Atopic sensitization in the first year of life

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Background: There is conflicting evidence on whether allergen-specific memory is primed prenatally, whether this priming affects persistent immunologic effects, and whether it is modulated by the first environmental exposures in infancy.
Objective: We sought to explore the course of atopic sensitization between birth and 12 months of age.
Methods: Specific IgE levels for 6 food and 13 common inhalant allergens were assessed in cord blood and 1-year blood samples in the Protection against Allergy–Study in Rural Environments (PASTURE) birth cohort including 793 children from rural regions of 5 European countries. Detailed information on children's health, nutrition, and farm-related exposures was gathered by using a pregnancy questionnaire, 2 questionnaires at 2 and 12 months of age, and a diary covering the time in between.
Results: Sensitization was more common at 12 months of age than at birth for almost all specificities. On an individual level, persistent sensitization to the same allergens was rare (1%), whereas transient (only at birth, 11%) and incident (only at 12 months, 34%) sensitization was seen in substantial proportions of children. Associations of transient sensitization with maternal sensitization differed with the allergen specificities, with the strongest associations for food allergens (odds ratio [OR], 10.6; 95% CI, 6.0-18.6) and the weakest associations for seasonal allergens (OR, 1.64; 95% CI, 0.94-2.86). Associations of maternal sensitization with incident sensitization were also seen. Incident sensitization was related to distinct prenatal and postnatal environmental exposures of mother and child, such as consumption of cereals for incident sensitization to seasonal allergens (OR, 0.66; 95% CI, 0.50-0.88).
Conclusion: IgE sensitization patterns change between birth and 12 months and are related to maternal and environmental influences. (J Allergy Clin Immunol 2013;■■■:■■■-■■■.)

Key words: *Atopic sensitization, early life, environmental exposures*

A central hallmark of an immune response to allergens is the detection of IgE antibodies. Hence it is crucial to study atopic sensitization by measuring specific IgE levels at various stages of development, including the prenatal period.

Currently, it is not known whether the immune system can mount a prenatal IgE response to specific allergens. Detection of allergen-specific IgE in cord blood is a subject of debate because contamination of cord blood by maternal IgE remains an open question.[1-4]

Furthermore, the effect of maternal exposure to allergens and other environmental stimuli on the fetal immune response has to be evaluated, and its persistence beyond the perinatal period remains to be determined. In the context of farm-related exposures, we have previously found an inverse association of maternal farm exposure during pregnancy and an IgE response to seasonal allergens in cord blood,[5] which has also been observed at school age.[6] However, the development of IgE production in the interval between birth and school age and its effect on disease manifestation remain unclear.

The aim of the present analysis of the Protection against Allergy–Study in Rural Environments (PASTURE) birth cohort is to characterize atopic sensitization within the first year of life. We focus on this time window because fundamental changes in allergen exposure take place during this period. First, direct exposure to inhalant allergens occurs with the first breaths and is followed by sequential introduction of different foods and their

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Abbreviations used
aOR: Adjusted odds ratio
PARSIFAL: Prevention of Allergy Risk factors for Sensitization
PASTURE: Protection against Allergy–Study in Rural Environments
OR: Odds ratio

allergens. We hypothesized that maternal and environmental factors influenced the development of atopic sensitization already in this early period of life.

In this analysis we classify sensitization patterns with respect to changes between birth and the age of 12 months. We distinguish between incident sensitization, persistent sensitization, and transient sensitization (Table I). Furthermore, we assess the relation between sensitization and environmental exposures, as determined by using a pregnancy questionnaire and weekly or monthly diaries during the first year of life.

METHODS

Study design and population

PASTURE has been set up to study the development of childhood asthma and allergies in a prospective birth cohort, including children from rural areas in 5 European countries: Austria, Finland, France, Germany, and Switzerland. The study design has been described earlier.[7] In 2002-2005, pregnant women were contacted in the third trimester of pregnancy. Women who lived on family-run livestock farms were assigned to the farm study group. For the reference study group, women from the same rural areas but not living on a farm were recruited. The study was approved by the ethics committees of the participating institutions, and written informed consent was obtained from the children's parents or guardians.

Specific IgE in serum samples

Specific IgE for 6 food and 13 common inhalant allergens was assessed in cord blood samples and at the age of 12 months in peripheral blood by using the semiquantitative Allergy Screen test panel for atopy (Mediwiss Analytic, Moers, Germany) in a central laboratory. This method has previously been validated against the *in vitro* IgE CAP system (Pharmacia, Freiburg, Germany) and the skin prick test, with a low intra-assay imprecision.[8] Cord blood and 12-month blood samples were not measured at 2 different time points but rather in the sequence they arrived at the central laboratory (see Fig E1, *A*, in this article's Online Repository at www.jacionline.org). Because some centers had started earlier with recruitment, they already had their 12-month specimens measured when other centers were just submitting their cord blood specimens. For each subject, a positive control was measured to standardize the biotin/streptavidin reaction. Food allergens included hen's egg, cow's milk, peanut, hazelnut, carrot, and wheat flour; inhalant allergens comprised *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, cat, horse, and dog as perennial allergens and *Alternaria* species, mugwort, plantain, alder, birch pollen, hazel pollen, rye pollen, and a grass pollen mix as seasonal allergens. In addition, peripheral blood samples of the mothers were taken at birth (in Switzerland and Finland) or at a home visit when the child was 2 months old (Austria, France, and Germany) and were assessed for the same IgE specificities.

Diary and questionnaires

Questionnaires were based on items from the Asthma Multicenter Infants Cohort Study (AMICS),[9] the Allergy and Endotoxin (ALEX) study,[10] the Prevention of Allergy Risk Factors for Sensitization In Children Related to Farming and Anthroposophic Lifestyle (PARSIFAL) study,[11] and the American Thoracic Society questionnaire.[12]

Questionnaires were administered at the end of pregnancy and when the children were 2 and 12 months of age. The questions referred to the general health of the children's families, with a focus on respiratory and atopic diseases and maternal health during pregnancy. Additional detailed information on the children's health, nutrition, and farm-related exposures was gathered by using a diary covering the 9th to 52nd weeks of life. Questions about the children's health included occurrence and quantity of infections or symptoms (cough, wheeze, runny nose, fever, otitis, pneumonia, diarrhea, urinary tract infection, and rash). Farm-related exposures referred to the contact with animals and their feed and litter material. The children's diet was assessed with respect to the type of supplemental food and the time point of its regular introduction (ie, at least weekly consumption of the respective food). Furthermore, the parents were asked whether they bought the food in a shop or directly from a farm and whether they prepared the meals themselves or used convenience food.

Statistical analysis

Statistical analysis was performed with SAS 9.2 software (SAS Institute, Cary, NC). Specific IgE levels in children and mothers were dichotomized at the detection limit of 0.2 IU/mL to compare IgE levels in peripheral blood with the low IgE levels in cord blood. A higher cutoff of 0.35 IU/mL was also explored. In addition to specific IgE to the particular allergens, combinations of specific IgE were defined for IgE to seasonal, perennial, and food allergens. Combined variables were created to reflect the time course in atopic sensitization between birth and 12 months (Table I). *Never sensitized* was defined as the absence of detectable IgE to the same allergen specificity at birth and at 12 months. *Incident sensitization* was defined as detectable IgE at 12 months but not at birth. *Transient sensitization* was defined as detectable specific IgE at birth but not at 12 months. *Persistent sensitization* was defined as detectable specific IgE for the same allergen specificity at birth and at 12 months. When testing associations or concordances, the never sensitized category served as a reference category for all others.

