

Sensitization to grass pollen allergen molecules in a birth cohort—natural Phl p 4 as an early indicator of grass pollen allergy



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Background: Grass pollen allergy is one of the most common allergies worldwide.

Objective: The aim of this study was to evaluate the usefulness of grass pollen allergen molecules for prediction of grass pollen allergy during childhood and up to adolescence.

Method: Questionnaire data and sera obtained from the study subjects at the ages of 4, 8, and 16 years from the population-based Barn/Children Allergy Milieu Stockholm Epidemiology birth cohort were used. Sera from 763 representative subjects with serum samples available at all 3 ages were analyzed for IgE reactivity to 8 *Phleum pratense* (Phl p) allergens (MeDALL [Mechanisms for the Development of Allergies] chip) and to timothy grass extract (ImmunoCAP). Allergic rhinitis to grass pollen (ARg) was defined as upper airway symptoms during grass pollen exposure.

Results: The prevalence of sensitization to any Phl p molecule was higher compared with that to timothy extract at all 3 ages: at the age of 4 years, 9.7% versus 6.8%; at the age of 8 years, 28.4% versus 15.3%; and at the age of 16 years, 37.1% versus 27.1%. General estimating equations analyses revealed that among children sensitized at the age of 4 years, the overall odds ratio (OR) of later ARg (up to 16 years) was increased only for IgE reactivity to Phl p 1 (OR = 4.9) and natural Phl p 4 (OR = 6.9). The likelihood of later symptoms increased with the number of allergen molecules; at the age of 4 years, 2 or more molecules predicted ARg to 78% and 3 or more molecules predicted ARg to 95%. A positive test result for timothy extract predicted ARg to 70%.

Conclusions: Natural Phl p 4 is a hitherto unrecognized early indicator of grass pollen allergy, in addition to Phl p 1. To identify grass pollen sensitization and predict later ARg, allergen molecules are of added value to timothy extract alone and may help clinicians improve prediction of grass pollen allergy. (J Allergy Clin Immunol 2020;145:1174-81.)

Key words: Allergen molecules, allergic rhinitis, BAMSE, grass pollen, cohort, IgE, MeDALL, microarray, Phl p 4

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Grass pollen is a ubiquitous allergen source in temperate, subtropic, and tropic regions of the world and the most common sensitizing allergen source in Europe.¹ In temperate climates, grasses from the Pooideae subfamily, including timothy grass (*Phleum pratense*), are dominating.¹⁻³ Allergen molecules from the Pooideae subfamily are highly cross-reactive, and *P. pratense* can be used for diagnostic and therapeutic purposes among patients with grass pollen allergy in the temperate regions of the world.^{3,4}

At present, 13 allergen molecules from *P. pratense* (Phl p) have been identified: Phl p 1 to Phl p 13.² Phl p 1 has been

Abbreviations used

ARg: Allergic rhinitis to grass pollen
BAMSE: Barn/Children Allergy Milieu Stockholm Epidemiology
CCD: Cross-reacting carbohydrate determinant
ISAC: Immuno-solid-phase allergen chip
ISU-E: ISAC standardized units for IgE-detection
MeDALL: Mechanisms for the Development of Allergies
nPhl p: Natural *Phleum pratense*
OR: Odds ratio
Phl p: *Phleum pratense*

reported to be the most prevalent sensitizing allergen among patients allergic to grass pollen, and it is suggested for use as a marker for primary grass sensitization. Phl p 2, Phl p 5, and Phl p 6 are other major allergens specific for the Pooideae subfamily, of which Phl p 5 seems to be the most potent grass pollen allergen because of structural features facilitating effector cell activation.⁵ Phl p 7, a polcalcin, and Phl p 12, a profilin, are minor grass pollen allergens but are highly cross-reactive, and Phl p 11, a minor allergen, shows some sequence homology with soybean trypsin inhibitor-like pollen; however, there is only limited IgE cross-reactivity among the members of this protein family.⁶ Natural Phl p 4 (nPhl p 4) is a glycosylated protein with cross-reacting carbohydrate determinants (CCDs), which may lead to IgE cross-reactivity to other plants. Although nPhl p 4 is a major allergen with a high prevalence of IgE reactivity, its clinical relevance seems to be low because it induces only poorly effector cell degranulation and immediate-type symptoms in patients.⁷⁻⁹ It seems that the majority of Phl p 4-specific IgE is directed to poorly allergenic carbohydrate epitopes.^{7,10}

Previous data have demonstrated that grass sensitization is heterogeneous,¹¹⁻¹³ and several molecular profiles have been presented.^{13,14} In a cross-sectional study Cipriani et al proposed Phl p 1, 7 and 12 to be relevant biomarkers for grass pollen allergy among Italian children with defined grass pollen allergy.¹¹ Furthermore, among 82 adult patients from Austria with grass pollen allergy, the relevant allergen molecules for allergen immunotherapy for grass pollen allergy were shown to be Phl p 1, Phl p 2, Phl p 5, and Phl p 6.⁸

The aim of this study was to evaluate the clinical usefulness of molecular-based allergy diagnostics for grass pollen allergy in childhood up to the age of 16 years in a population-based setting. In particular, we address the association of early sensitization and later onset of symptoms of allergic rhinitis to grass pollen (ARg).

METHODS

The BAMSE cohort

We used data from the population-based BAMSE (Barn/Children, Allergy, Milieu, Stockholm, Epidemiology) birth cohort. The details of the study have been published previously.¹⁵ In brief, the cohort consists of 4089 children recruited shortly after birth, when baseline data were obtained; parental questionnaires on symptoms of allergy-related disorders were used repeatedly thereafter to obtain data until the children were 16 years old. The response rate at the latest follow up at the age of 16 years was 78% from baseline. At the ages of 4, 8, and 16 years, participants who completed the questionnaire were invited for blood sampling. The number of children with blood samples obtained at all 3 time points was 1699. In previous studies, no significant differences were found regarding background characteristics between those 1699 children and the

BAMSE cohort.^{16,17} The BAMSE cohort is part of the MeDALL (Mechanisms for the Development of Allergies) collaboration, a European Union-funded project. Permission for the study was obtained from the Regional Ethical Review board at Karolinska Institutet, Stockholm, Sweden, throughout the study years, and parents gave informed consent for each follow-up.

Study population

Of the 1699 children, 800 with available sera from all 3 time points were randomly sampled for microarray testing.^{16,18} Children with results from the microarray testing and complete information from the parental questionnaires on symptoms of allergic rhinitis at exposure to grass pollen at the ages of 4, 8, and 16 years were included in the study (N = 763).

Definition of symptoms

Allergic rhinitis to grass pollen (ARg) was defined as sneezing, runny, itchy, or blocked nose, or itchy eyes when exposed to grass pollen, as reported in the parental questionnaires when the children were 4, 8, and 16 years of age.¹⁶

Incident symptoms were defined as the aforementioned symptoms reported for the first time at the particular age, but not at previous time points.

