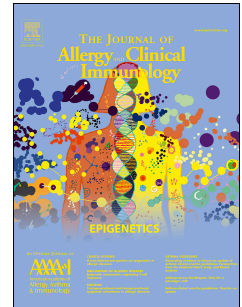


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Sensitization to grass pollen allergen molecules in a birth cohort – Phl p 4 as early indicator of grass pollen allergy

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Abstract

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

Objective: To evaluate the usefulness of grass pollen allergen molecules for prediction of grass pollen allergy during childhood up to adolescence.

Method: Questionnaire data and sera obtained at 4, 8 and 16 years from the population-based BAMSE birth cohort were used. Sera from 763 representative subjects with serum samples available at all three ages were analysed for IgE-reactivity to 8 *Phleum pratense* allergens (MeDALL-chip) and to timothy 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 to timothy extract at all three ages; 4 (9.7% vs. 6.8%), 8 (28.4% vs 15.3%) and 16 (37.1% vs. 27.1%) years. General estimating equations (GEE) analyses revealed that among children sensitized at 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 nPhl p 4 (OR=6.9). The likelihood of later symptoms increased with the number of allergen molecules; at 4 years ≥ 2 molecules predicted ARg to 78% and ≥ 3 molecules 95%. A positive test for timothy extract predicted ARg to 70%.

Conclusions: nPhl 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 and may help clinicians improve prediction of grass pollen allergy.

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.

Capsule summary: Early IgE detection is important for prediction of grass pollen allergy. 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.

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

Abbreviations:

- ARg – Allergic rhinitis to grass pollen
- BAMSE – Barn/Children Allergy/Asthma Milieu Stockholm Epidemiologic study
- CCD – cross-reacting carbohydrate determinants
- IgE – Immunoglobulin E
- ISU-E – ISAC standardized units for IgE-detection
- MeDALL - Mechanisms for the Development of ALLergies
- GEE analysis - general estimating equations analysis
- OR – odds ratio

Word count: 3900

Introduction

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 like timothy (*Phleum pratense*) are dominating (1-3). Allergen molecules from the Pooideae subfamily are highly cross-reactive and *Phleum 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 have been identified from *Phleum pratense* (Phl p), Phl p 1-13 (2). Phl p 1 has been reported to be the most prevalent sensitizing allergen among grass pollen allergic patients and it is suggested to be used as a marker for primary grass sensitization. Phl p 2, 5 and 6 are other major allergens specific for the Pooideae subfamily of which Phl p 5 seems to be the most potent grass pollen allergen due to structural features facilitating effector cell activation (5). Phl p 7, a polcalcin, 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 but 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 only poorly induces 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 grass pollen allergic adult patients from Austria the relevant allergen molecules for allergen immunotherapy for grass pollen allergy were shown to be Phl p 1, 2, 5 and 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 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 birth cohort BAMSE (Barn/Children, Allergy, Milieu, Stockholm, Epidemiology). The details of the study have been previously published (15). In brief, the cohort consists of 4089 children recruited shortly after birth when baseline data was obtained and repeatedly thereafter up to 16 years using parental questionnaires on symptoms of allergy-related disorders. The response rate at the latest follow up at 16 years was 78% from baseline. At ages 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 three time points was 1699. In previous studies, no significant differences have been found regarding background characteristics between the 1699 children and the BAMSE cohort (16, 17). The BAMSE cohort is part of the MeDALL collaboration, the European Union funded project Mechanisms for the Development of ALLergies. 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

Eight hundred of the 1699 children with available sera from all three 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 AR symptoms at exposure to grass pollen at 4, 8 and 16 years, were included in the study (N=763).

Definition of symptoms

Grass pollen-related AR (ARg) was defined as sneezing, runny, itchy or blocked nose, itchy eyes when exposed to grass pollen, reported in the parental questionnaires at ages 4, 8 and 16 years (16).