Agreement between detectable IgE levels between birth and 12 months was assessed by using the McNemar test. Farm and reference children were compared for detectable IgE by using the Fisher exact test. Concordances between detectable IgE levels at birth and 12 months were calculated by using the Kendall τ partial on study center and study group.

The data provided by questionnaires and diaries were reduced by combination variables, reflecting quarterly or annual exposure and subsequent variable cluster analysis. Early exposure was defined by the time point of first contact to an exposure (food, day care, infections, or a farm-related exposure) and coded by using 5 categories (never or in the fourth, third, second, or first quarters). Infections were also assessed with respect to the frequency of episodes and the diversity of disease entities. The 152 variables representing environmental determinants and potential confounders were first tested for associations with incident sensitization in models only adjusted for farming and study center. The 31 variables with P values of less than .05 were subsequently entered in multiple logistic regression by using stepwise selection. In addition to the core model from the stepwise regression, an extended model was established, including potential standard confounders and variables on maternal IgE to the respective allergens. Because the study was based on previous knowledge and the variables were intercorrelated, corrections for multiple testing were not performed, which is well accepted in classical epidemiology.[13] Nevertheless, a conservative significance level of 1% was chosen for the stepwise regression models. In addition, a factor analysis based on the correlation matrix of all exposure variables was performed with varimax rotation, extracting 15 factors. Because exposure variables were continuous, categorical, or dichotomous, all variables were rescaled to a range of 0 to 1, and the Kendall τ value was used to calculate the correlation matrix. The respective factors were then entered into logistic regression models for incident sensitization to seasonal, perennial, and food allergens. These models were adjusted for center and maternal IgE level to the same allergen. The resulting P values were corrected for 15 tests.

Center homogeneity was assessed based on interaction terms in logistic regression models. Models for persistent sensitization could not be established because of its low frequency. The models for transient sensitization did not

TABLE I. Patterns of atopic sensitization over time

Pattern	IgE at birth	IgE at 12 mo
Never sensitized*	No	No
Incident sensitization	No	Yes
Transient sensitization	Yes	No
Persistent sensitization	Yes	Yes

**Never sensitized* was used as the reference category of the mutually exclusive categories of incident, transient, and persistent sensitization.

differ from those for sensitization in cord blood published earlier,[5] and hence they are not reported in this article.

RESULTS

The selection of the present study sample is shown in Fig 1. Of the original birth cohort (n = 1133), 793 children were included. Differences between the subpopulations included and not included in the present analysis are shown in Table E1 in this article's Online Repository at www.jacionline.org. The participation rates differed between the centers.

Allergen-specific IgE levels rarely exceeded 3.5 IU/mL (<3% of all children) at 12 months and never at birth. The 95th percentiles at 12 months were consistently less than 0.7 IU/mL (RAST class 2) for any IgE specificity, except cat (1.3 IU/mL). As shown in Fig E1, *A*, there was a slight trend toward lower levels of positive controls over time both in cord blood and at 12 months. However, the levels of positive controls at both time points did not differ between children with and without any specific IgE at 12 months (see Fig E1, *B* and *C*); hence a systemic bias of time point of measurement on changing IgE levels at birth and at 12 months was excluded.

At 12 months, the prevalences of a doctor's diagnosis of atopic eczema and food allergy were 9.8% and 2.1%, respectively. These prevalences did not differ significantly between farm and reference children, although atopic eczema was less common in farm children (odds ratio [OR], 0.64; 95% CI, 0.39-1.04; $P = .0713$). There were no significant associations between the above diagnoses and incident sensitization to seasonal, perennial, and food allergens. Significant associations for the above diagnoses were only found with relatively rare IgE specificities (<3%), such as hen's egg and wheat flour.

When comparing the frequencies of sensitization between birth and 12 months, specific IgE to any allergen except hen's egg and grass pollen was more common at the latter time point, irrespective of the detection limit applied (Fig 2). The most pronounced increase in IgE prevalence was seen for perennial allergens (particularly dog and cat) similarly across all study centers (see Fig E2 in this article's Online Repository at www.jacionline.org).

Persistent sensitization was very uncommon for all allergen specificities studied (Fig 3). This is also reflected by very low concordances between the 2 time points, as determined based on Kendall τ values of less than 0.15 for all specificities.

The overall changes in sensitization patterns are summarized in Fig 4. Of all 793 children included in the present analysis, almost half (45%) were never sensitized. Incident sensitization was seen in 34%, transient sensitization in 11%, and persistent sensitization in 10%. Only 8 (1%) children were sensitized against exactly the same allergen specificities at both time points. Restriction of the models to children whose mothers were not sensitized to the same allergens as their children did not reveal substantial changes (see

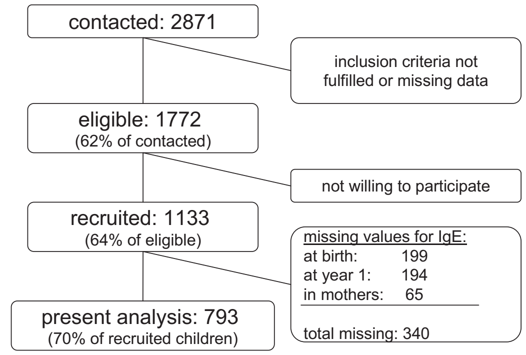

FIG 1. Selection of the study population. In the recruited sample of 1133 children, complete IgE data at birth, at 12 months, and in mothers were available for 793 (378 farm and 415 nonfarm) children.

Fig E3 in this article's Online Repository at www.jacionline.org). Separate analyses for farm and reference children revealed similar path diagrams (see Fig E4 in this article's Online Repository at www.jacionline.org). When assessing the allergen categories of seasonal, perennial, and food allergens separately, the overall picture was similar (see Fig E5 in this article's Online Repository at www.jacionline.org), although incident sensitization was less common for seasonal (8%) and food (19%) allergens, and transient sensitization was less common for perennial allergens (2%).

When comparing the 2 study groups of farm and reference children, only a few differences were found: transient IgE to grass and tree pollen was almost only seen in reference children (Fig 5, *C*). However, the most evident disparity was seen in specific IgE to cow's milk and to any food allergen, which were more common in farm children at 12 months with respect to both prevalence and incidence (Fig 5, *A* and *B*). We assumed that IgE to cow's milk was related to cow's milk consumption, which is more common in farm children. Hence we explored the association of farm milk consumption with cow's milk IgE levels at 12 months. After adjustment for farming and center, farm milk consumption was still positively related to cow's milk IgE levels (adjusted odds ratio [aOR], 1.35; 95% CI, 0.83-2.19). However, an in-depth analysis revealed that consumption of boiled (aOR, 1.71; 95% CI, 1.03-2.84) but not unboiled (aOR, 0.76; 95% CI, 0.40-1.44) farm milk conferred the effect.

To assess the effect of maternal sensitization on the children's sensitization patterns, we tested their associations adjusted for farming and center. For transient and persistent sensitization, a gradient emerged for the strength of the association with maternal sensitization over the allergen specificities with strongest associations with food allergens, followed by perennial allergens, and weak associations with seasonal allergens (Fig 6). For incident sensitization, the associations were equally weak but definitively present for perennial and food allergens (Fig 6).