Specific IgE reactivity

Serum samples were analyzed for IgE reactivity to microarrayed allergen molecules by using the MeDALL chip, which was based on the immuno-solid-phase allergen chip (ISAC) microarray platform (Phadia Multiplexing/Thermo Fisher Scientific, Uppsala, Sweden) but differed from the commercially available ISAC from the standpoints of layout and number of allergen molecules. The technical details and features of the MeDALL chip, together with the cutoff at least 0.3 ISU-E were considered positive. Briefly, aliquots of 35 μ L of serum were incubated on the microarray, and after 120 minutes of incubation at room temperature, the slides were washed, fluorescence-labeled anti-IgE antibodies (Thermo Fisher) were added, and the slides were incubated for 30 minutes. The chips were then washed, dried, and analyzed by using a Laser Scan Confocal microarray reader (LuxScan 10K/A, Capital-Bio, Beijing, China). The results were evaluated by using Phadia Microarray Image Analysis software and are reported in ISU-E. Levels of IgE to timothy extract were measured with ImmunoCAP (ImmunoCAP System, Thermo Fisher Scientific). A level of 0.35 kU_A/L was considered positive.

Sensitization refers to IgE reactivity to either grass allergen molecules (≥ 0.3 ISU-E) or timothy extract (≥ 0.35 kU_A/L).

Monosensitization refers to IgE reactivity to a single allergen molecule (≥ 0.3 ISU-E).

Statistical analyses

The prevalence of IgE reactivity to the Phl p molecules is expressed as a percentage of the total number of available observations at each time point (N = 763). The specific IgE levels for the different Phl p molecules are presented as box plots of ISU-E values with 25th, 50th, and 75th percentiles, calculated on values greater than the cutoff (≥ 0.3), as well as median ISU-E values and range. For Phl p 1 and Phl p 4, the change in median IgE levels in children from the age of 4 to the age of 16 years was calculated with quantile regression. A Venn diagram was created to see the overlap of prevalence of symptoms of ARg and IgE reactivity to Phl p 1, Phl p 4, or Phl p 5 as well as to timothy extract.²⁰ The associations between the different Phl p molecules in children at the ages of 4 or 8 years and subsequent onset of incident ARg up to age 16 years were calculated as proportions, odds ratios (ORs), and overall risk (general estimating equations). Crude ORs were calculated for each Phl p molecule. Molecules showing a significantly increased crude odds ratio (OR

>1 [95% CI]) were included in the multivariate model. In addition, absolute risks were calculated as the number of subjects among those at risk, with the outcome (symptoms of ARg) at the age of 16 years divided by the total number of subjects at risk (IgE reactivity to allergen molecules/timothy extract but without symptoms at the ages of 4 and 8 years, respectively). Predicted probability estimates were plotted according to the number of IgE-reactive (≥ 0.3 ISU-E) allergen molecules based on results from a logistic regression.

RESULTS

Study population

Comparison of baseline characteristics between the study population (N = 763) and the original cohort of 4089 children revealed no major differences (Table E1, Online Repository).

IgE reactivity from childhood to adolescence

Sensitization to any Phl p molecule increased with increasing age (Fig 1). Among the molecules analyzed, the most prevalent sensitizing allergen molecule was Phl p 1, irrespective of age (Fig 1) (6.6% at the age of 4 years, 22.8% at the age of 8 years, and 31.2% at the age of 16 years), followed in order by Phl p 4 (3.9%, 13.0%, and 19.0%, respectively), Phl p 5 (1.4%, 6.4%, and 14.8%, respectively), Phl p 6 (0.3%, 2.8%, and 10.4%, respectively), Phl p 2 (0%, 1.7%, and 6.8%, respectively), Phl p 7 (0%, 0.7%, and 1.2%, respectively), Phl p 11 (0%, 0.3%, and 0.8%, respectively), and Phl p 12 (0%, 0.3%, and 0.7%, respectively). The highest IgE levels were seen for Phl p 1 and 5 (see Fig E1 in this article's Online Repository at www.jacionline.org). Monosensitization was noted for Phl p 1, Phl p 4, and Phl p 5 at 4 and 8 years of age (Fig 2). At 16 years of age, monosensitization was also observed for Phl p 6 in 1 child. This child did not have a positive reaction to timothy extract and did not report symptoms of ARg (data not shown). IgE reactivity to Phl p 2, Phl p 7, Phl p 11, and Phl p 12 was observed only as concomitant IgE reactivity to Phl p 1, Phl p 4, or Phl p 5, and with lower ISU-E levels than those of Phl p 1, Phl p 4, and Phl p 5.

Among children monosensitized to Phl p 1 at 4 years of age, 89% stayed sensitized up to 16 years of age and 73% developed new sensitizations (Fig 3, A). Their median levels of Phl p 1-specific IgE increased from 1.2 ISU-E at the age of 4 years to 24.6 ISU-E at the age of 16 years ($P = .001$). Among children monosensitized to nPhl p 4, 75% stayed sensitized up to 16 years of age and 70% developed new sensitizations (Fig 3, B). However, their median levels of Phl p 4-specific IgE remained low (0.7-4.1 ISU-E [$P = .027$]). At the ages of 4, 8, and 16 years, respectively, 50%, 52%, and 42% of children sensitized to nPhl p 4 had IgE reactivity to 1 or more of the other CCD-containing molecules Cup a 1, Cry j 1, Pla a 2, Cyn d 1, and Jug r 2. Details of the sensitization pattern for the CCD-containing molecules are presented in Fig E2 (available in the Online Repository at www.jacionline.org).

The prevalence of sensitization to Phl p 1, Phl p 4, and/or Phl p 5 was significantly higher than sensitization to timothy extract at the ages of 4 ($P = .041$), 8 ($P < .001$), and 16 years ($P < .001$) (Fig 4). At 8 and 16 years of age, more than 98% of children with IgE antibodies to timothy extract had IgE reactivity to Phl p 1, Phl p 4, and/or Phl p 5. At 4 years of age this proportion was lower (82.7%).

IgE reactivity and current symptoms

The prevalence of symptoms in response to grass increased with increasing age and was 1.2%, 7.2%, and 15.2%, at 4, 8, and 16 years of age, respectively (Fig 4). At 4 years of age, only 6% of

the grass-sensitized children had developed symptoms. The proportion of children reporting symptoms increased with the number of sensitizing molecules. Among children with sensitization to at least 2 allergen molecules the proportion with symptoms in response to grass was comparable to that of sensitization to timothy extract (Table E2).