Incident symptoms were defined as symptoms as above 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 using the MeDALL chip which is based on the ISAC microarray platform (Phadia

Multiplexing/Thermo Fisher Scientific, Uppsala, Sweden), but differed from the commercially available ISAC regarding layout and the number of allergen molecules. The technical details and features of the MeDALL chip together with the cut off ≥ 0.3 ISAC Standardized Units (ISU-E) are described in detail by Lupinek et al (19). IgE-reactivity profiles and levels were measured for allergen molecules from *Phleum pratense* (Phl p 1, Phl p 2, Phl p 4, Phl p 5, Phl p 6, Phl p 7, Phl p 11, Phl p 12), but also the CCD-containing molecules Cup a 1 (*Cupressus arizonica*), Cry j 1 (*Cryptomeria japonica*), Pla a 2 (*Platanus acerifolia*), Cyn d 1 (*Cynodon dactylon*), Jug r 2 (*Juglans regia*). A level of ≥ 0.3 ISU-E was considered positive. Briefly, aliquots of 35 μ l of serum were incubated on the microarray and after 120 min of incubation at room temperature, slides were washed, and fluorescence-labelled anti-IgE antibodies (Thermo Fisher) were added and incubated for 30 min. Chips were then washed, dried and analyzed using a Laser Scan Confocal microarray reader (LuxScan 10K/A; Capital-Bio, Beijing, China). The results were evaluated using Phadia Microarray Image Analysis (MIA) software and are reported in ISU-E. IgE to timothy extract was measured with ImmunoCAP (ImmunoCAP System, Thermo Fisher Scientific). A level of 0.35 kUA/l was considered positive.

Sensitization refers to IgE-reactivity to either grass allergen molecules (≥ 0.3 ISU-E) or timothy extract (≥ 0.35 kU/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 are expressed as percent 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 cut-off (≥ 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 from 4 to 16 years were calculated with quantile regression. A Venn diagram was performed to see the overlap of prevalence of symptoms of ARg, IgE-reactivity to Phl p 1, 4 or 5, and to timothy extract (20). The association between the different Phl p molecules at age 4 or 8 years and subsequent onset of incident ARg up to age 16 years was calculated as proportions, odds ratios (OR) and overall risk (General estimating equations, GEE). Crude ORs were calculated for each Phl p molecule. Molecules showing a significantly increased crude odds ratio (OR >1, 95% confidence level) 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

215 ARg) at 16 years, divided by the total number of subjects at risk (IgE-reactivity to allergen
216 molecules/timothy extract but without symptoms, at 4 and 8 years, respectively). Predicted
217 probability estimates were plotted according to the number of IgE-reactive (≥ 0.3 ISU-E)
218 allergen molecules, based on results from a logistic regression.

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Results

Study population

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

IgE reactivity from childhood to adolescence

Sensitization to any Phl p molecule increased with increasing age (Figure 1). Among the molecules analyzed, the most prevalent sensitizing allergen molecule was Phl p 1, irrespective of age (Figure 1), (at 4 years 6.6%, 8 years 22.8% and 16 years 31.2%), followed in order by Phl p 4 (3.9%, 13.0%, 19.0%), Phl p 5 (1.4%, 6.4%, 14.8%), Phl p 6 (0.3% 2.8%, 10.4%), Phl p 2 (0%, 1.7%, 6.8%), Phl p 7 (0%, 0.7%, 1.2%), Phl p 11 (0%, 0.3%, 0.8%) and Phl p 12 (0%, 0.3%, 0.7%). The highest IgE levels were seen for Phl p 1 and 5 (Figure E1).

Monosensitization was noted for Phl p 1, 4 and 5 at 4 and 8 years of age (Figure 2). At 16 years of age, monosensitization was also observed for Phl p 6 in one child. This child was not positive to timothy extract and did not report symptoms of ARg (data not shown). IgE-reactivity to Phl p 2, 7, 11 and 12 was only observed as concomitant IgE-reactivity to Phl p 1, 4 or 5, and with lower ISU-E levels compared to those of Phl p 1, 4, and 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 (Figure 3A). Their median levels of Phl p 1-specific IgE increased from 1.2 ISU-E at 4 years to 24.6 ISU-E at 16 years ($p=0.001$). Among children monosensitized to nPhl p 4, 75 % stayed sensitized up to 16 years of age, and 70% developed new sensitizations (Figure 3B). However, their median levels of Phl p 4-specific IgE remained low (0.7–4.1 ISU-E, $p=0.027$). At 4, 8 and 16 years, respectively, 50%, 52% and 42% of children sensitized to nPhl p 4 had IgE reactivity to any of the other CCD-containing molecules Cup a 1, Cry j 1, Pla a 2, Cyn d 1, Jug r 2. Details of the sensitization pattern for the CCD-containing molecules are presented in Figure E2.

