was measured according to the American Thoracic Society (ATS)/European Respiratory Society recommendations as a marker of airway inflammation.[16]

Medical records data. We extracted data from children's primary care medical records, including oral corticosteroid prescriptions, emergency department admissions, and asthma/wheeze-related hospitalizations.[17]

Definition of variables

We defined clinical outcomes from the data collected at age 11 years. Severe asthma/wheeze exacerbations during childhood were ascertained from medical records data.

Current wheeze was defined as a positive answer to the following question: "Has your child had wheezing or whistling in the chest in the last 12 months?" *Current asthma* was defined as the presence of any 2 of the 3 features: (1) current wheeze; (2) current use of asthma medication; and (3) physician-diagnosed asthma ever. *Current rhinitis* was defined as a positive answer to the following question: "In the past 12 months, has your child had a problem with sneezing or a runny or blocked nose when he/she did not have a cold or the flu?"[18] *Current eczema* was defined as a positive answer to the following question: "Has your child had an itchy rash that comes and goes in the last 12 months?" *Severe wheeze/asthma exacerbation* was defined as receipt of oral corticosteroids for at least 3 days or admission to the hospital or emergency department visit because of asthma/wheeze requiring oral steroids.[19,20] FEV_1 was expressed as percent predicted[21]; we also recorded FEV_1/forced vital capacity (FVC) ratio. Children were categorized as having *airway hyperreactivity* after a 20% decrease in FEV_1 by the final stage of the challenge. We calculated the *dose-response ratio*[22] to include all evaluable data as a continuous variable.

CRD

ImmunoCAP ISAC (Thermo Fisher Scientific, Uppsala, Sweden) was used to measure sIgE levels to 112 allergenic molecules. IgE antibodies to 15 allergen components were considered in this study: 8 timothy grass *(Phleum pratense)* components (Phl p 1, 2, 4, 5, 6, 7, 11, and 12) and 7 mite (*Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, *Blomia tropicalis*, and the storage mite *Lepidoglyphus destructor*) components (Der f 1, 2, Der p 1, Der p 2, Der p 10, Lep d 2, and Blo t 5). The level of component-specific IgE antibodies was reported in ISAC standardized units (ISU). We categorized the raw data into 4 sIgE semiquantitative discrete groups, according to the manufacturer's guidelines: no (<0.3 ISU), low (0.3-1 ISU), medium (1-15 ISU), and high (>15 ISU) sensitization.

Statistical methods

We designed a nested, 2-stage latent class analysis (LCA) to identify cross-sectional sensitization patterns and their longitudinal time trajectories. LCA is a statistical method for finding subgroups (ie, latent classes or clusters) of related cases from multivariate discrete data based on the patterns of their associated variables. In the current study LCA enabled exploration as to whether in our study population there are subgroups into which study participants cluster based on the cross-sectional and longitudinal patterns of sIgE responses to different allergen components (ie, 8 components for timothy grass and 7 components for mite).

Fig 1 shows a schematic description of our nested modeling approach. We first applied LCA on sIgE data at each time point to identify cross-sectional sensitization classes and then used such inferred class membership in a further LCA on the longitudinal data to analyze the evolution of IgE profiles over time. Thus a first-level LCA inferred cross-sectional sensitization classes at each of the 3 time points (sensitization profiles at ages 5, 8, and 11 years); we then proceeded to perform a second-level LCA to establish longitudinal time trajectories of these sensitization profiles (ie, sensitization trajectories). LCA was performed with R software (http://www.r-project.org/). For more details on statistical methods, please see the Methods section in this article's Online Repository at www.jacionline.org.

To make full use of the ISAC data available for regression analysis, we allocated children who had specific IgE measurements taken at 2 of 3 time points to the trajectory that had the most corresponding combinations of class memberships in it. We related trajectories to clinical outcomes through logistic regression.[23,24]

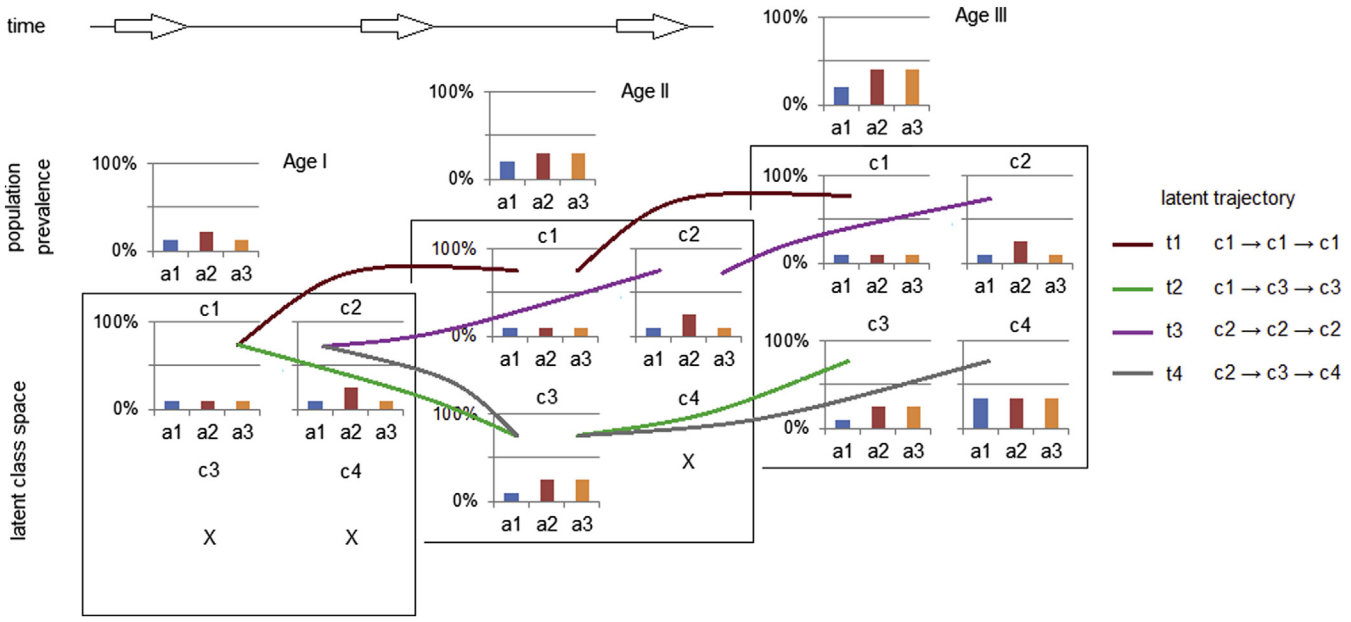

FIG 1. Use of nested LCA to infer sensitization patterns and time trajectories. At 3 time points (*Age I*, *Age II*, and *Age III*), sensitizations to 3 allergens (*a1*, *a2*, and *a3*) are measured in the population. The prevalence of these allergens in the study population per each time point is shown below each age landmark outside the boxes. When LCA is applied, at each time point, 1 or more allergen patterns can be found. The *square boxes below each age landmark* represent the latent space and include sensitization patterns *(classes c1-c4)* found at each time point. If the same allergen patterns are found at different ages, they are labeled consistently. Subjects can be assigned to a particular class at each time point by looking at their allergen profiles. After this first LCA, the prevalence of classes in the population at each time point can be calculated (not shown for simplicity). A second-stage LCA can be applied to the classes across time points to identify longitudinal patterns of transition/stasis among classes. These are called time trajectories and are shown as lines that connect one class to another in a subsequent age point *(t1-t4)*. Trajectories will have assigned probabilities as well (not shown).