Phl p 1 and nPhl p 4 are early indicators of grass pollen allergy

We estimated the impact of asymptomatic sensitization in childhood on the risk of symptoms at 8 and 16 years of age, respectively. Among children sensitized to any grass allergen molecule at 4 years, the OR for ARg at 16 years was 8.9 (96% CI = 5.2-15.0). The overall OR from the longitudinal general estimating equations model of reporting ARg up to 16 years of age, increased significantly for IgE reactivity to Phl p 1, Phl p 4, and Phl p 5 at the age of 4 years. However, after adjustment for other allergen molecules, the ORs remained increased solely for Phl p 1 (4.9 [95% CI = 2.7-8.7]) and nPhl p 4 (6.9 [95% CI 3.5-13.7]) (Table I). Comparable results were seen from age 8 years up to age 16 years (Table II). The proportion of children with Phl p 7 and Phl p 12 sensitization at 8 years of age and symptoms of ARg at 16 years of age were 100%, but the number of children was very low (Table II).

As a comparison, we calculated the absolute risk of having incident symptoms at age 16 years for different sensitization patterns (Table E3 and E4). The highest proportion of symptoms of ARg up to 16 years of age were seen among children with IgE reactivity to 2 or more Phl p molecules (Table E3) or among those with sensitization to Phl p 1, Phl p 4, or Phl p 5 and timothy extract (Table E4) (ie, 76.9% or 72.5% at 4 years of age and 50.9% or 50.0% at 8 years of age). Among children with IgE reactivity to Phl p 1, Phl p 4, and/or Phl p 5 but not to timothy extract at 8 years of age, a significantly higher proportion (16.8% [95% CI = 10.5-25.9]) than among those not sensitized (4.5% [95% CI = 3.1-6.7]) had symptoms of ARg up to 16 years of age. Regarding those with monosensitization to Phl p 5 or sensitization to timothy extract only, no strong conclusions can be made on account of a very low number of children with these sensitization patterns.

The probability of reporting incident symptoms of ARg up to 16 years of age increased with increasing number of IgE reactivities to Phl p molecules at both 4 and 8 years of age (Fig 5). At the age of 4 years, sensitization to 3 or more grass allergen molecules led to a 95% likelihood for grass pollen symptoms at age 16 years. However, the number of children with sensitization to 3 or more allergen molecules was low. For sensitization to timothy extract, the likelihood was 70%. At 8 years of age, the likelihood of later symptoms was less than 20% if sensitized to only 1 or no allergen molecule. For timothy extract, the likelihood of later symptoms was 50% if sensitized.

DISCUSSION

Here we used a molecular allergen approach to detect grass pollen sensitization and to predict, early in life, symptoms up to adolescence in the population-based BAMSE birth cohort. The results revealed that grass pollen sensitization was significantly more prevalent when detected with allergen molecules than with timothy extract alone. At 4 years of age, the majority of the sensitized children were still asymptomatic and the probability of later symptoms increased with increasing number of allergen

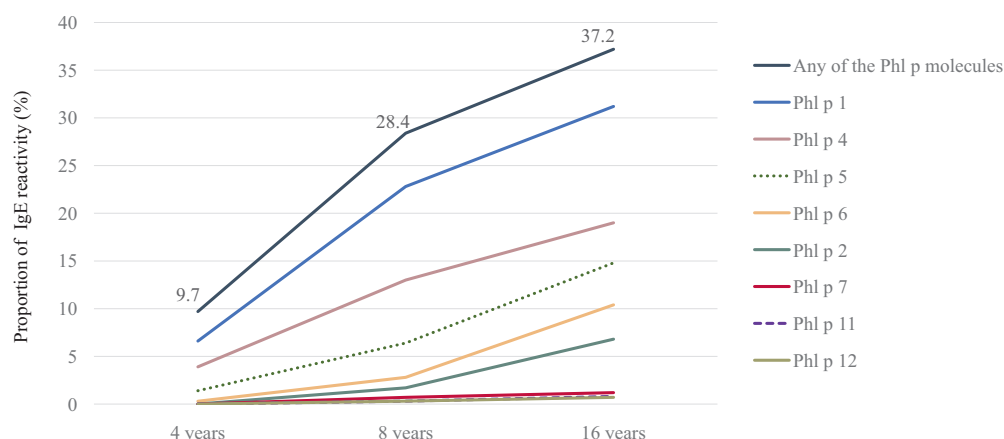


FIG 1. Proportion (%) of children with IgE reactivity to each of the *P pratense* allergen molecules, as well as to any of the grass allergen molecules, among 763 children from the BAMSE birth cohort at the ages of 4, 8, and 16 years, respectively.

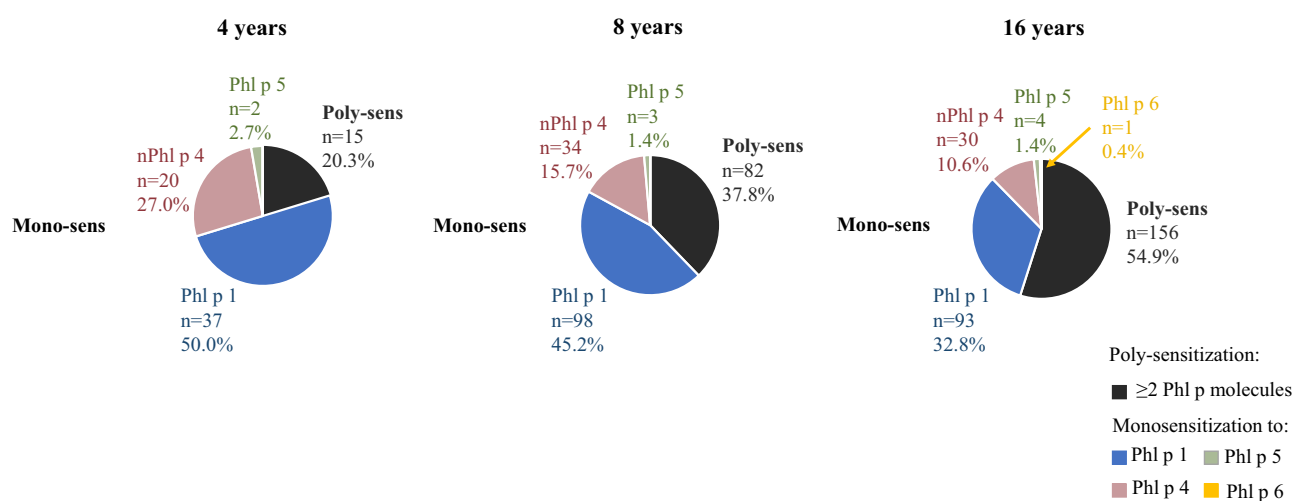


FIG 2. Proportion of children, among those sensitized to any grass molecule, with monosensitization (Mono-sens) to the different Phl p molecules or polysensitization (Poly-sens) (≥ 2 Phl p molecules). Monosensitization to Phl p 2, Phl p 7, Phl p 11, or Phl p 12 was not observed at any age.

molecules. Furthermore, in addition to Phl p 1, the CCD-containing molecule nPhl p 4 could be identified as a hitherto unknown early indicator of grass pollen allergy. The latter result was surprising because the majority of Phl p 4-specific IgE is directed against CCDs, and thus, the allergenic activity is probably low. We conclude that early IgE detection is important for prediction of grass pollen allergy and that testing for IgE reactivity to allergen molecules may add important information to a test for timothy extract positivity.