Next, the environmental determinants of incident sensitization to seasonal, perennial, and food allergens were explored in multivariable models (Table II). Consumption of fruit during pregnancy was found to be positively related to seasonal IgE levels, whereas early contact with silage and early consumption of cereals were inversely related. Consumption of cereals was also inversely associated with IgE levels to perennial allergens. A potential risk factor for perennial IgE was early contact with farm animals other than cows, pigs, horses, or poultry, such as sheep, goats, and hares. Contact with dogs, a relevant source of

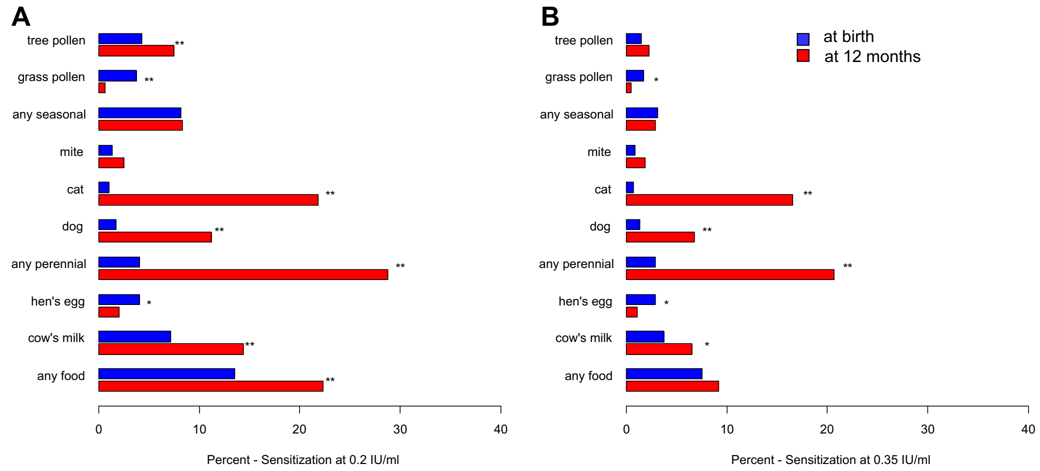

FIG 2. Detectable specific IgE at birth and at 12 months. Prevalences of detectable specific IgE are given in percentage at 2 cutoffs: **A,** IgE level of 0.2 IU/mL or greater; **B,** IgE level of 0.35 IU/mL or greater. Significant differences between the 2 time points (at birth and in the first year) are marked as follows: $*P < .05$ and $**P < .01$.

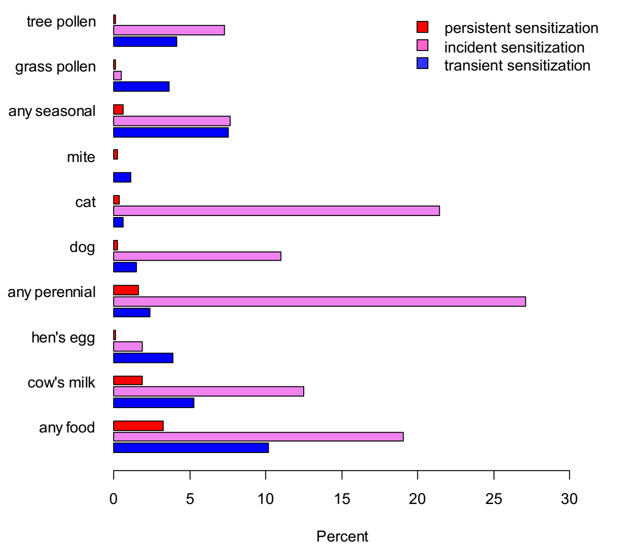

FIG 3. Proportions of persistent, incident, and transient sensitization to several allergen specificities for IgE levels of 0.2 IU/mL or greater.

perennial allergen, was inversely related to perennial IgE levels, but did not enter the models because of marginally exceeding the predefined significance level. In contrast, contact with cats was not related to perennial IgE levels.

The only determinant for food allergy was the study group variable of farm children versus reference children. When additionally adjusting the models in Table II for the respective maternal IgE specificities and further confounders, the models remained stable, and the estimates of the determinants did not change (Table II). As shown in Table E2 in this article's Online Repository at www.jacionline.org, restriction of the models to children whose mothers were not sensitized to the same allergens as their children hardly altered any estimate presented in Table II.

The associations of the IgE outcomes and their determinants were consistent across the study centers, as determined based on P values of interaction terms of greater than .15 in logistic regression models. Similar models were established when applying a higher cutoff for IgE of 0.35 IU/mL (data not shown).

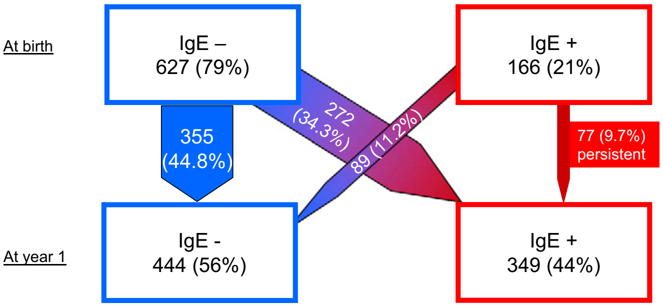

FIG 4. Pathway diagram of incident (n = 272), transient (n = 89), and persistent (n = 77) sensitization for IgE levels of 0.2 IU/mL or greater. Absolute numbers (or percentages) are given for children who stay negative for IgE production (IgE−), start, abolish, or continue IgE production (IgE+) within the first year.

Finally, 15 factors were extracted from a factor analysis on the 152 exposure variables. For IgE to seasonal allergens, an inverse association with factor 7 was found (aOR, 0.20; 95% CI, 0.07-0.56; $P = .0023$; Bonferroni-corrected $P = .0345$), and for IgE to food allergens, a positive association with factor 1 was found (aOR, 2.08; 95% CI, 1.35-3.21; $P = .0010$; Bonferroni-corrected $P = .0150$). Adjustment for maternal IgE to the same allergens did not change the estimates of the associated factors (data not shown). None of the 15 factors was significantly associated with IgE levels to perennial allergens. Factor 1 was related to farming and contact with cattle, and factor 7 was related to consumption of cereals (see Table E3 in this article's Online Repository at www.jacionline.org).

DISCUSSION

Between birth and age 12 months, substantial fluctuation of specific IgE levels was seen. Persistent sensitization was rare, whereas incident postnatal and transient perinatal sensitization were found in substantial proportions of children. Incident postnatal sensitization was seen for perennial allergens in particular, followed by food allergens. However, food allergens were also the most common elicitors of transient sensitization. Positive associations of