The prevalence of sensitization to any of Phl p 1, 4 or 5 was significantly higher than to timothy extract at 4 ($p=0.041$), 8 ($p<0.001$) and 16 years ($p<0.001$) (Figure 4). At 8 and 16 years of age, >98% of children with IgE antibodies to timothy extract had IgE reactivity to any of Phl p 1, 4 or 5. At 4 years of age this proportion was lower, 82.7%.

IgE-reactivity and current symptoms

The prevalence of symptoms to grass increased with increasing age and was 1.2%, 7.2% and 15.2%, at 4, 8 and 16 years, respectively (Figure 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 ≥ 2 allergen molecules the proportion with symptoms 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, 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 GEE model of reporting ARg up to 16 years, significantly increased for IgE-reactivity to Phl p 1, 4 and 5 at 4 years. However, after adjustment for other allergen molecules 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). Comparable results were seen from 8 up to 16 years (Table 1). The proportion of children with Phl p 7 and Phl p 12 sensitization at 8 years and symptoms of ARg at 16 years were 100%, but the number of children was very low (Table 1).

As a comparison, we calculated the absolute risk of having incident symptoms at 16 years for different sensitization patterns (Table E3 and E4). The highest proportion of symptoms of ARg up to 16 years were seen among children with IgE-reactivity to ≥ 2 Phl p molecules (Table E3) or among those with sensitization to Phl p 1, 4 or 5 and timothy extract (Table E4), i.e. 76.9% or 72.5% at 4 years and 50.9% or 50.0% at 8 years. Among children with IgE-reactivity to Phl p 1/4/5 but not to timothy extract at 8 years, 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. Regarding those with monosensitization to Phl p 5 or sensitization to timothy extract only, no strong conclusions can be made due to a very low number of children with these sensitization patterns.

The probability of reporting incident symptoms of ARg up to 16 years increased with increasing number of IgE-reactivities to Phl p molecules at both 4 or 8 years (Figure 5). At 4 years, sensitization to three or more grass allergen molecules led to a 95% likelihood for grass pollen symptoms at 16 years. However, the number of children with ≥ 3 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 one 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 timothy extract alone. At 4 years, the majority of the sensitized children were still asymptomatic and the probability of later symptoms increased with increasing number of allergen 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 since 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 using only three allergen molecules, Phl p 1, 4 and 5, IgE-reactivity was significantly more often detected than using timothy extract. More than 98% of children with IgE antibodies to timothy extract also had IgE reactivity to any of Phl p 1, 4 or 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, 4 or 5. Hence, at preschool age, allergen molecules and timothy extract both contributed with information about grass pollen sensitization. These results may be due to the fact that two 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, 4 and 5 to appear early in the sensitization process (12, 21). In our study, IgE-reactivity to Phl p 2, 7, 11 and 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 only found in one 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 both in 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, since nPhl p 4 contains CCDs (2, 3) and has shown to have much lower allergenic activity compared to Phl p 1, 2 and 5 (8). We noted that monosensitization to nPhl p 4 was found in 27.0% of the children at 4 years. Among these children 70% acquired additional sensitizations to grass molecules up to 16 years and the overall OR of reporting symptoms up to 16 years 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 glycoallergens (10) and/or 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 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 SPT (8). Furthermore, studies have suggested that the addition of rPhl p 4 only slightly improved the sensitivity (11, 13, 29). However, all studies mentioned above are performed among already symptomatic subjects. In addition, the importance of nPhl p 4 among grass allergic patients 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, proportions of IgE sensitizations to Phl p 5 may vary in different populations due to exposure to different grasses containing group 5 allergens.

The probability of current symptoms to grass was associated with the number of allergen molecules, as seen for other inhalant allergens (16, 18). With sensitization to ≥ 2 allergen molecules approximately the same proportion of children had symptoms as with a positive IgE response to timothy extract. A small proportion of children with sensitization to Phl p 1/4/5, 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 1/4/5 reported current symptoms. Therefore, we conclude that using allergen molecules is more informative than 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 nonsensitized children reflecting 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 > 90% for later symptoms was seen with IgE-reactivity to ≥ 3 allergen molecules. Few children were polysensitized, why the result should be interpreted with caution. However, a larger number of children had IgE-reactivity to ≥ 2 allergen molecules or to allergen molecules in combination with timothy extract both of which corresponded to an absolute risk of > 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, for prediction of later symptoms, allergen molecules as well as timothy extract seems to be needed.