RESULTS

Participant flow and demographic data

Of 1184 children prenatally enrolled, ImmunoCAP ISAC data were available for 226 children at age 1 year, 248 at age 3 years, 588 at age 5 years, 543 at age 8 years, and 461 at age 11 years. Data at ages 1 and 3 years were very sparse (eg, only 5 children were sensitized to any of the timothy grass allergens at age 1 year). Therefore we made an *a priori* decision to use data from ages 5, 8, and 11 years in the analysis. We inferred longitudinal trajectories among 235 children who had ISAC data at all 3 time points and allocated trajectories to investigate their clinical relevance among 534 children with ISAC data from at least 2 time points. Demographic characteristics are summarized in Table I, with details on by-age prevalence of sensitizations to specific components shown in Table E1 in this article's Online Repository at www.jacionline.org. Maternal atopy and asthma were more common among children without ISAC data; there were no significant differences in demographic data or clinical outcomes between children included and those excluded from this analysis (Table I).

Cross-sectional sensitization profiles

Timothy grass. Three cross-sectional classes were identified at age 5 years, and 4 classes were identified at ages 8 and 11 years (see Fig E1 in this article's Online Repository at www.jacionline.org). The first 3 classes were virtually identical across all ages, whereas the fourth class emerged at ages 8 and 11 years. On the basis of the number of allergen components to which sensitization occurred and on the levels of component-specific IgE antibodies, we qualitatively labeled the 4 cross-sectional sensitization profiles as no, mild, moderate, and severe sensitization. The mild sensitization profile was characterized by a greater than 40% probability of IgE antibody response to Phl p 1, 4, and 5 (but not other components) at all ages, with most children belonging to this profile having low or medium sIgE levels. In contrast, children in the severe sensitization profile at age 11 years had greater than 40% probability of IgE response to all 8 components, with a majority of sIgEs having medium or high levels (see Fig E1, *C*). The proportion of children within each of the sensitization profiles at different ages is presented in Table E2 in this article's Online Repository at www.jacionline.org. With increasing age, there was a shift toward a higher proportion of children belonging to more severe sensitization profiles.

Dust mite. A 5-class model was optimal at each of the 3 time points (see Fig E2 in this article's Online Repository at www.jacionline.org). On the basis of the allergen components that elicited IgE responses and the levels of component-specific IgE antibodies, we qualitatively labeled the profiles as no sensitization, group 1 sensitization, group 2 sensitization, complete moderate sensitization, and complete high sensitization. It is noteworthy that at each time point, we found that some children were sensitized to 1 allergen component group only. The proportion of children within each of the sensitization profiles at different ages is

TABLE I. Population characteristics of children with or without ISAC data available for at least 2 time points or all 3 ages (5, 8, and 11 years)

	ISAC data on ≥2 time points (n = 534), no. (%)	ISAC data, ages 5, 8, and 11 y (n = 235), no. (%)	No ISAC data (n = 650), no. (%)	*P* value
Sex (male)	292/534 (54.7)	132/235 (56.1)	350/650 (53.9)	.774
Older siblings	309/533 (58.0)	134/235 (57.0)	290/543 (53.4)	.132
Maternal smoking (pregnancy)	62/530 (11.7)	29/235 (12.3)	57/495 (11.5)	.927
Maternal asthma	87/534 (16.3)	41/235 (17.5)	148/650 (22.8)	.005
Paternal asthma	81/534 (15.2)	32/235 (13.6)	82/648 (12.7)	.212
Maternal atopy	292/522 (55.9)	124/230 (53.9)	390/624 (62.5)	.024
Paternal atopy	328/518 (63.3)	136/229 (59.4)	388/619 (62.7)	.824
Sensitization (skin prick test), age 3 y	102/478 (21.3)	40/215 (18.6)	123/505 (24.4)	.260
Sensitization (skin prick test), age 5 y	149/512 (29.1)	57/232 (24.6)	145/451 (32.2)	.305
Sensitization (skin prick test), age 8 y	165/520 (31.7)	63/231 (27.3)	149/407 (36.6)	.119
Sensitization (skin prick test), age 11 y	161/470 (34.3)	70/232 (30.2)	119/325 (36.6)	.493
Current asthma, age 11 y	93/498 (18.7)	31/200 (15.5)	97/416 (23.3)	.085
FEV_1 (% predicted), age 11 y	98.92 (97.87-99.96)	99.54 (98.08-101.01)	97.60 (96.31-98.90)	.120

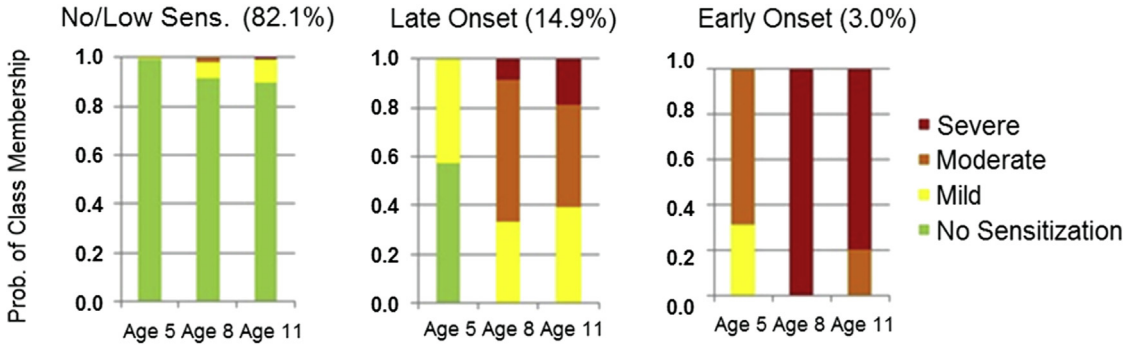

FIG 2. Latent classes inferred for timothy grass allergens to identify trajectories of cross-sectional sensitization classes over time.

presented in Table E3 in this article's Online Repository at www.jacionline.org. In contrast to timothy grass, there was little evidence for a shift toward a higher proportion of children belonging to the severe sensitization profile with increasing age.

Longitudinal sensitization trajectories

Timothy grass. We identified 3 sensitization trajectories in the longitudinal LCA. Cross-sectional sensitization profiles within each of the longitudinal sensitization trajectories at different ages are shown in Fig 2. On the basis of their characteristics, we qualitatively labeled them as follows: no/low grass sensitization trajectory (82.1%), late-onset trajectory (14.9%), and early-onset trajectory (3.0%). Fig E3 in this article's Online Repository at www.jacionline.org shows sIgE levels to individual allergenic components in different trajectories and their evolution over time. Children in the early-onset trajectory had high levels of sIgE to grass allergens at age 5 years, whereas those in the late-onset trajectory had IgE responses between ages 5 and 11 years. However, the progression of individual component responses appeared similar in the early- and late-onset trajectories, following the sequence of Phl p 1/5 → Phl p 2/4/6 → Phl p 7/11/12. IgE antibody levels specific to the components that induced sensitization early on increased in concentration with increasing age.