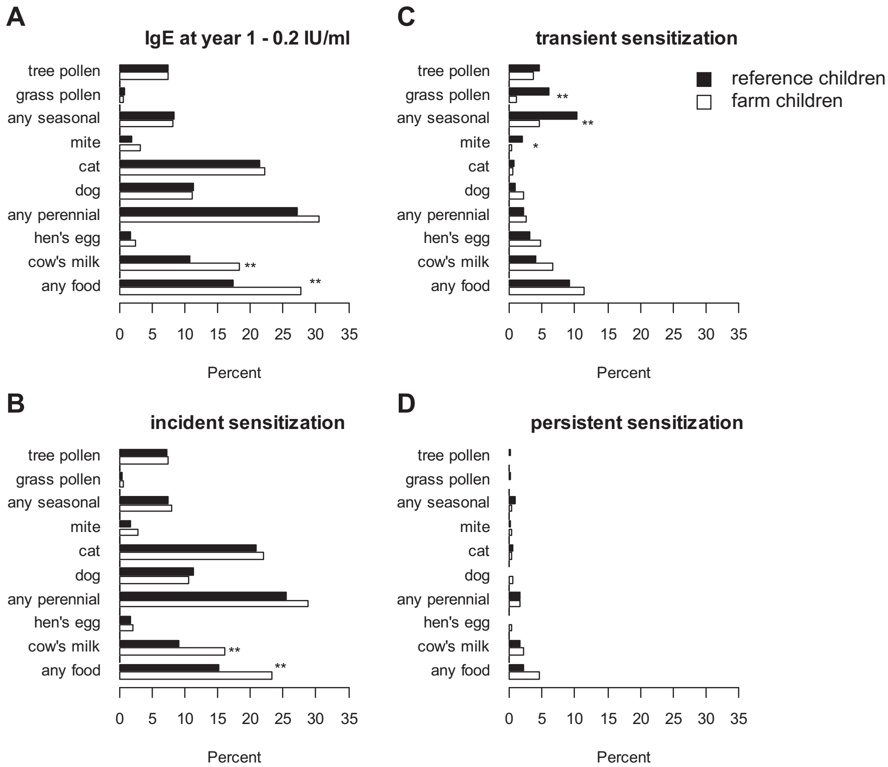

FIG 5. Detectable specific IgE stratified by farm and reference children. **A,** Prevalences of detectable specific IgE given in percentages at IgE levels of 0.2 IU/mL or greater. **B,** Prevalences of new cases of specific IgE, ie, IgE-positive children at 12 months who were negative for the respective IgE specificities at birth. **C,** Prevalences of lost IgE, ie, IgE-negative children at 12 months who were positive for the respective IgE specificities at birth. **D,** Prevalences of persistent IgE cases, ie, IgE-positive children at 12 months who were positive for the respective IgE specificities at birth. Significant differences between farm *(white bars)* and reference *(black bars)* children are marked as follows: $^{*}P < .05$ and $^{**}P < .01$.

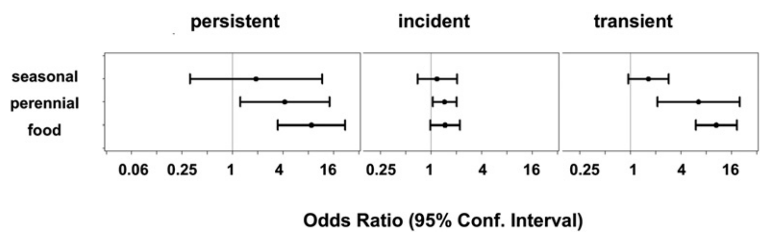

FIG 6. Associations between child's and maternal sensitization.

maternal IgE and child IgE levels to the same allergen were found for transient and incident sensitization. The strength of these associations differed with allergen specificities; the associations were strongest for food allergens and weakest for seasonal allergens. Incident sensitization was related to distinct prenatal and postnatal environmental exposures of the mother and child.

Potential contamination of cord blood by maternal IgE

The interpretation of these data first requires some reflection on the question of whether cord blood is contaminated by maternal IgE. One approach to this issue is comparing IgE and IgA levels in cord blood. IgA is believed not to be synthesized by the fetus; hence detection of IgA in cord blood is considered indicative of maternofetal transfusion. Indeed, correlations between cord blood IgE and IgA levels have been described, although only in a minority of subjects.[1,2] In those cases transplacental transfusion of IgE cannot be excluded; however, in the majority of children, cord blood IgE levels are not associated with transfused maternal IgA levels, ultimately supporting the notion of a proper fetal origin of IgE. In the PASTURE cohort we had tested 60 randomly selected IgE-positive cord blood samples and detected IgA at a cutoff level of 32 mg/L in 3 subjects,[4] indicating genuine atopic sensitization in the vast majority of newborns with specific IgE in cord blood. This is supported by Kamemura et al,[14] who excluded contamination of cord blood by maternal IgA using a highly sensitive method. A further argument against contamination of cord blood is the detection of specific IgE in the cord blood of children of mothers with negative results for the same IgE

TABLE II. Determinants of incident sensitization to inhalant or food allergens at 12 months (IgE ≥0.2 IU/mL)

	IgE to seasonal allergens (n = 728)		IgE to perennial allergens (n = 761)		IgE to food allergens (n = 686)	
	Core model	Extended model	Core model	Extended model	Core model	Extended model
Farming	1.17 (0.65-2.11); P = .6016	1.40 (0.73-2.68); P = .3067	1.17 (0.84-1.64); P = .3445	1.28 (0.86-1.90); P = .2332	**1.90 (1.31-2.75); P = .0007**	**2.11 (1.33-3.34); P = .0015**
Early contact with silage	**0.56 (0.39-0.81); P = .0020**	**0.57 (0.40-0.82); P = .0024**		0.91 (0.78-1.06); P = .2227		0.98 (0.83-1.16); P = .7966
Early consumption of purchased cereals*	**0.67 (0.50-0.90); P = .0072**	0.68 (0.50-0.91); P = .0105		1.00 (0.85-1.18); P = .9671		1.00 (0.83-1.21); P = .9857
Early consumption of homemade cereals†		0.82 (0.41-1.65); P = .5763	**0.56 (0.37-0.86); P = .0087**	0.59 (0.38-0.92); P = .0194		0.81 (0.50-1.33); P = .4101
Consumption of fruit in pregnancy	**2.71 (1.44-5.07); P = .0019**	**2.64 (1.39-5.04); P = .0032**		0.80 (0.55-1.15); P = .2224		0.98 (0.65-1.49); P = .9292
Early contact with sheep, goats, and hares		0.98 (0.73-1.32); P = .9034	**1.30 (1.10-1.54); P = .0019**	**1.30 (1.10-1.54); P = .0026**		0.92 (0.75-1.13); P = .4171
Sex (male)		0.71 (0.40-1.25); P = .2339		0.86 (0.61-1.21); P = .3796		1.13 (0.76-1.66); P = .5519
Family history of atopy		1.37 (0.74-2.52); P = .3121		0.67 (0.47-0.95); P = .0253		1.10 (0.73-1.66); P = .6398
High parental education		1.15 (0.40-3.30); P = .7900		0.47 (0.26-0.87); P = .0155		0.73 (0.38-1.44); P = .3661
At least 2 older siblings		0.68 (0.35-1.31); P = .2447		0.74 (0.51-1.09); P = .1271		1.10 (0.72-1.67); P = .6601
Cesarean section		1.02 (0.47-2.22); P = .9605		0.73 (0.44-1.20); P = .2132		1.18 (0.69-2.03); P = .5466
Breast-feeding		1.05 (0.98-1.14); P = .1779		0.98 (0.94-1.03); P = .4286		0.96 (0.91-1.01); P = .0937
Maternal IgE to same allergens		0.98 (0.54-1.79); P = .9510		1.51 (1.06-2.13); P = .0218		1.61 (1.05-2.46); P = .0281
Center: Switzerland vs Austria	3.09 (1.30-7.35); P = .0105	2.83 (1.15-6.97); P = .0233	1.56 (0.91-2.68); P = .1032	1.58 (0.89-2.80); P = .1157	1.52 (0.85-2.72); P = .1602	1.48 (0.78-2.81); P = .2291
Center: France vs Austria	1.41 (0.53-3.73); P = .4934	1.63 (0.59-4.53); P = .3475	**2.64 (1.53-4.53); P = .0005**	**2.31 (1.29-4.14); P = .0048**	1.74 (0.99-3.06); P = .0552	1.15 (0.61-2.20); P = .6663
Center: Germany vs Austria	2.29 (0.93-5.63); P = .0720	2.03 (0.79-5.23); P = .1434	1.38 (0.80-2.38); P = .2422	1.28 (0.72-2.28); P = .4041	0.87 (0.47-1.60); P = .6468	0.71 (0.36-1.40); P = .3239
Center: Finland vs Austria	0.90 (0.33- 2.46); P = .8338	0.85 (0.28-2.63); P = .7808	**2.38 (1.33-4.27); P = .0037**	**2.91 (1.56-5.43); P = .0008**	0.99 (0.55-1.76); P = .9644	1.04 (0.52-2.10); P = .9071