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

The strength of this study is the population-based design and the high response rate, i.e. 78% from baseline at the 16-year follow up. The use of the MeDALL chip has allowed us to perform a comprehensive analysis of IgE-reactivities to multiple *Phleum pratense* allergen molecules with only a limited amount of sera which is beneficial in studies of children. A limitation is 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 as 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, 18-25 years old, the question of symptoms after exposure to an allergen had a positive predictive value (PPV) of 75% in relation to doctor's diagnosis of symptoms in combination with a positive skin prick test (33). In our

study the sensitivity, specificity and PPV of the questionnaire-based definition, symptoms of ARg, in comparison to symptoms of ARg in combination with sensitization to timothy extract, were at 16 years 100%, 97% and 84%, respectively, at 8 years, 100%, 97% and 64% and at 4 years 100%, 99% and 44%. Furthermore, the two 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/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 non-differential.

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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409

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Table and Figure legends

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

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

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

Figure 4: Proportional Venn diagram of IgE reactivity to Phl p 1, 4 or 5, timothy extract and reported symptoms (AR) at exposure to grass pollen. Proportions (%) with 95% confidence intervals (CI) and numbers (n) are given for the prevalence of each entity.

Table 1: Proportions, odds ratios (OR) and overall risk (GEE) of incident symptoms of allergic rhinitis to grass pollen (ARg) up to 16 years, among children with IgE-reactivity to the different Phl p molecules at A) 4 years and B) 8 years of age. Crude ORs were calculated for each Phl p molecule. Molecules showing a significantly increased crude odds ratio (OR >1 , 95% confidence level) were included in the multivariate model.

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

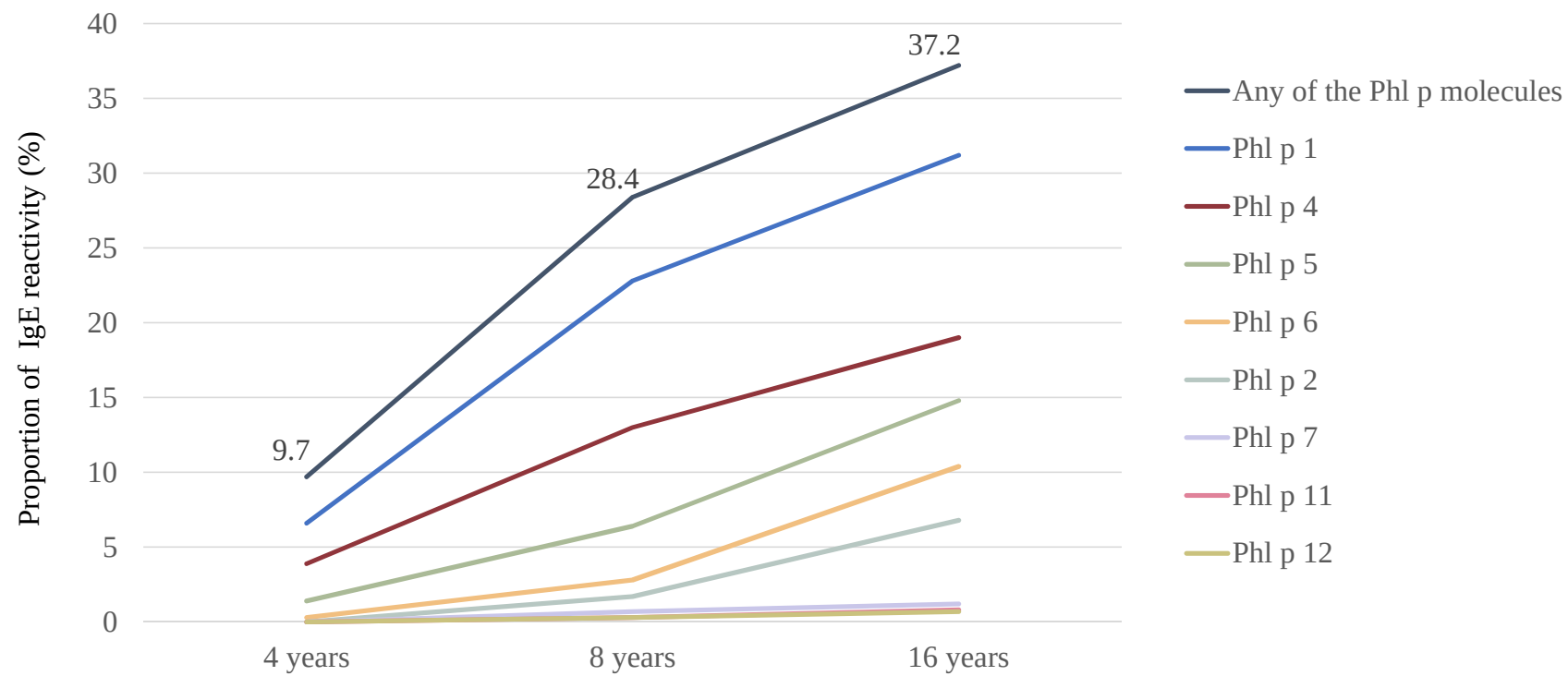
Symptoms of AR _g												
A)	8 years				16 years				Overall risk up to 16 years			
	n	%	OR	95% CI	n	%	OR	95% CI	OR	95% CI	OR _a	95% CI
IgE 4 yrs: (N)												
Phl p 1 (47)	12	25.5	6.4	3.1-13.3	23	48.9	6.9	3.7-12.8	6.7	3.9-11.6	4.9	2.7-8.7
Phl p 4 (29)	10	34.5	9.5	4.1-21.9	17	58.6	9.7	4.5-21.1	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	n.a.	n.a.