Dust mite. We identified 4 sensitization trajectories in the longitudinal LCA. Cross-sectional sensitization profiles within each of the longitudinal sensitization trajectories at different ages are shown in Fig 3. We qualitatively labeled these as follows: no/low mite sensitization trajectory (83.8%), group 1 allergens trajectory (4.3%), group 2 allergens trajectory (3.8%), and complete mite sensitization trajectory (8.1%). Fig E4 in this article's Online Repository at www.jacionline.org shows sIgE levels to individual allergenic components in different trajectories and their evolution over time. High levels of sIgE antibodies at any time point were observed only for Der f 1/Der p 1 and Der f 2/Der p 2, and the trajectories suggested that children might either have an IgE response to only one of these 2 allergen groups or become cosensitized to both at an early age; very rarely could a spreading of IgE response to the other allergen group be observed.

Association between longitudinal sensitization trajectories and clinical outcomes

There were marked differences between different trajectories in relation to asthma, rhinitis, eczema, lung function, airway hyperreactivity, and airway inflammation. Table II lists odds ratios (ORs) relating to the incidence of clinical outcomes for the various timothy grass and dust mite trajectories, whereas Table III provides summary statistics for lung function measurements.

Timothy grass sensitization trajectories. The early-onset trajectory was associated with a significantly higher risk of asthma, comorbidity of allergic diseases (asthma, rhinitis, and

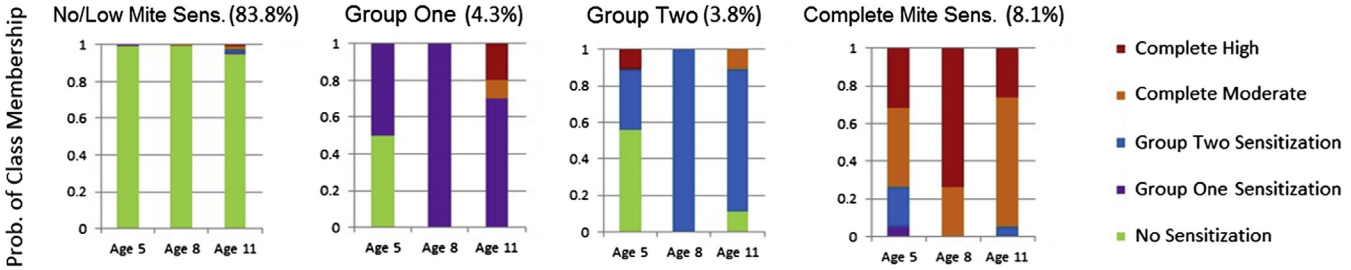

FIG 3. Latent classes inferred for house dust mite allergens to identify trajectories of cross-sectional sensitization classes over time.

TABLE II. Relationship between longitudinal trajectories for timothy grass and house dust mite allergens in relation to clinical outcomes of asthma, rhinitis, and eczema

	Timothy grass longitudinal sensitization trajectories		Dust mite longitudinal sensitization trajectories		
	Late onset (n = 97), OR (95% CI), *P* value	Early onset (n = 23), OR (95% CI), *P* value	Group 1 allergens (n = 23), OR (95% CI), *P* value	Group 2 allergens (n = 22), OR (95% CI), *P* value	Complete mite sensitization (n = 54), OR (95% CI), *P* value
Positive methacholine challenge	1.68 (0.97-2.91), .062	2.86 (0.99-8.28), .052	6.90 (2.17-21.97), .001	2.30 (0.84-6.32), .106	6.44 (3.00-13.82), <.001
Current wheeze	1.31 (0.73-2.35), .364	3.63 (1.49-8.86), .005	4.24 (1.69-10.63), .002	4.24 (1.69-10.63), .002	7.13 (3.81-13.34), <.001
Current asthma	1.53 (0.85-2.76), .158	6.20 (2.56-15.01), <.001	4.60 (1.83-11.56), .001	3.02 (1.12-8.10), .029	7.15 (3.80-13.44), <.001
Current rhinitis	5.84 (3.59-9.50), <.001	11.84 (4.25-33.01), <.001	6.11 (2.42-15.38), <.001	2.37 (0.99-5.66), .051	4.07 (2.23-7.42), <.001
Current eczema	1.15 (0.64-2.08), .637	3.71 (1.50-9.18), .004	3.21 (1.28-8.06), .013	0.82 (0.24-2.87), .761	2.17 (1.12-4.20), .021
Current asthma, rhinitis, and eczema	2.20 (0.65-7.46), .207	17.91 (5.55-57.74), <.001	4.43 (0.90-21.88), .068	2.11 (0.26-17.45), .488	5.91 (2.01-17.37), .001
Severe asthma exacerbation ever among wheezy children	1.96 (1.03-3.74), .041	3.91 (1.39-11.02), .010	1.82 (0.68-4.89), .233	1.30 (0.44-3.88), .636	3.29 (1.62-6.67), .001

ORs, 95% CIs, and *P* values are from univariate logistic regression. The reference category is the no/low grass sensitization trajectory.

eczema; OR, 17.9), diminished lung function, and more hyperreactive airways compared with the late-onset and no/low grass sensitization trajectories (Tables II and III). The late-onset trajectory was not significantly associated with asthma, but there was a strong association with rhinitis (Table II).

Among children with a history of wheezing, both children in the early-onset and those in the late-onset trajectories had notably higher risk of severe asthma exacerbations during childhood (Table II); however, the magnitude of risk was significantly higher in the early-onset trajectory (ORs of 3.91 [95% CI, 1.39-11.02; $P = .01$] and 1.96 [95% CI, 1.03-3.74; $P = .04$]), early-onset and late-onset trajectories, respectively.

Dust mite sensitization trajectories. Both the group 1 allergens and complete mite sensitization trajectories were strongly associated with asthma, eczema, and rhinitis, but the children in the complete mite sensitization trajectory had the highest ORs for asthma (OR, 7.15; 95% CI, 3.80-13.44) and were the only group significantly associated with asthma, with rhinitis and eczema as comorbidities (OR, 5.91; 95% CI, 2.01-17.37; Table II). Children belonging to the group 2 allergens trajectory generally had lower odds of clinical symptoms, except for a significant association with asthma. Children in the complete mite sensitization trajectory had significantly higher eNO levels ($P < .05$) compared with those in the group 1 allergens and group 2 allergens trajectories (Table III).

Among children with a history of wheezing, those in the complete mite sensitization trajectory (but not in other mite longitudinal trajectories) had significantly higher risk of severe asthma exacerbations during childhood (OR, 3.29; 95% CI, 1.62-6.67).