ORs are given with 95% CIs and *P* values (Wald test). *P* values of less than .01 are shown in boldface. All models are adjusted mutually for all the variables listed in the respective columns. The "core models" result from stepwise regression with the farming and center variables forced in; the "extended models" are the core models plus potential confounders plus maternal IgE to the same allergen specificity as assessed in the child.
*Consumption of purchased cereals in 5 categories: not introduced until the end of the first year or introduced in the fourth, third, second, and first quarters.
†Consumption of homemade cereals in 2 categories: not introduced versus introduced until the end of the first year.

specificity. In PASTURE mothers had negative IgE results in up to 65% of IgE-positive cord blood samples for the same allergens, and a substantial proportion of IgE-positive children had higher IgE scores than their mothers.[4] Moreover, substantial associations of maternal sensitization with persistent and incident sensitization were found, although both persistent and incident sensitization patterns are defined by detectable IgE levels at 12 months of age. However, the latter observation cannot be explained by maternofetal transfusion of IgE because maternal IgE would have already been cleared from the child's blood 12 months after transfusion. Furthermore, the strength of the association of transient and maternal sensitization differed considerably across the allergen specificities, which is consistent with a recent publication.[14]

This gradient also challenges the concept of maternofetal IgE transfer: Why should the placental barrier be selective for the antigen specificity of an IgE molecule? Several options of IgE transfer through the syncytiotrophoblast, the first hurdle of the placental barrier, have been suggested, among them immune complexes comprising specific antigens, IgG, and IgE.[15] In an *ex vivo* placental perfusion model, transfer of IgE-allergen complexes has been described for food allergens, but inhalant allergens were not transferred.[16] It remains unclear whether maternal IgE is trapped in the villous stroma beyond the syncytiotrophoblast (eg, for fetal protection from maternal immune attacks) or whether it might definitively enter the fetal circulation. It is conceivable that small amounts of IgE-antigen immune complexes can cross both elements of the placental barrier. In small doses the antigen might induce a measurable immune response. This putative fetal immune response to transferred allergens might last throughout the first year of life and explain why we detected an association of persistent and incident sensitization with maternal sensitization. An alternative explanation of this association might be found in genetic or epigenetic phenomena, which might also be accountable for the residual correlation between transient and maternal sensitization.

Trajectories of specific sensitization in the first year

Among the many birth cohorts established for the assessment of asthma and atopy, only a few have gathered data on sensitization patterns already at birth. The investigations published thus far display a complex pattern of IgE production within the first year of life.[1,17-19] The common denominator of all those studies seems to be a certain fluctuation of specific IgE to food and inhalant allergens at low levels, with a tendency to decrease until the age of 12 months; only beyond that age is a steady increase seen in atopic subjects.

The results of our study fit well into this picture because persistent sensitization was very uncommon. On the basis of the observed inconsistencies, the first year might be interpreted as a time period rather insusceptible to atopic sensitization. In contrast, the development of atopic sensitization might be promoted in this very period, but a consistent IgE response might become measurable only with some delay. The identification of environmental determinants for incident postnatal sensitization argues in favor of early postnatal adaptation of the immune system. In this context the postnatal fluctuation of specific IgE levels might appear as "the signature of an underlying process involving initial T_H2-cell activation followed by development of protective immunologic tolerance," as suggested by Rowe et al.[20] Determinants for this process might be identified by comparing transient and persistent sensitization, which failed in our dataset because of the limited numbers of children with persistent sensitization. However, it is conceivable that this underlying process might already be terminated in some subjects at the age of 12 months, whereas in others it is still ongoing. Whether these early signals of atopic sensitization are related to manifestation of disease in later life remains elusive. Currently, the only clues to disease manifestation known from other studies are the presence of persistent[21] and multiple early[22] sensitization.

Environmental determinants of new IgE at 12 months

PASTURE was mainly set up to study the influences of farm-related exposures on asthma and atopy; therefore farm exposures are quite accurately covered. The many questions asked on farm-related exposures and maternal and child nutrition required data reduction. Hence we grouped variables using a variable cluster procedure and extracted only the most informative variables for further use, which represented also other closely related variables. Nevertheless, a multitude of tests had to be performed. In a confirmatory setting neglecting any previous knowledge correcting for multiple testing of 152 variables would have been necessary, and the α level should have been decreased to 0.0003 when applying Bonferroni correction to an effective α level of 5%. Hence all associations presented in Table II would have missed significance. As a sensitivity analysis, we performed a factor analysis, extracting 15 factors, and corrected the association P values for 15 tests. Despite this conservative approach, significant associations were detected for 2 of the 3 outcomes (seasonal and food IgE levels). The associations were in the expected direction, and the variables loading on the respective factors reflected the determinants of the stepwise regression models presented in Table II. In the context of both the exploratory and confirmatory analysis strategy, we conclude that there are relevant environmental influences on production of specific IgE in the first year of life.

In an earlier analysis of cord blood IgE, the mother's exposure to animal sheds during pregnancy was a major protective factor for seasonal IgE (OR, 0.45; 95% CI, 0.25-0.82; $P = .009$).[5] When applying the same model to incident sensitization, the association was attenuated by approximately 30% but was still present (OR, 0.57; 95% CI, 0.32-1.04; $P = .069$). Because of slightly missing the significance threshold, this exposure variable was replaced in the final model (Table II) by the variable "early contact with silage," which might capture the actual exposure more precisely. In addition, silage has already been identified as a protective determinant for asthma in other studies, and its relation to fermentation and the presence of Lactobacillus, Aspergillus, and Listeria species is known.[23] The latter have been related to asthma protection.[24]

For perennial IgE, early contact with goats, sheep, or hares was a risk factor, which is well in line with a previous study.[23]

Among nutrition-related variables covered by the questionnaires, the early introduction of cereals emerged as a protective factor for sensitization to inhalant allergens. It has been speculated that the antioxidant properties of cereals might explain the lower mortality of inflammatory diseases in subjects consuming a diet rich in cereals.[25] In this context consumption of cereals might indeed modify the immune response to allergens and protect against manifestation of atopic disease. In contrast, the consumption of fruits during pregnancy appeared to be a risk factor for IgE to seasonal allergens. An explanation might be found in cross-reactivity of pollen allergens with the Bet v 6–related allergens or profilins.[26] The somewhat higher frequency of the latter variables in the analysis sample was attributable to varying participation rates across centers (see Table E1), for which all models were adjusted.

At first glance, the higher rate of sensitization to cow's milk in farm children seems counterintuitive. However, an in-depth analysis showed that this positive association was restricted to consumption of boiled farm milk. In contrast, consumption of unboiled farm milk exhibited a slightly inverse association. Analogous relations were found for cord blood IgE, as reported earlier.[5] School-aged children can consume unboiled milk more frequently than babies. This might explain the inverse association of farm milk consumption and food sensitization detected in schoolchildren in the PARSIFAL study.[27]

From the above findings, we conclude that there is a mixed picture of sensitization patterns between birth and age 12 months. Beyond the apparent disarrangement, however, some traces of systematic order emerge: the sensitization patterns vary (1) with the allergen specificity and (2) with the route of exposure and (3) are influenced by environmental determinants, the effect of which cannot merely be attributed to chance. In the face of the generally low allergen-specific IgE levels at both time points and the missing association with atopic eczema and food allergy, it remains open whether this "signature" of atopic sensitization will finally affect the development of atopic disease in later life.