The present study shows that in our population with use of only 3 allergen molecules, Phl p 1, Phl p 4, and Phl p 5, IgE reactivity was detected significantly more often than with use of timothy extract. More than 98% of children with IgE antibodies to timothy extract also had IgE reactivity to Phl p 1, Phl p 4, and/or Phl p 5. Thus, there was no added value of using timothy extract to detect sensitization. At 4 years, however, only 82.7% of those with IgE against timothy extract had IgE reactivity to Phl p 1, Phl p 4, or Phl p 5. Hence, at preschool age, allergen molecules and timothy extract both contributed information about grass pollen sensitization. These results may be due to the fact that 2 different methods were used; a singleplex assay with allergen extract and a

multiplex assay with individual allergen molecules. However, this may also be the case in a clinical situation. Other longitudinal cohorts have also found Phl p 1, Phl p 4, and Phl p 5 to appear early in the sensitization process.^{12,21} In our study, IgE reactivity to Phl p 2, Phl p 7, Phl p 11, and Phl p 12 was not observed in any child at 4 years of age. Monosensitization to these molecules was not noted at any age, and monosensitization to Phl p 6 was found only in 1 child at 16 years of age. Thus, these molecules seem not to appear early in the sensitization process.

Phl p 1 and nPhl p 4 were found to be important early indicators of grass pollen allergy. In line with previous studies, Phl p 1 was the most prevalent sensitizing molecule in our population,^{2,8,11,22-25} but a high proportion also had IgE reactivity to nPhl p 4. In its natural glycosylated form, this allergen molecule has been classified as a major allergen in both children^{12,13,21,26} and adults according to frequency of IgE recognition,^{2,22,27,28} but IgE reactivity to recombinant rPhl p 4 is seen much less frequently.¹¹ The clinical value of sensitization to nPhl p 4 has been questioned because nPhl p 4 contains CCDs^{2,3} and has been shown to have much lower allergenic activity compared with Phl p 1, Phl p 2, and Phl p 5.⁸ We noted that

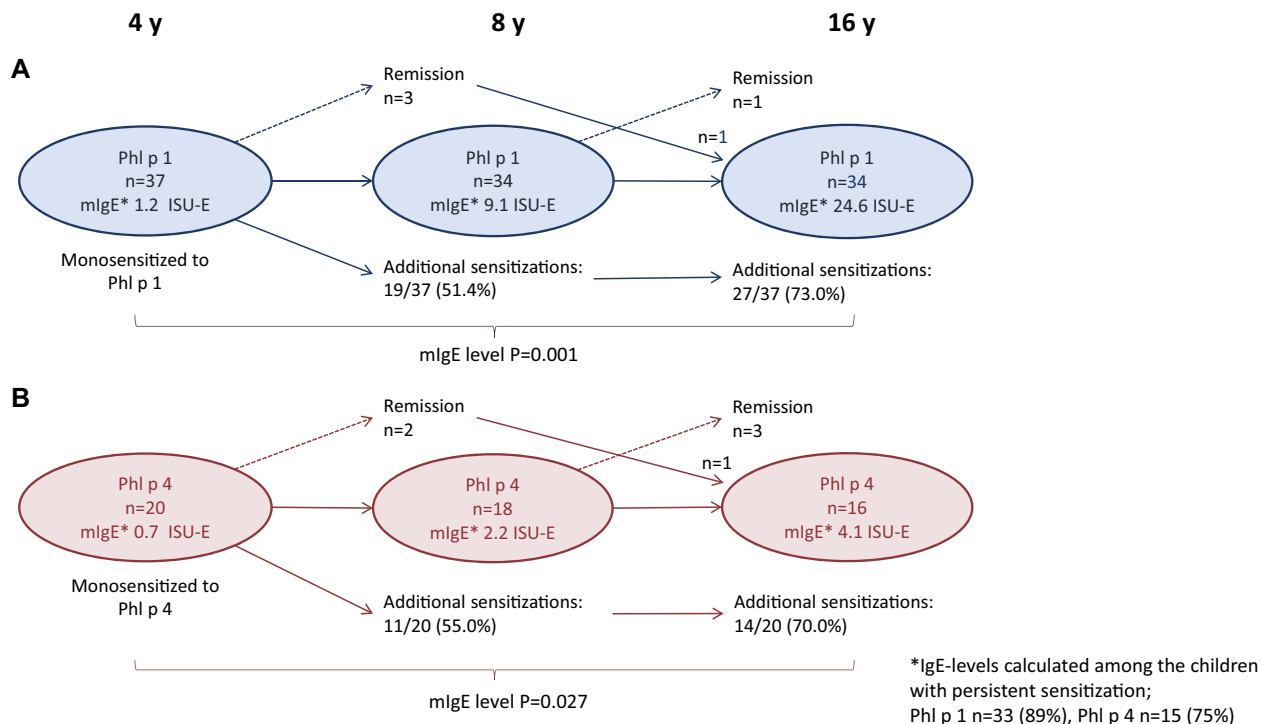


FIG 3. Development of status of sensitization in children with monosensitization at 4 years of age to Phl p 1 (**A**) or nPhl p 4 (**B**). Shown are the numbers of children with remission, the median levels of allergen-specific IgE (mlgE), and the numbers and proportions of children with additional sensitizations to Phl p molecules at the ages of 8 and 16 years, respectively.