DISCUSSION

Key findings

Our findings suggest that the nature of the development of IgE responses during childhood to multiple allergen components differs between timothy grass and dust mite allergens. Our data indicate that IgE reactivity profiles to timothy grass components diversify and increase in concentration and molecular heterogeneity over time, confirming the "molecular spreading" hypothesis.[12] We have shown that this process can be initiated at different times in childhood, giving rise to an early-onset or late-onset longitudinal trajectory. Different pathways emerged for dust mite components in that the dominant longitudinal pattern of progression from individual component responses to multiple sensitization did not emerge. Instead, children either remained firmly sensitized to one of the component groups (group 1 or 2 allergens) throughout childhood or there was a cosensitization to these major allergen groups, with little progression from one developmental pathway to the other. Thus for dust mite, the key discriminating factor appears to be the panel of allergen components that elicit an IgE response, rather than temporal factors (as is the case for grass).

We demonstrated that different longitudinal patterns of IgE responses to grass and mite allergen components during childhood bear different associations with clinical outcomes. For

TABLE III. Relationship between time trajectories for timothy grass and dust mite allergens in relation to lung function, airway hyperreactivity, and airway inflammation (eNO)

	Timothy grass longitudinal sensitization trajectories			Dust mite longitudinal sensitization trajectories			
	No/low (n = 414), mean (95% CI)	Late onset (n = 97), mean (95% CI), *P* value	Early onset (n = 23), mean (95% CI), *P* value	No/low (n = 435), mean (95% CI)	Group 1 allergens (n = 23), mean (95% CI), *P* value	Group 2 allergens (n = 22), mean (95% CI), *P* value	Complete mite sensitization (n = 54), mean (95% CI), *P* value
FEV_1 (% predicted)	99.38 (98.24-100.52), baseline	98.80 (96.11-101.49), .682	91.29 (85.57-97.01), .002	99.65 (98.53-100.76), baseline	92.28 (84.95-99.61), .004	96.15 (90.68-101.62), .183	97.34 (94.17-100.51), .180
FEV_1/FVC ratio (%)	87.92 (86.28-87.57), baseline	85.79 (83.95-87.61), .172	80.70 (77.37-84.04), <.001	87.02 (86.34-87.70), baseline	82.55 (79.24-85.85), .003	84.11 (81.19-87.03), .061	84.71 (82.74-86.68), .024
Normalized methacholine dose-response ratio	4.31 (4.00-4.62), baseline	3.96 (3.25-4.67), .345	2.60 (1.36-3.84), .017	4.56 (4.25-4.87), baseline	2.42 (1.65-3.19), .001	3.25 (1.97-4.53), .051	2.43 (1.61-3.26), <.001
eNO	10.90 (10.02-11.85), baseline	18.93 (15.42-23.22), <.001	29.33 (16.72-51.43), <.001	10.25 (9.54-11.01), baseline	26.55 (16.38-43.04), <.001	17.05 (10.51-27.66), .005	40.95 (32.22-52.06), <.001

Means, 95% CIs, and *P* values were calculated by using regression analysis.

timothy grass, the early-onset longitudinal trajectory was predictive of asthma, rhinitis, and eczema (as well as being associated with diminished lung function in school age), whereas the late-onset trajectory was significantly associated with rhinitis. For dust mite, children in the complete mite sensitization trajectory had the highest risk of asthma and significantly higher eNO levels compared with those in all other trajectories. Children in the group 2 allergens trajectory tended to have a lower risk of clinical disease compared with those in the group 1 allergens or complete mite sensitization trajectories. Finally, the grass early-onset and complete mite sensitization trajectories were the only longitudinal trajectories significantly associated with asthma with comorbid rhinitis and eczema; furthermore, among children with a history of wheezing, those belonging to these 2 longitudinal trajectories had markedly increased risk of severe exacerbations during childhood. The risk of exacerbation was also increased among children in the late-onset grass trajectory. However, we note that our results need to be interpreted with caution and should not be used to make definitive conclusions given the relatively modest sample size and wide CIs we observed when we examined the relationship between longitudinal trajectories and various clinical outcomes.

Limitations

There are several potentially important mite allergens that are not included on the ISAC chip. We cannot exclude the possibility that Der f 1/Der p 1 and Der f 2/Der p 2 might both act as lead allergens in a molecular spreading process that involves such allergens. For example, previous studies have demonstrated that although the majority of IgE binding to *D pteronyssinus* allergens was accounted for by Der p 1 and Der p 2 across a large range of allergic subjects, a further 30% was due to Der p 4, Der p 5, and Der p 7 in equal amounts, establishing these as midpotency allergens.[25] Hence the combination of mite allergen components present on the ISAC chip might not be optimal for the investigation of molecular spreading. However, even if molecular spreading does occur, based on our data, it would be expected to follow at least 2 fundamentally different pathways characterized predominantly by IgE responses to group 1 or group 2 allergen components.

Another limitation relates to the availability of data in earlier life. Although ISAC data were available for 226 children at age 1 year and 248 children at age 3 years, this added little information because only 5 children were sensitized to any of the timothy grass allergens at age 1 year and only 6 children were sensitized to at least 1 of the dust mite allergens. However, we cannot exclude a possibility that the information of component IgE responses in early life would be valuable in a much larger sample. We ascertained sensitization trajectories in a subpopulation on children who attended follow-ups at ages 5, 8, and 11 years and gave assent for venipuncture and cannot unequivocally exclude the possibility of bias. However, it is unlikely that this would have affected the allocation to different cross-sectional sensitization profiles or longitudinal sensitization trajectories.

Interpretation

Atopic subjects frequently experience multiple sensitization events as time progresses.[26] Using a nested LCA model, we identified a clear diversification of the IgE responses to timothy grass allergens over time, which is suggestive of increasing

molecular heterogeneity as time progresses. This is reflected in the fact that a 3-class model best captured the underlying structure of the data at age 5 years, whereas 4 classes were more appropriate for ages 8 and 11 years. In addition to this cross-sectional observation, our longitudinal analysis confirmed the molecular spreading for timothy grass. However, it is of note that although there were differences in the time of onset of IgE reactivity, the 2 major grass longitudinal sensitization trajectories showed a similar pattern of progression of individual component responses: after initial sensitization to the lead allergens Phl p 1 and 5, one can observe additional IgE responses to Phl p 2, 4, and 6 at later times, with some children also having sIgE to Phl p 7, 11, and 12 thereafter.

Recently, by analyzing longitudinal skin prick test and sIgE data to whole allergen extracts collected from birth to school age in our birth cohort, we identified that atopy can encompass several different subtypes characterized by a unique pattern of response, both in terms of allergens and time course.[27] These subtypes differed in their associations with clinical outcomes; one of these classes (characterized by early responses to multiple allergens) was strongly associated with asthma. These findings were recently validated in an independent birth cohort.[28] Although these results highlight the deficiencies of the current interpretation of allergy tests, the above approach cannot as yet be applied in the clinic because it is reliant on the availability of longitudinal data.[1] Results of the current study indicate that characterization of atopic subtypes over time might help further elucidate the highly divergent development of clinical symptoms among atopic children.[29,30] This is supported by our finding of the different association between longitudinal trajectories of the component-specific IgE and clinical outcomes. For example, for timothy grass, the early-onset trajectory was predictive of lower airway-related clinical outcomes and comparatively poorer lung function, whereas the late-onset trajectory was predominantly associated with upper airway disease (rhinitis). For dust mite allergens, children in the complete mite sensitization trajectory tended to have the highest risk for asthma (in particular with comorbidities), higher airway inflammation (eNO), and, among asthmatic patients, higher risk of severe exacerbations compared with those from all other longitudinal trajectories.