B)	16 years					
	n	%	OR	95% CI	OR _a	95% CI
IgE 8 yrs: (N)						
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	-	-	-	-

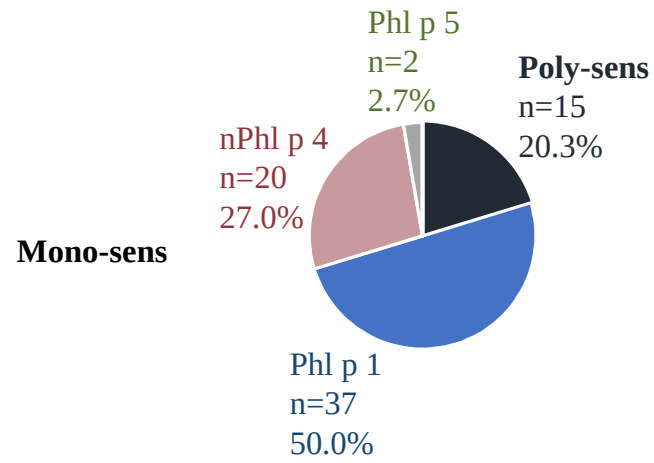
n.a: not applicable

ORa: Multivariate logistic regression model at 4 years (Phl p 1, 4, 5) and 8 years (Phl p 1, 2, 4, 5, 6), respectively.

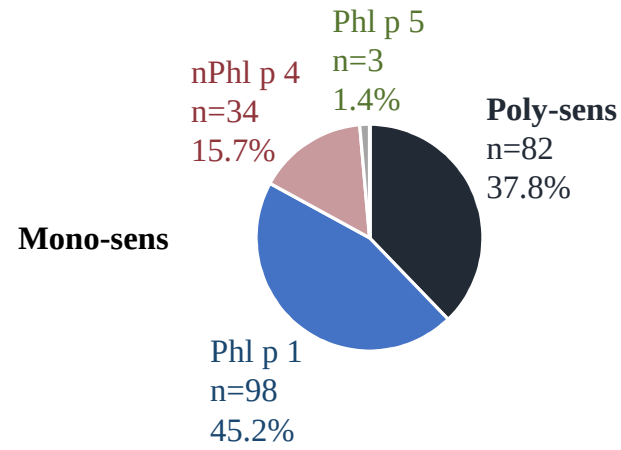
Bold font: Significantly increased odds ratios (OR>1, 95% confidence level).