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Clinical implications: Prenatal and early postnatal environmental and maternal factors determine atopic sensitization in the first year of life. Hence it might be advisable to consider potential preventive measures already in these periods.

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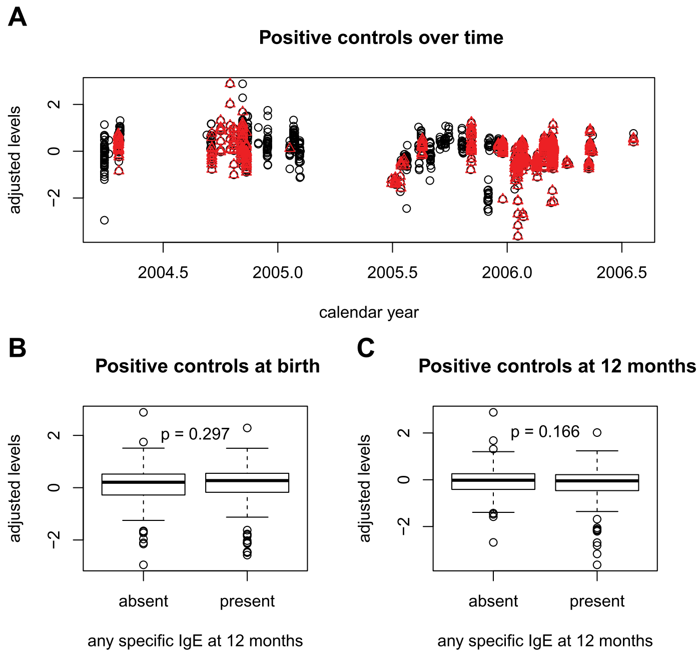

FIG E1. Levels of positive controls over time. Positive controls were used to standardize IgE measurements. **A,** Adjusted levels of positive controls are given over time for measurements in cord blood *(black circles)* and at 12 months *(red triangles)*. **B** and **C,** Adjusted levels of positive controls for IgE measurements at birth (Fig E1, *B*) and at 12 months (Fig E1, *C*) are given separately for children with and without any specific IgE at 12 months. Levels of positive controls were adjusted for study center, study group, and version of measurement software after logarithmic transformation.

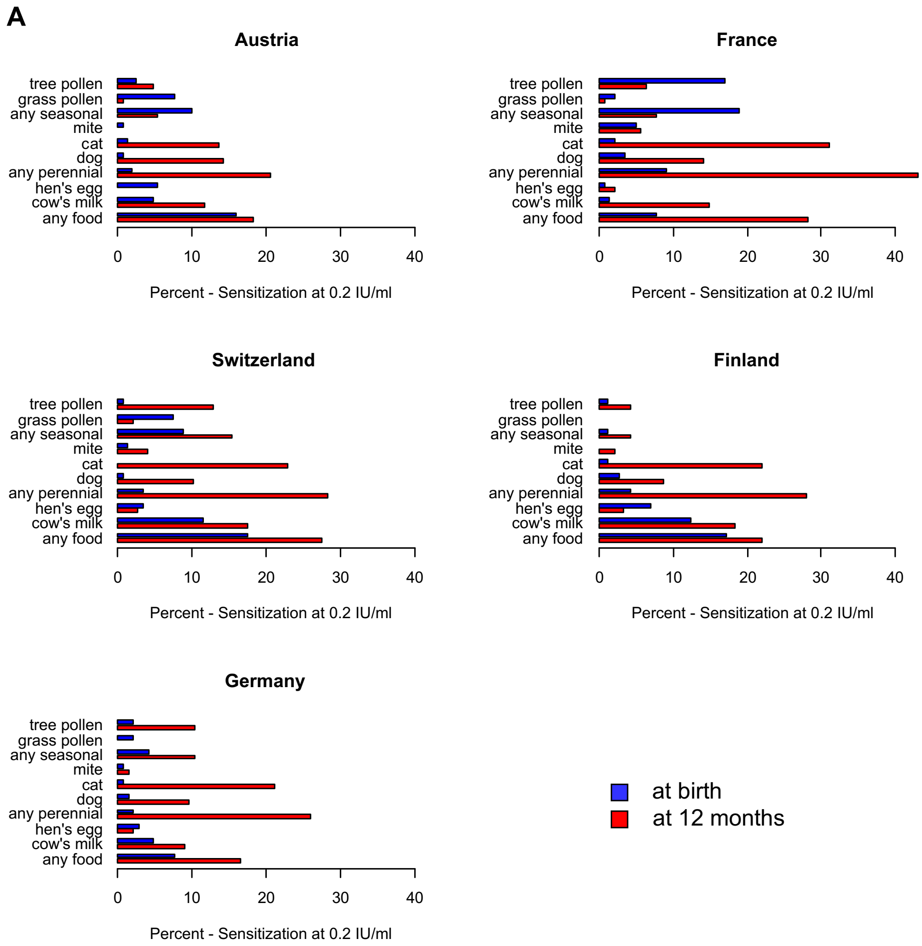

FIG E2. Detectable specific IgE at birth and at 12 months stratified by center. **A,** Prevalences of detectable specific IgE levels of 0.2 IU/mL or greater. **B,** Prevalences of detectable specific IgE levels of 0.35 IU/mL or greater.