N=763

- IgE-reactivity to any of Phl p 1/4/5
- IgE-reactivity to timothy extract
- Symptoms of AR at exposure to grass pollen

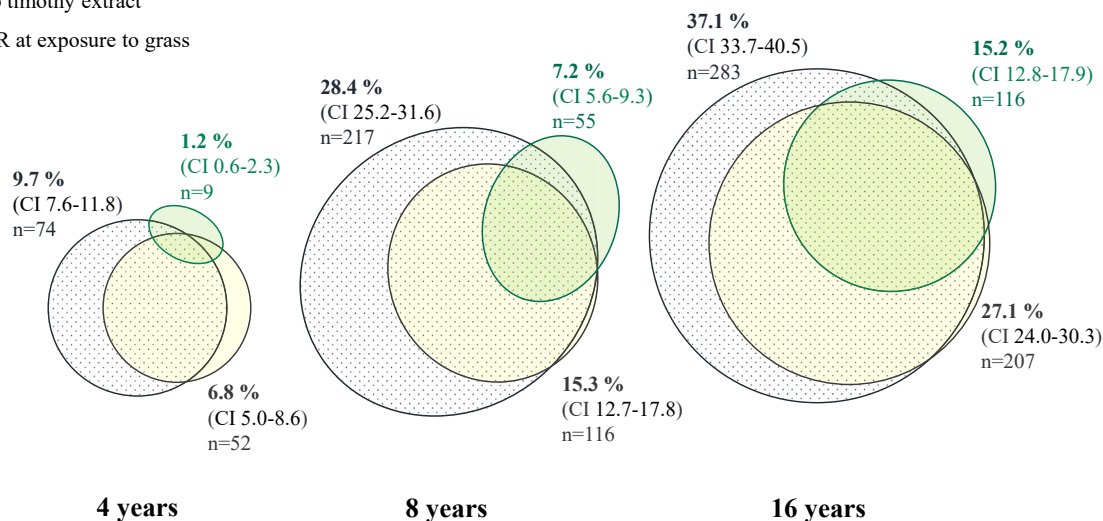


FIG 4. Proportional Venn diagram of IgE reactivity to Phl p 1, Phl p 4, or Phl p 5 and timothy extract and reported symptoms of allergic rhinitis at exposure to grass pollen (ARg). Proportions (%) with 95% CIs and numbers are given for the prevalence of each entity.

monosensitization to nPhl p 4 was found in 27.0% of the children at 4 years of age. Among these children 70% acquired additional sensitizations to grass molecules up to 16 years of age and the overall OR of reporting symptoms up to 16 years of age was 6.9. Between 48% and 58% did not have concomitant IgE

reactivity to other CCD-containing molecules. This may be due to the fact that Phl p 4 CCDs seem to dominate over CCDs of other glycoallergens¹⁰ and/or that the protein fraction of nPhl p 4 may be responsible for part of the IgE response in these patients. On the other hand, Westritschnig et al did not find any patients with

TABLE I. Proportions, ORs, and overall risk (GEE) of incident symptoms of allergic rhinitis to grass pollen (ARg) up to 16 years of age, among children with IgE reactivity to the different Phl p molecules, but without symptoms, at 4 years of age

IgE at age 4 y (N)	Symptoms of AR _g											
	At age 8 y				At age 16 y				Overall risk at age ≤16 y			
	n	%	OR	95% CI	n	%	OR	95% CI	OR	95% CI	OR _a	95% CI
Phl p 1 (47)	12	25.5	6.4	3.1-13.3	23	48.9	6.9	3.7-12.8	6.7	3.9-11.6	4.9	2.7-8.7
Phl p 4 (29)	10	34.5	9.5	4.1-21.9	17	58.6	9.7	4.5-21.1	9.6	5.1-18.3	6.9	3.5-13.7
Phl p 5 (9)	2	22.2	4.3	0.9-21.5	6	66.7	12.5	3.1-50.6	7.9	2.5-24.3	2.0	0.5-7.1
Phl p 6 (2)	1	50.0	15.0	0.9-243.6	1	50.0	6.0	0.4-96.0	9.4	0.9-98.6	na	na

na, Not applicable; OR, odds ratio; OR_a, adjusted odds ratio from the multivariate logistic regression model (at the age of 4 years including Phl p 1, Phl p 4, and Phl p 5). Crude ORs were calculated for each Phl p molecule. Molecules showing significantly increased crude ORs (OR > 1; 95% CI) were included in the multivariate model. Boldface indicates significantly increased ORs (OR > 1, 95% CI).

TABLE II. Proportions, ORs, and overall risk (GEE) of incident symptoms of allergic rhinitis to grass pollen (ARg) up to 16 years of age, among children with IgE reactivity to the different Phl p molecules, but without symptoms, at 8 years of age

IgE at age 8 y (N)	Overall risk at age ≤16 y					
	n	%	OR	95% CI	OR _a	95% CI
Phl p 1 (142)	49	34.5	9.4	5.7-15.5	6.4	3.7-11.0
Phl p 4 (66)	23	34.9	5.6	3.1-9.9	2.2	1.1-4.3
Phl p 5 (32)	17	53.1	11.2	5.3-23.5	1.9	0.7-5.5
Phl p 6 (11)	9	81.8	40.2	8.5-189.7	5.1	0.8-33.3
Phl p 2 (9)	6	66.7	17.1	4.2-69.8	1.3	0.3-7.3
Phl p 7 (4)	4	100	—	—	—	—
Phl p 11 (0)	0	—	—	—	—	—
Phl p 12 (1)	1	100	—	—	—	—

OR, Odds ratio; OR_a, adjusted odds ratio from the multivariate logistic regression model (at the age of 8 years including Phl p 1, Phl p 2, Phl p 4, Phl p 5, and Phl p 6). Crude ORs were calculated for each Phl p molecule. Molecules showing significantly increased crude ORs (OR > 1; 95% CI) were included in the multivariate model. Boldface indicates significantly increased odds ratios (OR>1, 95% confidence level).

exclusive IgE reactivity to nPhl p 4 among 82 adult Austrian patients with grass pollen allergy, and Phl p 4 had a very weak allergenic activity, as demonstrated by skin prick test.⁸ Furthermore, studies have suggested that the addition of rPhl p 4 improved the sensitivity only slightly.^{11,13,29} However, all the aforementioned studies are performed among already-symptomatic subjects. In addition, the importance of nPhl p 4 among patients with grass allergy may vary in different regions, most likely as a result of local allergen exposure. For example, IgE sensitization to Phl p 4 in subjects from a tropical region was completely asymptomatic, and no basophil activation was observed in response to extracts from tropical grasses. Early asymptomatic sensitization to Phl p 4 has been noted by others.¹² Our study suggests that IgE reactivity to nPhl p 4 in childhood, regardless of its low biological activity, serves as an early indicator of future grass pollen allergy in a population exposed to temperate grasses.

Other studies have shown Phl p 5 to be an important allergen for grass pollen allergy.³ However, we did not observe independent associations of Phl p 5 with symptoms, which may be due to the low number of children monosensitized to Phl p 5 in our population. In fact, the proportions of IgE sensitizations to Phl p 5 may vary in different populations because of exposure to different grasses containing group 5 allergens.

The probability of current symptoms in response to grass was associated with the number of allergen molecules, as seen for other inhalant allergens.^{16,18} With sensitization to 2 or

more allergen molecules, approximately the same proportion of children had symptoms as did those with a positive IgE response to timothy extract. A small proportion of children with sensitization to Phl p 1, Phl p 4, and/or Phl p 5, but without sensitization to timothy extract, had current symptoms of ARg. None of the few children with sensitization to timothy extract but without sensitization to Phl p 1, Phl p 4, and/or Phl p 5 reported current symptoms. Therefore, we conclude that using allergen molecules is more informative than is using timothy extract in relation to current symptoms at school age and adolescence.