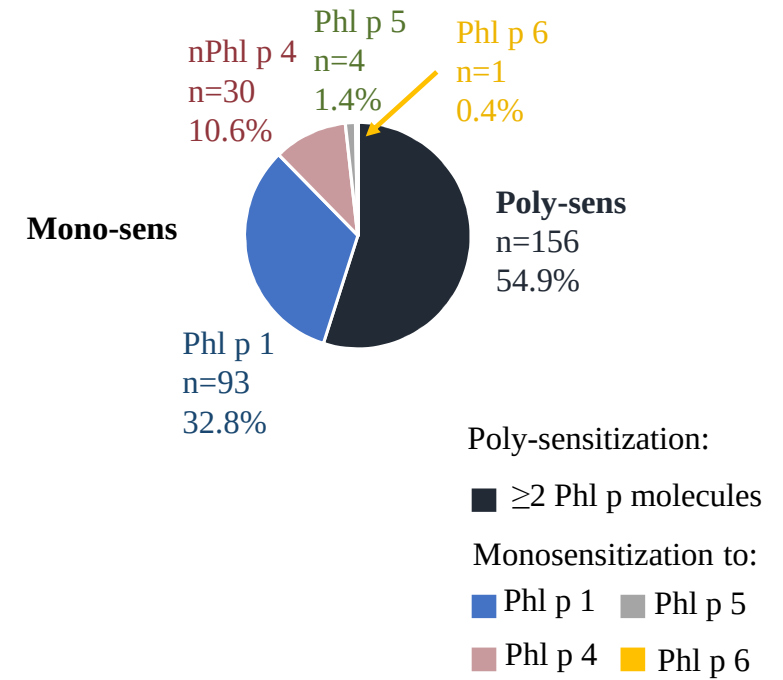
4 years

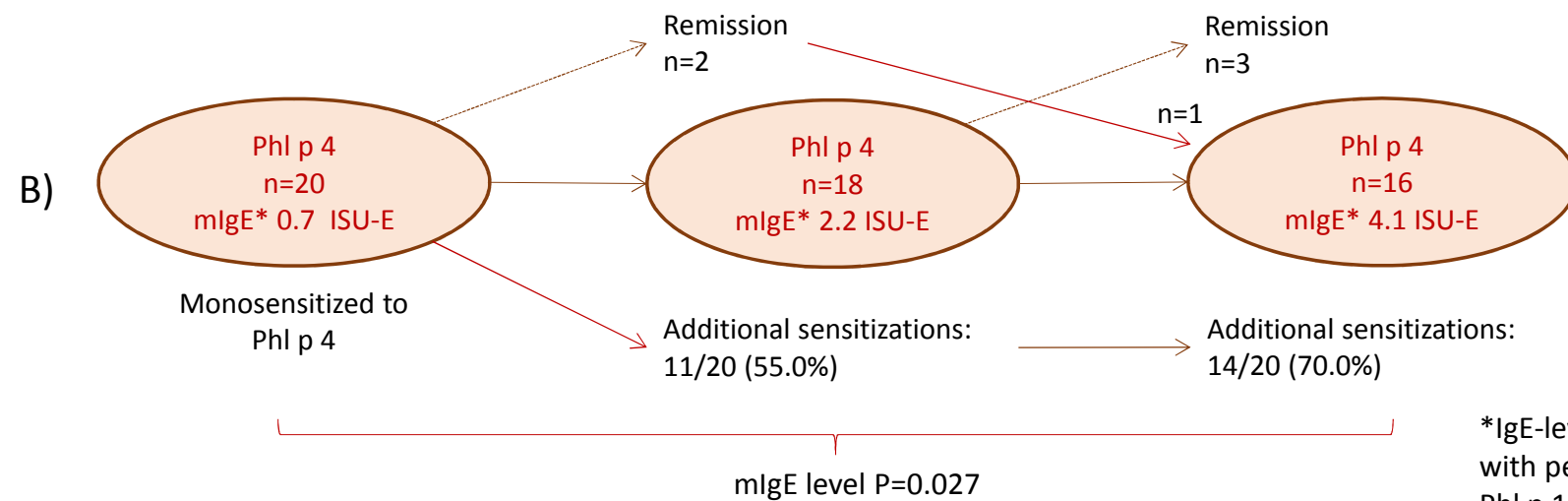
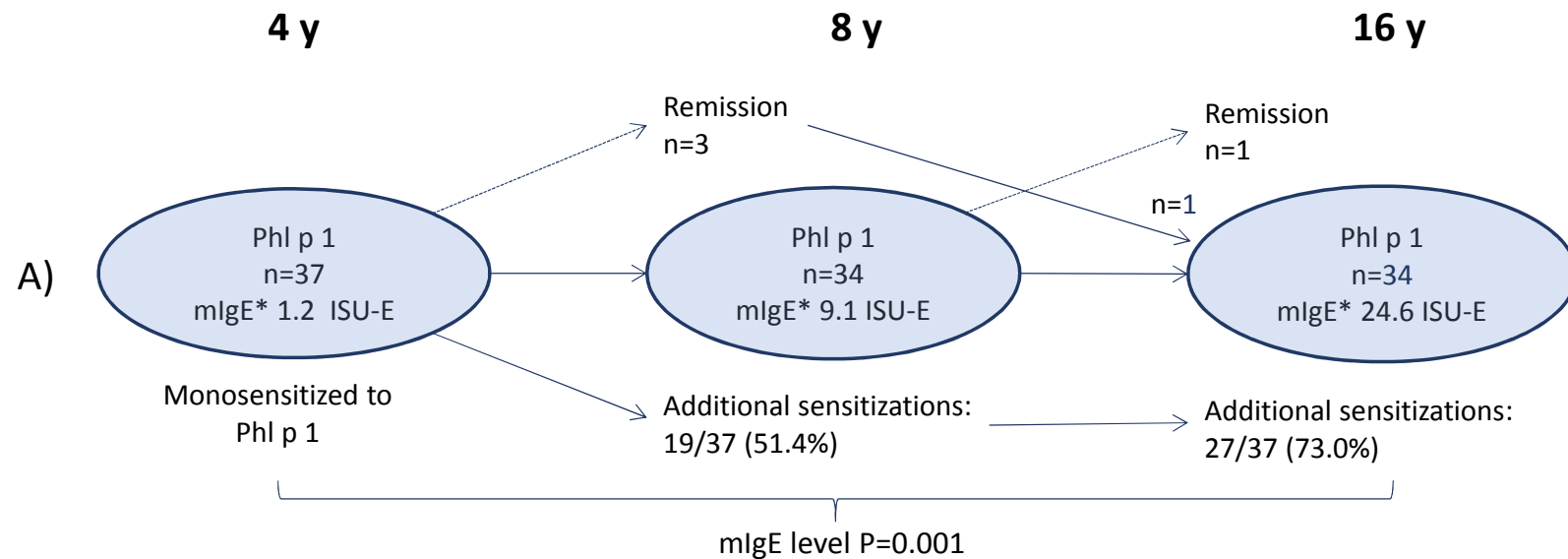