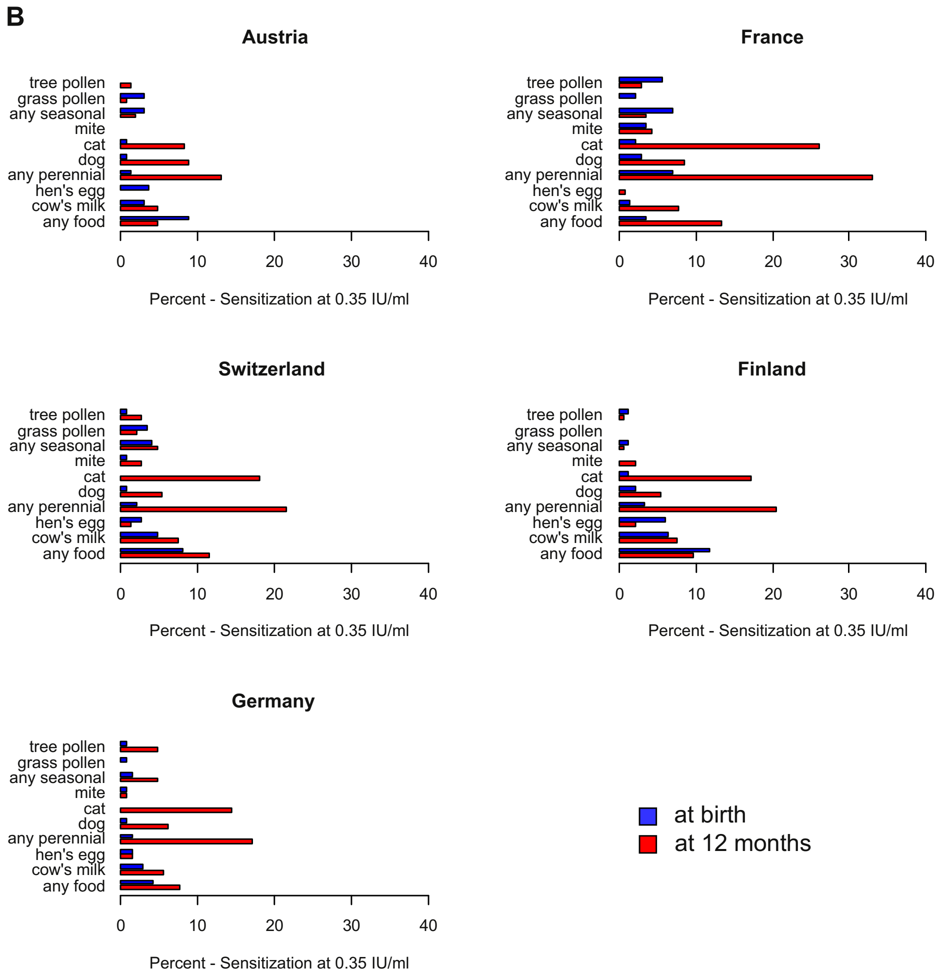

FIG E2. *(Continued)*

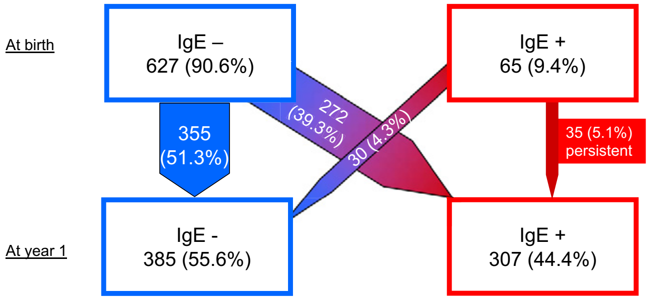

FIG E3. Paths of new onset, loss, or persistence of IgE production (cutoff, 0.2 IU/mL) restricted to children whose mothers were not sensitized to the same allergens. Absolute numbers (or percentages) are given for children who continue to have negative results for IgE production *(IgE−)* or start, abolish, or continue IgE production *(IgE+)* within the first year.

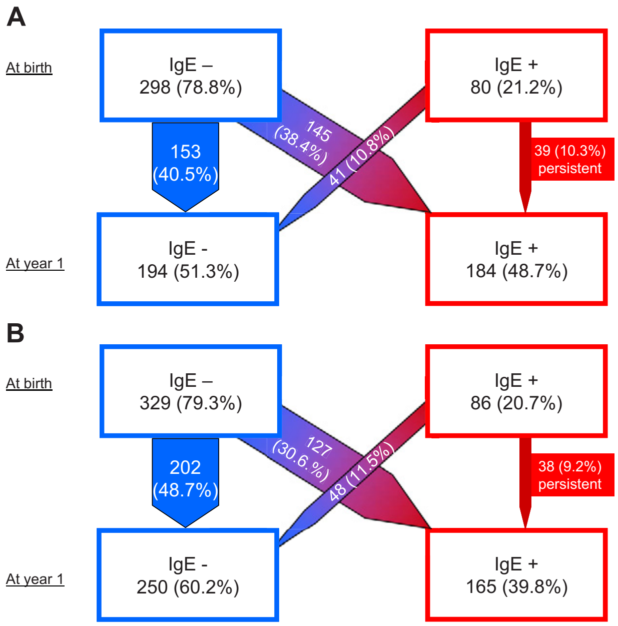

FIG E4. Paths of new onset, loss, or persistence of IgE production (cutoff, 0.2 IU/mL) stratified by the study groups farm children **(A)** versus reference children **(B)**. Absolute numbers (or percentages) are given for children who continue to have negative results for IgE production *(IgE−)* or start, abolish, or continue IgE production *(IgE+)* within the first year.

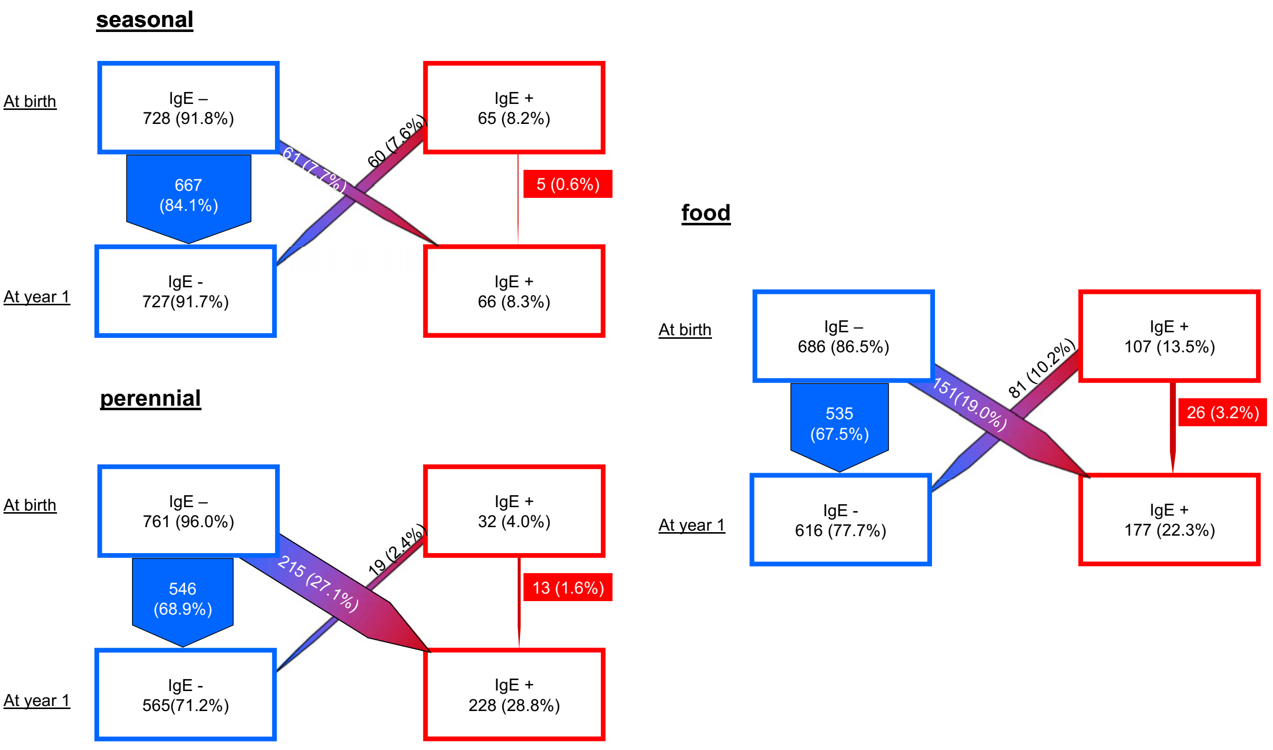

FIG E5. Paths of new onset, loss, or persistence of IgE production (cutoff, 0.2 IU/mL) stratified by the broad categories of sensitization against seasonal, perennial, and food IgE. Absolute numbers (or percentages) are given for children who continue to have negative results for IgE production *(IgE−)* or start, abolish, or continue IgE production *(IgE+)* within the first year.