In conclusion, our findings suggest that different longitudinal patterns in the development of IgE responses to grass and mite allergen components during childhood are associated with different clinical outcomes and confirm the hypothesis that atopic sensitization can be stratified into subtypes with a diversification of their evolutionary pathways over time.[1,27,28,31] Better resolution of such longitudinal patterns might help us better understand the pathophysiology underlying atopic diseases and might facilitate the development of biomarkers of allergic disease, which can be used for the prediction of future risk.

Clinical implications: Development of different clinical phenotypes of allergic diseases (asthma, eczema, and rhinitis), as well as asthma severity throughout childhood, is predicted by the molecular nature of IgE responses to individual allergen components.

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METHODS

Statistical analysis

We designed a nested, 2-stage LCA to identify sensitization trajectories. First, we looked at each time point (5, 8, and 11 years) individually and applied LCA with sensitization profiles for each of the allergens as manifest variables to reduce the data dimensionality and determine the major IgE reactivity profiles at each age. Next, we used the class memberships inferred from the first LCA as manifest variables in a further LCA to detect groups of children with a similar evolution in their sensitization profiles (as shown in Fig 1).

LCA was performed by using the R software (http://www.r-project.org/) through the package *poLCA*[E1] with the expectation maximization algorithm to estimate relevant parameters (with 10,000 iterations over 100 independent runs to check convergence).[E2] We determined the number of classes for each analysis using both the Akaike information criterion and Bayesian information criterion,[E3-E5] choosing the Akaike information criterion for results reported here because this provided a qualitatively better resolution at component level. The average sensitization intensity toward allergens at each age for the class trajectories was calculated as the mean of sensitization categories for all children in a particular trajectory. CIs were inferred by using bootstrapping[E6] with the R package *boot*.[E7]

Lung function measurement

Children were asymptomatic at the time of assessment of lung function. Short-acting β_2-agonists were withheld for at least 6 hours and long-acting β_2-agonists were withheld for at least 24 hours before testing.

Spirometry. Spirometry was performed at age 11 years according to ATS guidelines by using a Lilly pneumotachograph system with animated incentive software (Jaeger, Würzburg, Germany). FEV_1 and FVC values were recorded, and data were expressed as FEV_1 percent predicted and FEV_1/FVC ratio.

Airway hyperreactivity. At the 11-year follow-up, airway reactivity was assessed by means of methacholine challenge with a 5-step protocol performed according to ATS guidelines. Quadrupling doses of methacholine (0.0625-16.0 mg/mL) were delivered to subjects through a DeVilbiss 646 nebulizer (Sunrise Medical HHG, Somerset, Pa) and a KoKo dosimeter (Pulmonary Data Services, Doylestown, Pa) calibrated to deliver 0.009 mL/0.6 s actuation. The predicted FEV_1 was calculated, and if the measured value was less than 1.0 L or less than 60% of predicted value, the test was not performed. FEV_1 was measured 30 and 90 seconds after 5 inhalations of each dose of methacholine. The challenge was stopped when either a 20% decrease in FEV_1 was observed or the maximum methacholine concentration had been administered.

Data from primary care medical records

We extracted all data from electronic and paper-based primary care medical records, including oral corticosteroid prescriptions, emergency department admissions, and asthma/wheeze-related hospitalizations.[E8] Eligible practices were contacted and invited to participate in the study either by both postal information packs and telephone calls (general practitioner [GP] practices with ≥2 children in the study) or by post only (GP practices with a single study participant). Data access and manual extraction were performed during arranged visits to each GP practice. A trained pediatrician extracted data from electronic and paper-based primary care medical records, including prescriptions, acute wheeze episodes, oral steroid prescriptions, and hospital admissions for asthma or wheeze during the first 8 years of life. Timing, type of visit, symptoms, indication, and prescriptions for each encounter were noted. We calculated the child's age in days for each event.

CRD

We categorized the raw data into 4 sIgE semiquantitative discrete groups according to the manufacturer's guidelines: no (<0.3 ISU), low (0.3-1 ISU), medium (1-15 ISU), and high (>15 ISU) sensitization. Other discretization techniques were also tested, including a binary based on the threshold of 0.3 ISU; however, our previous results showed that categorization based on the manufacturer's guidelines compared favorably with alternatives.[E9]

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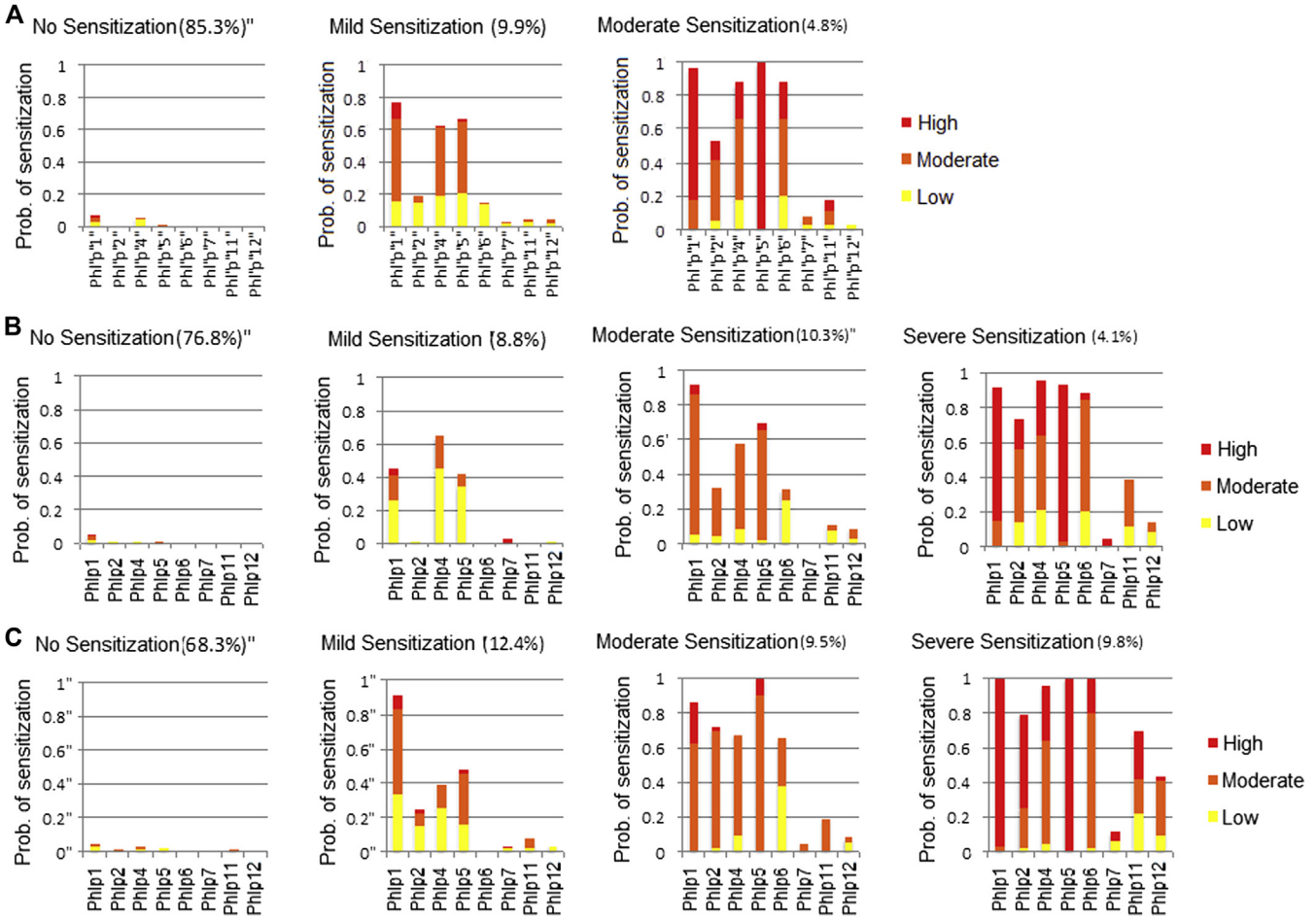