8 years



16 years





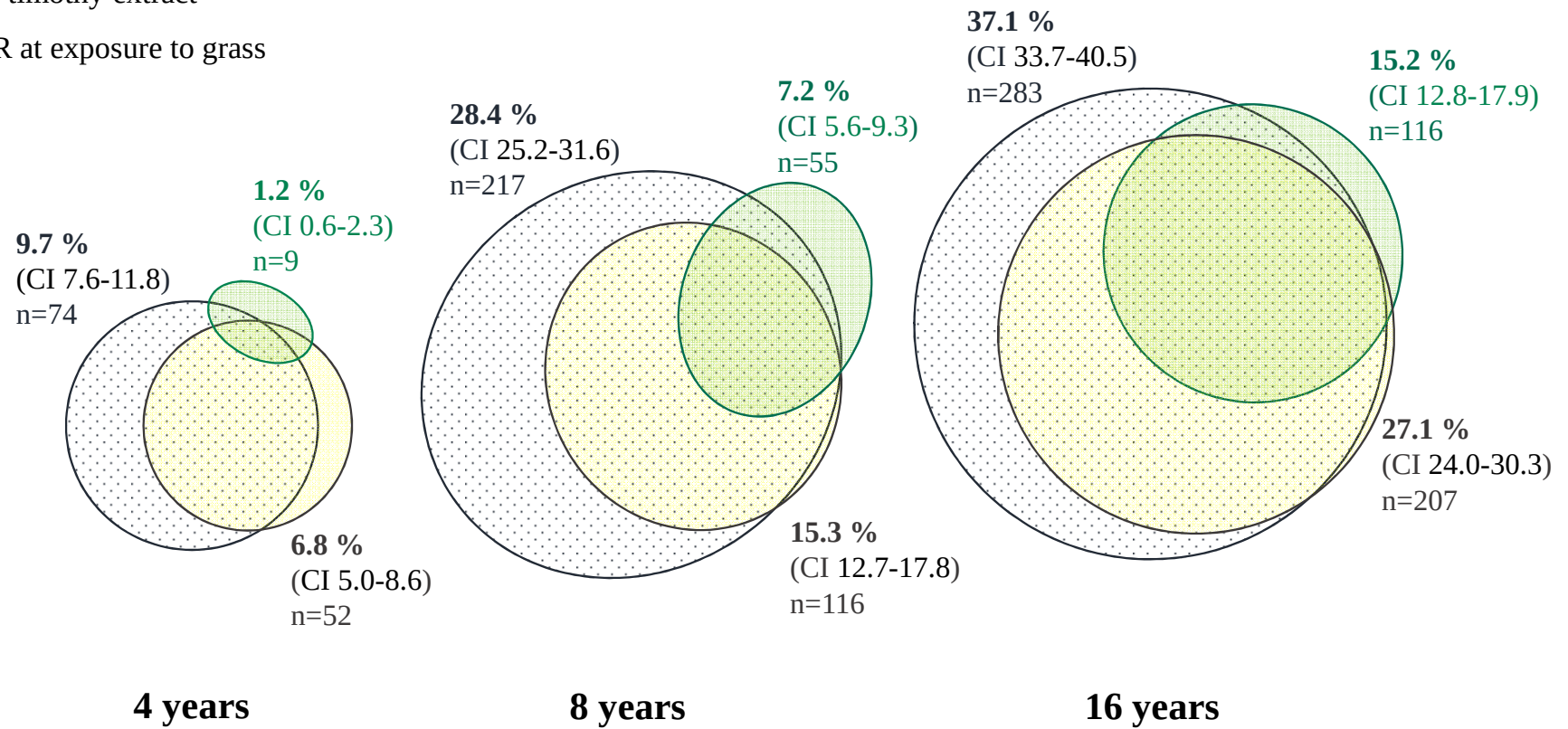
*IgE-levels calculated among the children with persistent sensitization;
Phl p 1 n=33 (89%), Phl p 4 n=15 (75%)