TABLE E1. Population's characteristics

	Not included (n = 340)		Included (n = 793)		
	No.	Percent	No.	Percent	*P* value, χ^2 test
Farming	152	44.7	378	47.7	.3599
Center					
Austria	50	14.7	170	21.4	<.0001
Switzerland	93	27.4	149	18.8	
France	61	17.9	142	17.9	
Germany	108	31.8	146	18.4	
Finland	28	8.2	186	23.5	
Sex (male)	160	53.9	400	50.4	.3130
Parental atopy	171	54.6	420	53.4	.7193
Maternal atopy	109	32.2	262	33.0	.7712
High parental education	289	88.4	719	91.4	.1226
At least 2 older siblings	112	32.9	267	33.7	.8118
Child in stable of cows, pigs, etc	171	62.2	496	64.3	.5405
Child in stable of poultry	38	13.8	140	18.1	.1018
Early contact with sheep, goats, and hares	47	17.1	120	15.5	.5474
Early contact with silage	74	27.0	193	25.0	.5127
Dogs in the first year of life	83	31.7	248	31.7	.9918
Cats in the first year of life	122	46.6	351	44.9	.6364
Early consumption of purchased cereals*	178	64.7	489	63.3	.6816
Early consumption of homemade cereals†	89	32.4	335	43.4	.0014‡
Consumption of fruit in pregnancy	167	49.4	454	57.5	.0119‡
Consumption of farm milk	69	25.1	196	25.4	.9223
Atopic dermatitis	27	10.3	76	9.8	.8109
Food allergy	8	3.1	16	2.1	.3464
Asthma (doctor's diagnosis)	7	2.7	39	5.0	.1147
Wheeze until month 2	14	4.7	42	5.3	.6981
Wheeze 3-12 mo	68	26.1	221	28.3	.4834

	Mean (SD)	Mean (SD)	*P* value, *t* test
Duration of breast-feeding in the first year (mo)	5.9 (4.0)	6.1 (4.1)	.5649

*Consumption of purchased cereals in 2 categories: not introduced versus introduced until the end of the first year.
†Consumption home-made cereals in 2 categories: not introduced versus introduced until the end of the first year.
‡These differences are caused by different participation rates across centers. If adjusting for center, the differences between the subsamples are no longer significant (P = .364 and P = .311, respectively).

TABLE E2. Determinants of incident sensitization to inhalant or food allergens at 12 months (IgE ≥0.2 IU/mL) restricted to children whose mothers were not sensitized to the same allergens

	IgE to seasonal allergens (n = 660)		IgE to perennial allergens (n = 678)		IgE to food allergens (n = 660)	
	Core model	Extended model	Core model	Extended model	Core model	Extended model
Farming	1.31 (0.71-2.43), *P* = .3895	1.60 (0.81-3.15), *P* = .1754	1.17 (0.82-1.66), *P* = .3862	1.24 (0.82-1.89), *P* = .3123	**1.97 (1.35-2.88); *P* = .0005**	**2.21 (1.37-3.55), *P* = .0011**
Early contact with silage	**0.46 (0.29-0.73), *P* = .0010**	**0.45 (0.28-0.72), *P* = .0009**		0.94 (0.80-1.10), *P* = .4321		0.98 (0.83-1.16), *P* = .7934
Early consumption of purchased cereals*	**0.67 (0.49-0.90), *P* = .0092**	**0.66 (0.48-0.91), *P* = .0100**		0.95 (0.80-1.13), *P* = .5324		0.98 (0.81-1.19), *P* = .8429
Early consumption of homemade cereals†		0.78 (0.36-1.68), *P* = .5254	**0.54 (0.34-0.86), *P* = .0089**	0.58 (0.36-0.93), *P* = .0234		0.79 (0.48-1.31), *P* = .3680
Consumption of fruit in pregnancy	**2.79 (1.45-5.35), *P* = .0021**	**2.75 (1.40-5.41), *P* = .0034**		0.81 (0.55-1.19), *P* = .2788		1.06 (0.68-1.64), *P* = .7999
Early contact with sheep, goats, and hares		1.00 (0.73-1.37), *P* = .9881	**1.29 (1.07-1.54), *P* = .0062**	**1.29 (1.07-1.55), *P* = .0081**		0.90 (0.73-1.13), *P* = .3666
Sex (male)		0.64 (0.35-1.19), *P* = .1569		0.84 (0.59-1.20), *P* = .3349		1.11 (0.74-1.65), *P* = .6192
Family history of atopy		1.58 (0.82-3.05), *P* = .1730		0.72 (0.49-1.05), *P* = .0865		1.15 (0.75-1.76), *P* = .5239
High parental education		1.35 (0.42-4.37), *P* = .6192		0.50 (0.26-0.96), *P* = .0380		0.86 (0.42-1.76), *P* = .6697
At least 2 older siblings		0.69 (0.34-1.36), *P* = .2804		0.69 (0.47-1.03), *P* = .0720		1.13 (0.74-1.74), *P* = .5676
Cesarean section		0.90 (0.39-2.05), *P* = .7931		0.68 (0.40-1.15), *P* = .1465		1.25 (0.73-2.15), *P* = .4239
Breast-feeding		1.09 (1.00-1.18), *P* = .0433		0.98 (0.93-1.03), *P* = .3755		0.95 (0.90-1.00), *P* = .0660
Maternal IgE to same allergens		0.87 (0.46-1.65), *P* = .6731		1.44 (1.00-2.08), *P* = .0528		1.53 (0.99-2.37), *P* = .0584
Center: Switzerland vs Austria	**4.79 (1.76-13.02), *P* = .0022**	**4.38 (1.56-12.30), *P* = .0051**	1.48 (0.82-2.66), *P* = .1938	1.56 (0.83-2.91), *P* = .1666	1.56 (0.86-2.83); *P* = .1431	1.62 (0.84-3.11), *P* = .1506
Center: France vs Austria	1.90 (0.64-5.62), *P* = .2471	2.51 (0.80-7.85), *P* = .1140	**2.61 (1.47-4.62), *P* = .0010**	**2.31 (1.25-4.25), *P* = .0073**	1.63 (0.90-2.93); *P* = .1044	1.08 (0.55-2.11), *P* = .8291
Center: Germany vs Austria	3.01 (1.07-8.44), *P* = .0368	2.75 (0.93-8.10), *P* = .0675	1.45 (0.81-2.57), *P* = .2086	1.36 (0.73-2.51), *P* = .3306	0.85 (0.45-1.59); *P* = .6099	0.74 (0.37-1.47), *P* = .3893
Center: Finland vs Austria	1.41 (0.46-4.33), *P* = .5459	1.30 (0.37-4.62), *P* = .6825	**2.60 (1.39-4.86), *P* = .0028**	**3.24 (1.66-6.33), *P* = .0006**	1.01 (0.56-1.81); *P* = .9737	1.10 (0.54-2.25), *P* = .8010

ORs are given with 95% CIs and *P* values (Wald test). *P* values of .01 or less are shown in boldface. All models are adjusted mutually for all the variables listed in the respective columns. The "core models" result from stepwise regression with the farming and center variables forced in; the "extended models" are the core models plus potential confounders plus maternal IgE to the same allergen specificity as assessed in the child.

*Consumption of purchased cereals in 5 categories: not introduced until the end of the first year or introduced in the fourth, third, second, and first quarters.

†Consumption of homemade cereals in 2 categories: not introduced versus introduced until the end of the first year.

TABLE E3. Correlations of factors with distinct variables

Factor	Variable*	Kendall τ
1	Farming	0.70
	Keeping cows during pregnancy	0.68
	Early contact with hay	0.66
	Contact with animal shed during pregnancy	0.62
	Forestry	0.61
	Exposure to animal shed in first year	0.55
	Early contact with silage	0.54
	Contact with barn during pregnancy	0.54
	Consumption of milk directly from a farm	0.54
	Presence while mother was dispersing straw	0.52
	Presence while mother was cleaning animals	0.51
7	Consumption of cereals other than wheat, rye, barley, and oat	0.67
	Consumption of purchased cereals	0.65
	Consumption of homemade cereals	0.62

*Only variables with Kendall τ values of greater than 0.5 are listed.