FIG E1. Nested latent classes inferred for the timothy grass allergens for cross-sectional sensitization profiles. Each class corresponds to a particular distribution of IgE categories in each allergen component (here the probability of being highly sensitized to a specific allergen by each class is depicted). This first-level analysis is carried out at the time points of 5 years **(A)**, 8 years **(B)**, and 11 years **(C)** of age, and resulting classes are matched across ages based on their IgE distribution patterns.

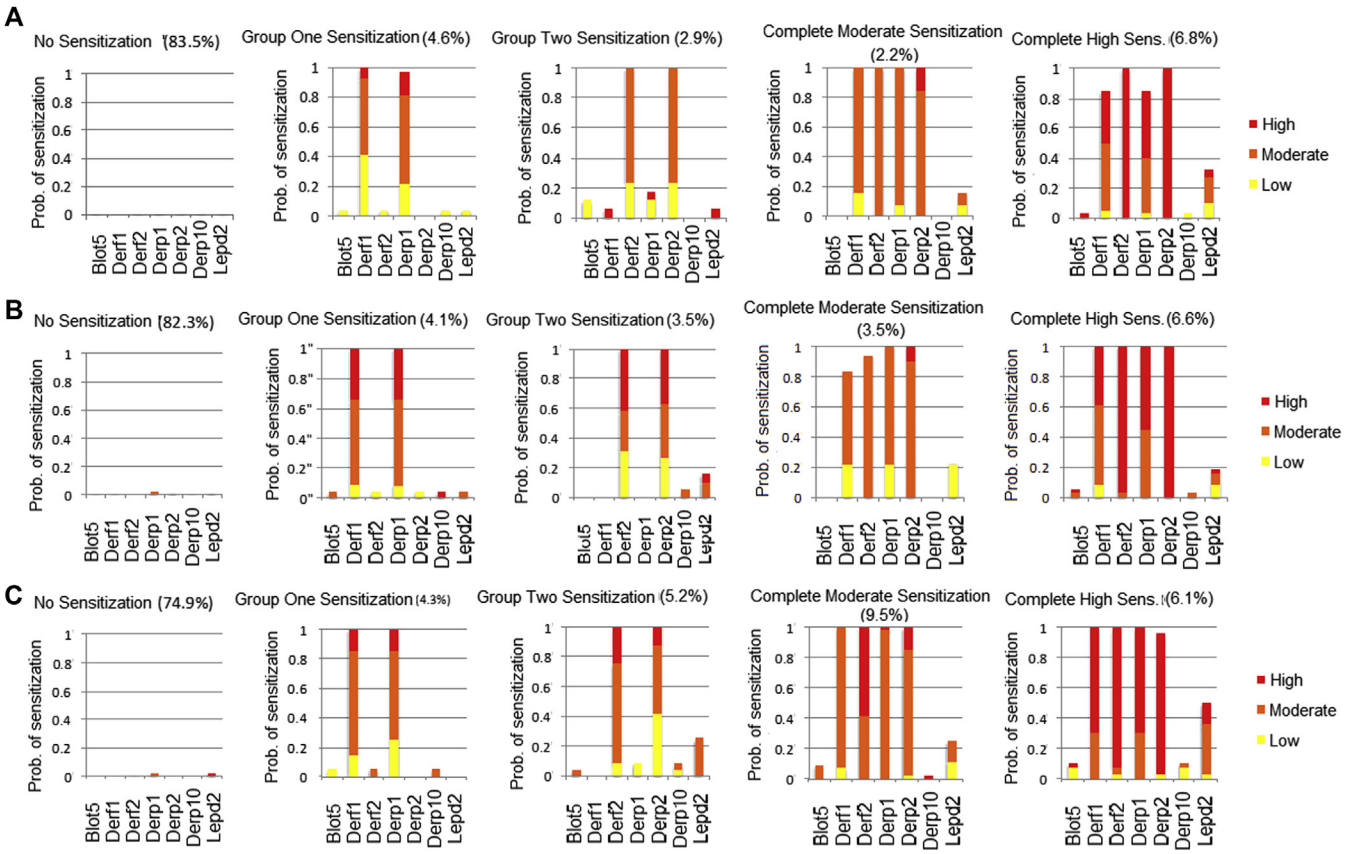

FIG E2. Nested latent classes inferred for dust mite allergens for cross-sectional sensitization profiles. Each class corresponds to a particular distribution of IgE categories in each allergen component (here the probability of being highly sensitized to a specific allergen by each class is depicted). This first-level analysis is carried out at the time points of 5 years **(A)**, 8 years **(B)**, and 11 years **(C)** of age, and resulting classes are matched across ages based on their IgE distribution patterns.