At 4 years of age, the vast majority of grass pollen-sensitized children were still asymptomatic, highlighting an important window of opportunity for prevention of grass pollen allergy at this time point.³⁰ Monosensitized children had a significantly higher risk for later symptoms than did nonsensitized children, reflecting the fact that sensitization *per se* is a risk factor for later symptoms.^{12,31,32} The probability of later symptoms increased with increasing number of allergen molecules, which is in line with other inhalant allergens.^{16,18} A likelihood of greater than 90% for later symptoms was seen with IgE reactivity to 3 or more allergen molecules. Few children were polysensitized, which is why the result should be interpreted with caution. However, a larger number of children had IgE reactivity to 2 or more allergen molecules or to allergen molecules in combination with timothy extract, both of which corresponded to an absolute risk of greater than 70% for later symptoms. Important to keep in mind is that sensitization to allergen molecules and timothy extract, respectively, did not completely overlap at preschool age. Thus, for detection of grass pollen sensitization in childhood and for prediction of later symptoms, allergen molecules as well as timothy extract seem to be needed.

At 8 years of age, the probability of having symptoms at 16 years of age also increased with the number of allergen molecules. However, the likelihood of symptoms at the age of 16 years among those with IgE reactivity to 3 or more allergen molecules at 8 years was lower (75%) than that observed for 4-year-old children with IgE reactivity to at least 3 allergens, but higher than for timothy extract (50%). On the other hand, having only 1 or no allergen molecule at the age of 8 years almost excluded the development of later ARg (<20%). Possible explanations for the higher likelihood of later symptoms when sensitized at 4 years of age than at 8 years of age may be (1) that early childhood seems to be the most dynamic period of IgE progression and (2) the longer time of exposure for development of symptoms from 4 to 16 years of age than from 8 to 16 years of age.^{12,30}

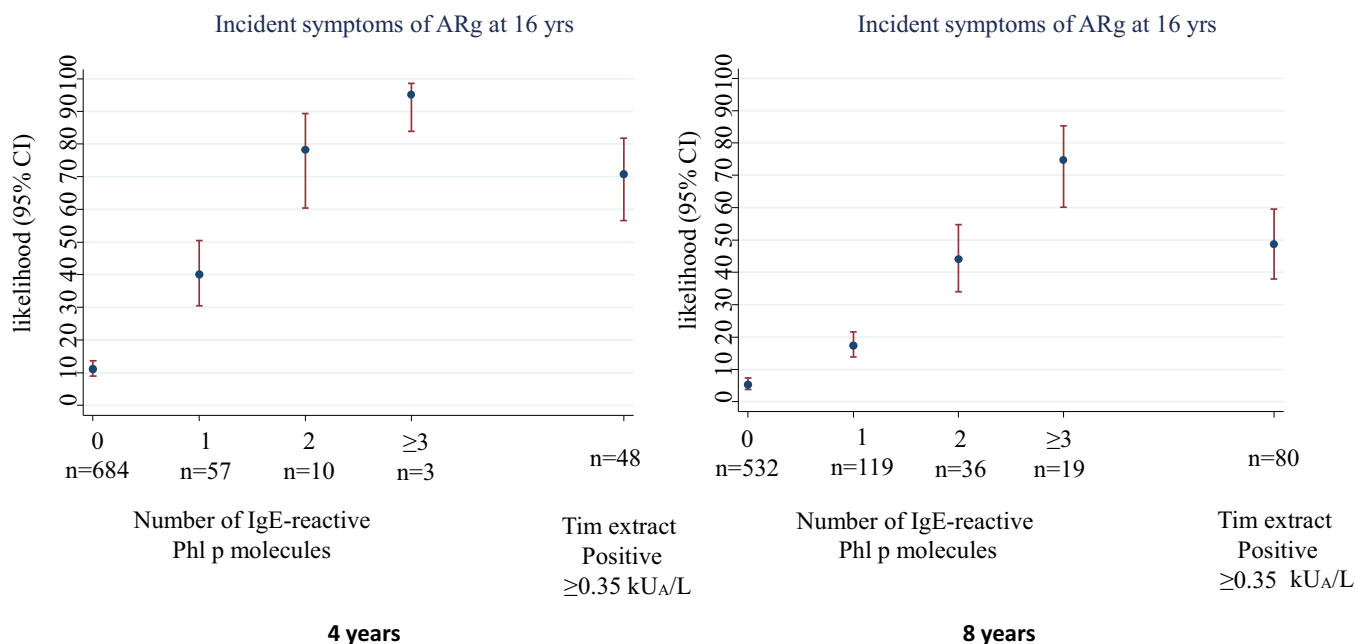


FIG 5. The probability of incident symptoms of ARg at 16 years of age in relation to the number of IgE reactivities to Phl p molecules or to timothy extract, among children without symptoms at baseline (at 4 years and 8 years of age, respectively). The predicted probability estimate was based on a logistic regression model.

The strength of this study is its population-based design and the high response rate (ie, 78% from baseline at the 16-year follow-up). Use of the MedALL chip has allowed us to perform a comprehensive analysis of IgE reactivities to multiple *P pratense* allergen molecules with only a limited amount of sera, which is beneficial in studies of children. A limitation of our study is the fact that the study population was a sample of the original cohort. A comparison of baseline characteristics between the study population and the original cohort showed no major differences. Another limitation is that ARg was defined on the basis of parental answers to symptoms at exposure to grass pollen, which may not be as accurate as in a clinical study. However, in a validation study from Finland among 290 students aged 18 to 25 years, the question of symptoms after exposure to an allergen had a positive predictive value of 75% in relation to a doctor's diagnosis of symptoms in combination with a positive skin prick test result.³³ In our study, the sensitivity, specificity, and positive predictive value of the questionnaire-based definition of symptoms of ARg, in comparison with symptoms of ARg in combination with sensitization to timothy extract, were as follows: at 16 years, 100%, 97%, and 84%, respectively; at 8 years, 100%, 97%, and 64%, respectively; and at 4 years, 100%, 99%, and 44%, respectively. Furthermore, the 2 major sources of pollen allergens in Scandinavia are grass and birch pollen and the peak of the grass pollen season does not overlap with the peak of birch pollen season, thus reducing the risk of misinterpreting the allergen source causative of symptoms. Using the children's answers of symptoms at exposure to grass pollen or defining the outcome as symptoms during June and/or July did not alter the associations (data not shown). The questionnaires were answered without parents or children knowing about the IgE reactivity; thus, any misclassification would be nondifferential.