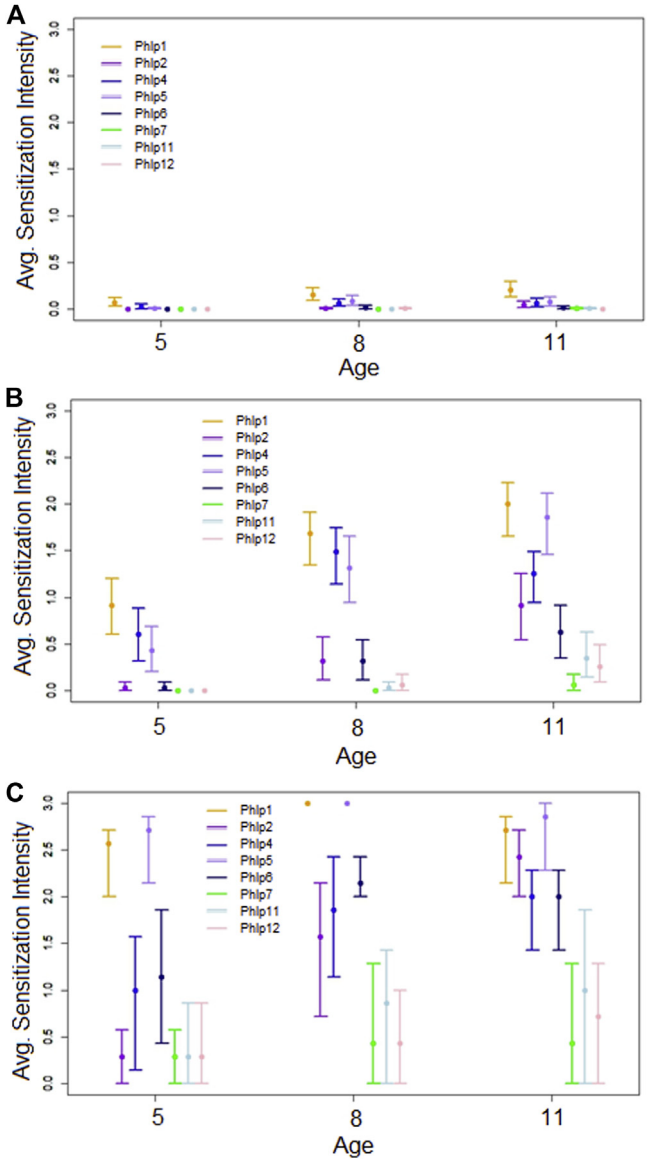

FIG E3. sIgE levels to timothy grass allergens in different trajectories and their evolution over time. Semiquantitatively discretized IgE levels have been allocated numeric values: 0, negative; 1, low; 2, medium; and 3, high. *Bars* indicate 95% bootstrap CIs around the mean for each allergen. **A,** No/low grass sensitization trajectory. **B,** Late-onset trajectory. **C,** Early-onset trajectory.

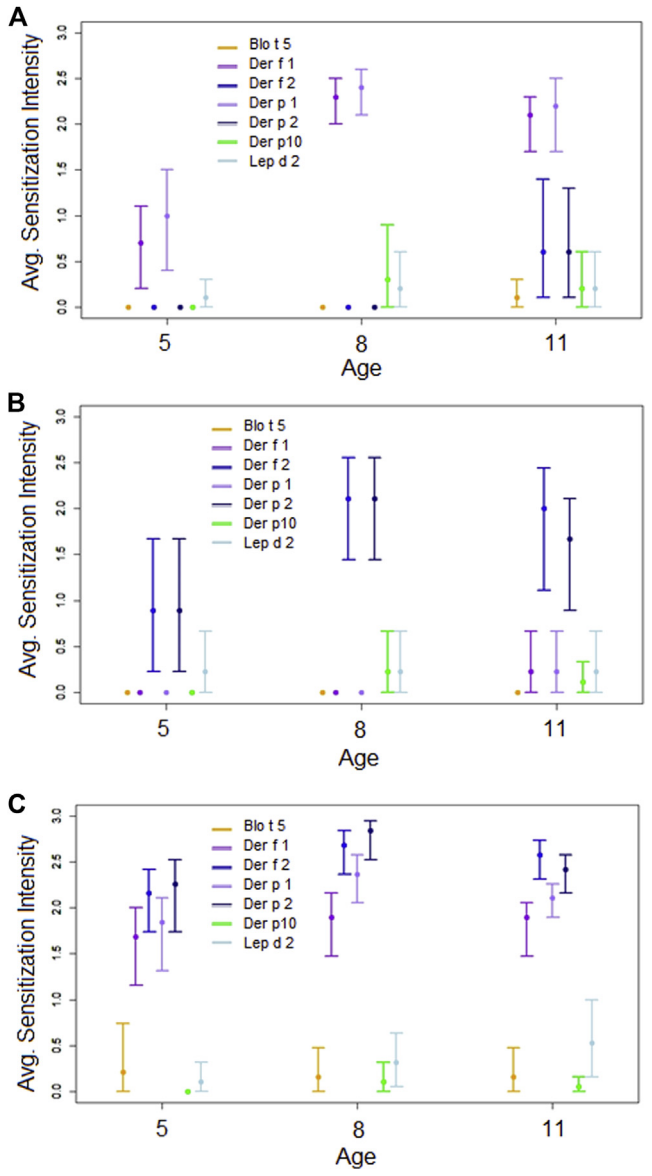

FIG E4. sIgE levels to house dust mite allergens in different trajectories and their evolution over time. Semiquantitatively discretized IgE levels have been allocated numeric values: 0, negative; 1, low; 2, medium; 3, high. *Bars* indicate 95% bootstrap CIs around the mean for each allergen. **A,** Group 1 allergens trajectory. **B,** Group 2 allergens trajectory. **C,** Complete mite sensitization trajectory.

TABLE E1. Prevalence of component-specific sensitizations (semiquantitative IgE level scale) across different ages

		Sensitization			
Age	Component	No (<0.3 ISU)	Low (0.3-1 ISU)	Medium (1-15 ISU)	High (>15 ISU)
1 y	Phl p 1	226 (100%)	0 (0%)	0 (0%)	0 (0%)
	Phl p 2	226 (100%)	0 (0%)	0 (0%)	0 (0%)
	Phl p 4	221 (97.79%)	5 (2.21%)	0 (0%)	0 (0%)
	Phl p 5	226 (100%)	0 (0%)	0 (0%)	0 (0%)
	Phl p 6	226 (100%)	0 (0%)	0 (0%)	0 (0%)
	Phl p 7	226 (100%)	0 (0%)	0 (0%)	0 (0%)
	Phl p 11	226 (100%)	0 (0%)	0 (0%)	0 (0%)
	Phl p 12	226 (100%)	0 (0%)	0 (0%)	0 (0%)
	Der f 1	223 (98.67%)	3 (1.33%)	0 (0%)	0 (0%)
	Der f 2	224 (99.12%)	1 (0.44%)	1 (0.44%)	0 (0%)
	Der p 1	224 (99.12%)	1 (0.44%)	1 (0.44%)	0 (0%)
	Der p 2	223 (98.67%)	2 (0.88%)	1 (0.44%)	0 (0%)
	Der p 10	226 (100%)	0 (0%)	0 (0%)	0 (0%)
	Lep d 2	226 (100%)	0 (0%)	0 (0%)	0 (0%)
	Blo t 5	226 (100%)	0 (0%)	0 (0%)	0 (0%)
3 y	Phl p 1	219 (88.31%)	15 (6.05%)	12 (4.84%)	2 (0.81%)
	Phl p 2	247 (99.6%)	1 (0.4%)	0 (0%)	0 (0%)
	Phl p 4	228 (91.94%)	12 (4.84%)	8 (3.23%)	0 (0%)
	Phl p 5	241 (97.18%)	3 (1.21%)	2 (0.81%)	2 (0.81%)
	Phl p 6	246 (99.19%)	1 (0.4%)	1 (0.4%)	0 (0%)
	Phl p 7	248 (100%)	0 (0%)	0 (0%)	0 (0%)
	Phl p 11	246 (99.19%)	1 (0.4%)	1 (0.4%)	0 (0%)
	Phl p 12	248 (100%)	0 (0%)	0 (0%)	0 (0%)
	Der f 1	226 (91.13%)	7 (2.82%)	14 (5.65%)	1 (0.4%)
	Der f 2	229 (92.34%)	5 (2.02%)	9 (3.63%)	5 (2.02%)
	Der p 1	221 (89.11%)	9 (3.63%)	16 (6.45%)	2 (0.81%)
	Der p 2	229 (92.34%)	5 (2.02%)	11 (4.44%)	3 (1.21%)
	Der p 10	248 (100%)	0 (0%)	0 (0%)	0 (0%)
	Lep d 2	248 (100%)	0 (0%)	0 (0%)	0 (0%)
	Blo t 5	248 (100%)	0 (0%)	0 (0%)	0 (0%)
5 y	Phl p 1	484 (82.31%)	26 (4.42%)	49 (8.33%)	29 (4.93%)
	Phl p 2	561 (95.41%)	11 (1.87%)	13 (2.21%)	3 (0.51%)
	Phl p 4	499 (84.86%)	37 (6.29%)	45 (7.65%)	7 (1.19%)
	Phl p 5	517 (87.93%)	13 (2.21%)	29 (4.93%)	29 (4.93%)
	Phl p 6	554 (94.22%)	14 (2.38%)	14 (2.38%)	6 (1.02%)
	Phl p 7	584 (99.32%)	2 (0.34%)	2 (0.34%)	0 (0%)
	Phl p 11	580 (98.64%)	3 (0.51%)	3 (0.51%)	2 (0.34%)
	Phl p 12	584 (99.32%)	2 (0.34%)	2 (0.34%)	0 (0%)
	Der f 1	513 (87.24%)	15 (2.55%)	43 (7.31%)	17 (2.89%)
	Der f 2	516 (87.76%)	6 (1.02%)	26 (4.42%)	40 (6.8%)
	Der p 1	506 (86.05%)	16 (2.72%)	43 (7.31%)	23 (3.91%)
	Der p 2	515 (87.59%)	7 (1.19%)	24 (4.08%)	42 (7.14%)
	Der p 10	585 (99.49%)	3 (0.51%)	0 (0%)	0 (0%)
	Lep d 2	570 (96.94%)	6 (1.02%)	9 (1.53%)	3 (0.51%)
	Blo t 5	583 (99.15%)	3 (0.51%)	1 (0.17%)	1 (0.17%)
8 y	Phl p 1	425 (78.27%)	25 (4.6%)	70 (12.89%)	23 (4.24%)
	Phl p 2	506 (93.19%)	7 (1.29%)	26 (4.79%)	4 (0.74%)
	Phl p 4	448 (82.5%)	39 (7.18%)	49 (9.02%)	7 (1.29%)
	Phl p 5	456 (83.98%)	21 (3.87%)	43 (7.92%)	23 (4.24%)
	Phl p 6	505 (93%)	19 (3.5%)	18 (3.31%)	1 (0.18%)
	Phl p 7	541 (99.63%)	2 (0.37%)	0 (0%)	0 (0%)
	Phl p 11	528 (97.24%)	7 (1.29%)	8 (1.47%)	0 (0%)
	Phl p 12	534 (98.34%)	5 (0.92%)	4 (0.74%)	0 (0%)
	Der f 1	468 (86.19%)	9 (1.66%)	44 (8.1%)	22 (4.05%)
	Der f 2	470 (86.56%)	7 (1.29%)	23 (4.24%)	43 (7.92%)
	Der p 1	463 (85.27%)	8 (1.47%)	44 (8.1%)	28 (5.16%)
	Der p 2	466 (85.82%)	7 (1.29%)	25 (4.6%)	45 (8.29%)
	Der p 10	539 (99.26%)	1 (0.18%)	2 (0.37%)	1 (0.18%)
	Lep d 2	525 (96.69%)	8 (1.47%)	8 (1.47%)	2 (0.37%)
	Blo t 5	539 (99.26%)	1 (0.18%)	2 (0.37%)	1 (0.18%)
11 y	Phl p 1	311 (67.46%)	29 (6.29%)	62 (13.45%)	59 (12.8%)
	Phl p 2	378 (82%)	11 (2.39%)	46 (9.98%)	26 (5.64%)
	Phl p 4	355 (77.01%)	24 (5.21%)	68 (14.75%)	14 (3.04%)
	Phl p 5	336 (72.89%)	17 (3.69%)	58 (12.58%)	50 (10.85%)
	Phl p 6	387 (83.95%)	18 (3.9%)	47 (10.2%)	9 (1.95%)
	Phl p 7	452 (98.05%)	4 (0.87%)	2 (0.43%)	3 (0.65%)
	Phl p 11	415 (90.02%)	12 (2.6%)	22 (4.77%)	12 (2.6%)
	Phl p 12	436 (94.58%)	9 (1.95%)	15 (3.25%)	1 (0.22%)
	Der f 1	369 (80.04%)	6 (1.3%)	63 (13.67%)	23 (4.99%)
	Der f 2	362 (78.52%)	5 (1.08%)	36 (7.81%)	58 (12.58%)
	Der p 1	364 (78.96%)	9 (1.95%)	64 (13.88%)	24 (5.21%)
	Der p 2	366 (79.39%)	12 (2.6%)	47 (10.2%)	36 (7.81%)
	Der p 10	454 (98.48%)	3 (0.65%)	3 (0.65%)	1 (0.22%)
	Lep d 2	426 (92.41%)	8 (1.74%)	22 (4.77%)	5 (1.08%)
	Blo t 5	452 (98.05%)	3 (0.65%)	5 (1.08%)	1 (0.22%)

Values are presented as numbers (percentages).

TABLE E2. Percentage share of children in each timothy grass cross-sectional sensitization profile at ages 5, 8, and 11 years

Age (y)	No sensitization	Mild sensitization	Moderate sensitization	Severe sensitization
5	85.3% (502/588)	9.9% (58/588)	4.8% (28/588)	NA
8	76.8% (417/543)	8.8% (48/543)	10.3% (56/543)	4.1% (22/543)
11	68.3% (315/461)	12.4% (57/461)	9.5% (44/461)	9.8% (45/461)

TABLE E3. Percentage share of children in each of the house dust mite cross-sectional sensitization profiles at ages 5, 8, and 11 years

Age (y)	No sensitization	Group 1 sensitization	Group 2 sensitization	Complete moderate sensitization	Complete high sensitization
5	83.5% (491/588)	4.6% (27/588)	2.9% (17/588)	2.2% (13/588)	6.8% (40/588)
8	82.3% (447/543)	4.1% (22/543)	3.5% (19/543)	3.5% (19/543)	6.6% (36/543)
11	74.9% (345/461)	4.3% (20/461)	5.2% (24/461)	9.5% (44/461)	6.1% (28/461)