In conclusion, we show that measuring IgE to grass pollen allergen molecules may add important information to a test of timothy extract sensitization and may help clinicians improve the diagnosis of grass pollen sensitization and prediction of grass pollen allergy in temperate regions. Phl p 1 and the low allergenic nPhl p 4 molecule were shown to be important early indicators for prediction of grass pollen allergy later in life.

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Clinical implications: Measuring IgE reactivity to grass allergen molecules during childhood may help clinicians improve detection of grass pollen sensitization and prediction of later grass pollen allergy.

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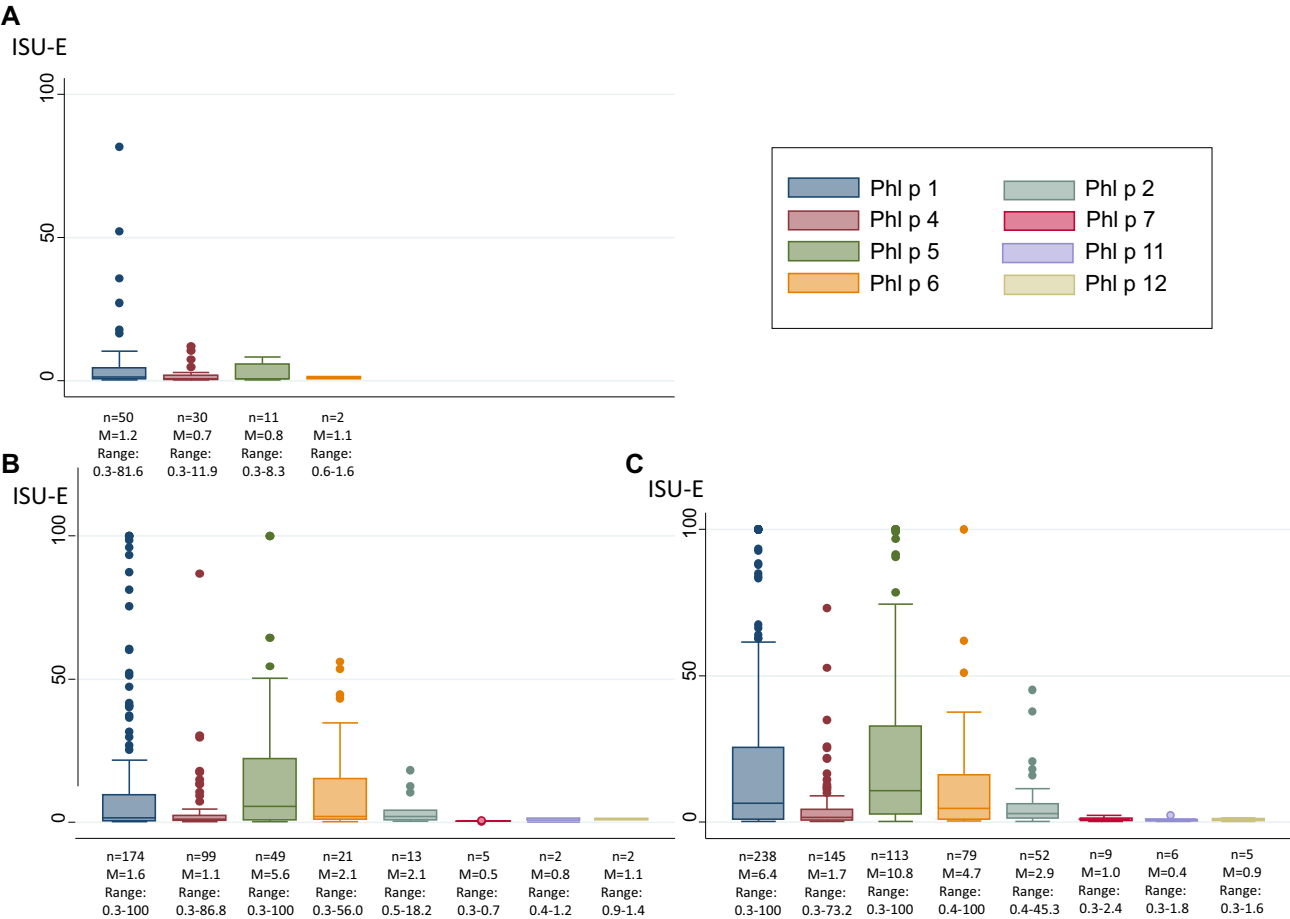


FIG E1. Levels of specific IgE (median [M] and range) at the ages of 4 years (A), 8 years (B), and 16 years (C) among 763 children from the BAMSE birth cohort.

N=763

4 YEARS	Phl p 4	Cup a 1	Cry j 1	Pla a 2	Cyn d 1*	Jug r 2	Any CCD**
	%	%	%	%	%	%	
Phl p 4 n=30	100	26.7	36.7	26.7	30.0	23.3	50.0
Cup a 1 n=13	61.5	100	92.3	84.6	38.5	38.5	
Cry j 1 n=16	68.8	75.0	100	62.5	37.5	31.3	
Pla a 2 n= 12	66.7	91.7	83.3	100	41.7	41.7	
Cyn d 1* n=11	81.8	45.5	54.6	45.5	100	45.5	
Jug r 2 n=7	100	71.4	71.4	71.4	71.4	100	

8 YEARS	Phl p 4	Cup a 1	Cry j 1	Pla a 2	Cyn d 1*	Jug r 2	Any CCD**
	%	%	%	%	%	%	
Phl p 4 n=99	100	33.3	30.3	32.3	19.2	13.1	51.5
Cup a 1 n=42	78.6	100	78.6	54.8	23.8	21.4	
Cry j 1 n=44	68.2	75.0	100	54.6	29.6	25.0	
Pla a 2 n= 35	91.4	65.7	68.6	100	40.0	37.1	
Cyn d 1* n=31	61.3	32.3	41.9	45.2	100	22.6	
Jug r 2 n=13	100	69.2	84.6	100	53.9	100	

16 YEARS	Phl p 4	Cup a 1	Cry j 1	Pla a 2	Cyn d 1*	Jug r 2	Any CCD**
	%	%	%	%	%	%	
Phl p 4 n=145	100	35.2	18.6	22.1	7.6	2.8	42.1
Cup a 1 n=62	82.3	100	51.6	50.0	6.5	3.2	
Cry j 1 n=38	71.1	84.2	100	68.4	15.8	7.9	
Pla a 2 n= 37	86.5	83.8	70.3	100	10.8	5.4	
Cyn d 1* n=19	57.9	21.1	31.6	21.1	100	10.5	
Jug r 2 n=4	100	50.0	75.0	50.0	50.0	100	

<10
10-20
20-30
30-40
40-50
50-60
60-70
70-80
80-90
90-100

*IgE-reactivity to Phl p 1 excluded
**Any of the CCD-containing molecules
Cup a 1, Cry j 1, Pla a 2, Cyn d 1, Jug r 2

FIG E2. The numbers of children with IgE reactivity to each of the CCD-containing molecules Phl p 4, Cup a 1, Cry j 1, Pla a 2, Cyn d 1, and Jug r 2 at age 4 years (*top*), 8 years (*middle*), and 16 years (*bottom*) (*left column*), and the percentages of children with additional sensitization to the other CCD-containing molecules (*top*, from left to right).

TABLE E1. Comparison between the study population (N = 763) and the original BAMSE cohort (N = 4089) regarding demographic and other characteristics

	Study population (N = 763)			BAMSE cohort (N = 4089)		
	n	%	95% CI	n	%	95% CI
Sex						
Male	381	49.9	46.4-53.5	2065	50.5	49.0-52.0
Family history of allergic rhinitis						
Yes	287	37.6	34.2-41.1	1397	34.2	32.7-35.6
Low socioeconomic status						
Yes	109	14.3	12.0-17.0	695	17.1	15.9-18.3
Birth month						
December-February	139	18.2	15.6-21.1	722	17.7	16.5-18.9
March-May	241	31.6	28.4-35.0	1201	29.4	28.0-30.8
June-August	223	29.2	26.1-32.6	1190	29.1	27.7-30.5
September-November	160	21.0	18.2-24.0	976	23.9	22.6-25.2
Mother's age						
<26 y	58	7.6	5.9-9.7	319	7.8	7.0-8.7
Parent born outside Scandinavia						
Yes	105	13.8	11.5-16.4	543	16.0	14.8-17.3
Older siblings						
Yes	380	49.8	46.3-53.4	1980	48.4	46.9-50.0
Breast feeding exclusively for ≥ 4 mo						
Yes	596	79.2	76.1-81.9	3116	79.5	78.2-80.7
Furred animals at home						
Yes	120	15.7	13.3-18.5	629	15.4	14.3-16.5
Mother smoking						
Yes	93	12.2	10.0-14.7	563	13.8	12.8-14.9
Smell of mildew in the home						
Yes	54	7.1	5.5-9.1	324	7.9	7.1-8.8
Daycare attendance						
Yes	568	74.5	71.3-77.5	2773	72.2	70.8-73.6
Fish intake at the age of 1 y						
≥ 2 times/mo	611	81.0	78.1-83.7	3143	80.1	78.8-81.3

TABLE E2. Proportion of children with current symptoms of AR_g among those not sensitized, monosensitized to Phl p 1, Phl p 4, or Phl p 5, or with polysensitization (N = 763)

Sensitization	Symptoms of AR _g								
	At age 4 y			At age 8 y			At age 16 y		
	n/N*	%	95% CI	n/N*	%	95% CI	n/N*	%	95% CI
No sensitization	5/689	0.7	0.3-1.7	13/546	2.4	1.4-4.1	13/479	2.7	1.6-4.6
Monosensitization									
Phl p 1	2/37	5.4	1.3-20.3	7/98	7.1	3.4-14.4	12/93	12.9	7.4-21.6
Phl p 4	0/20	0.0	na	8/34	23.5	11.8-41.5	7/30	23.3	11.0-42.8
Phl p 5	0/2	0.0	na	0/3	0.0	na	1/4	25.0	0.5-95.9
Polysensitization									
≥2 Phl p molecules	2/15	13.3	2.8-45.4	27/82	32.9	23.5-44.0	83/156	53.2	45.3-61.0
≥3 Phl p molecules†	0/3	0.0	n.a	14/33	42.4	26.2-60.4	61/99	61.6	51.5-70.8
Timothy extract–neg	5/711	0.7	0.3-1.7	20/644	3.1	2.0-4.8	19/556	3.4	2.2-5.3
Timothy extract–pos	4/52	7.7	2.8-19.3	35/116	30.2	22.4-39.3	97/207	46.9	40.1-53.7

na, Not applicable; neg, negative; pos, positive.

Shown for comparison is the proportion of children with symptoms when sensitized or not sensitized to timothy extract (N = 763). The total numbers of children with symptoms were as follows: at age 4 years, 9; at age 8 years, 55; and at 16 years, 116.

*n/N = number of children with symptoms/number of children with sensitization to the allergen molecule(s) at the particular time point.

†Subgroup of children with polysensitization, not mutually exclusive.

TABLE E3. Absolute risk of having incident symptoms of ARg at 16 years of age among children without sensitization to Phl p molecules, with monosensitization to Phl p 1, Phl p 4, or Phl p 5, or with polysensitization (≥ 2 Phl p molecules) at the ages of 4 years (top) and 8 years (bottom)

Sensitization by age	N	ARg at age 16 y		
		n	%	95% CI
At age 4 y				
No sensitization	684	73	10.7	8.6-13.2
Monosensitization				
Phl p 1	35	14	40.0	24.7-57.6
Phl p 4	20	11	55.0	31.8-76.2
Phl p 5	2	1	50.0	0-100
Polysensitization				
≥2 Phl p molecules	13	10	76.9	42.8-93.7
At age 8 y				
No sensitization	532	24	4.6	3.0-6.7
Monosensitization				
Phl p 1	91	23	25.3	17.3-35.4
Phl p 4	25	4	16.0	5.7-37.5
Phl p 5	3	0	0.0	na
Polysensitization				
≥2 Phl p molecules	55	28	50.9	37.5-64.2

na, Not applicable.

Sensitization to timothy extract is not taken into account. The groups are mutually exclusive.

TABLE E4. Absolute risk of having incident symptoms of ARg at 16 years of age for the group of children without sensitization to Phl p 1, Phl p 4, or Phl p 5 or timothy extract, for the group sensitized to Phl p 1, Phl p 4, or Phl p 5 but not to timothy extract, for the group only sensitized to timothy extract and for the group with sensitization to Phl p 1, Phl p 4, or Phl p 5 and timothy extract at the ages of 4 years (top) and 8 years (bottom)

Sensitization by age		N	ARg at age 16 y		
			n	%	95% CI
At age 4 y					
Phl p 1, Phl p 4, or Phl p 5	Timothy extract				
–	–	676	68	10.1	8.0-12.3
+	–	30	7	23.3	11.0-42.8
–	+	8	5	62.5	20.8-91.3
+	+	40	29	72.5	56.1-84.5
At age 8 y					
Phl p 1, Phl p 4, or Phl p 5	Timothy extract				
–	–	528	24	4.5	3.1-6.7
+	–	95	16	16.8	10.5-25.9
–	+	2	0	na	na
+	+	78	39	50.0	38.8-61.2

na, Not applicable.

The groups are mutually exclusive. The CIs are calculated as compared with no sensitization.