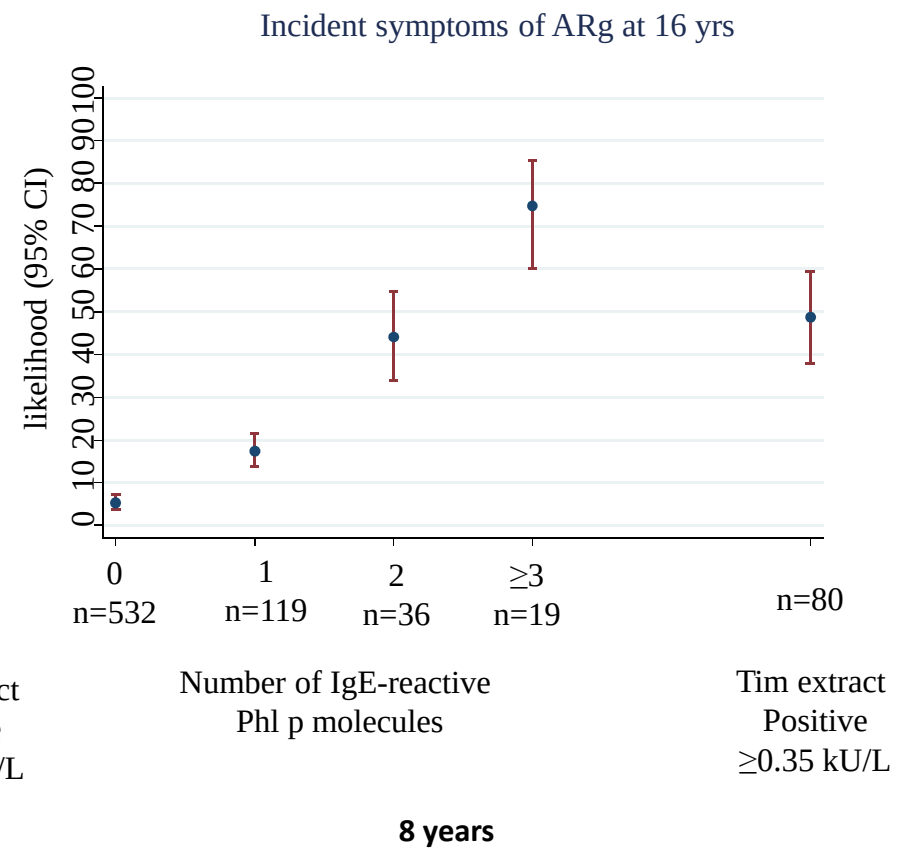
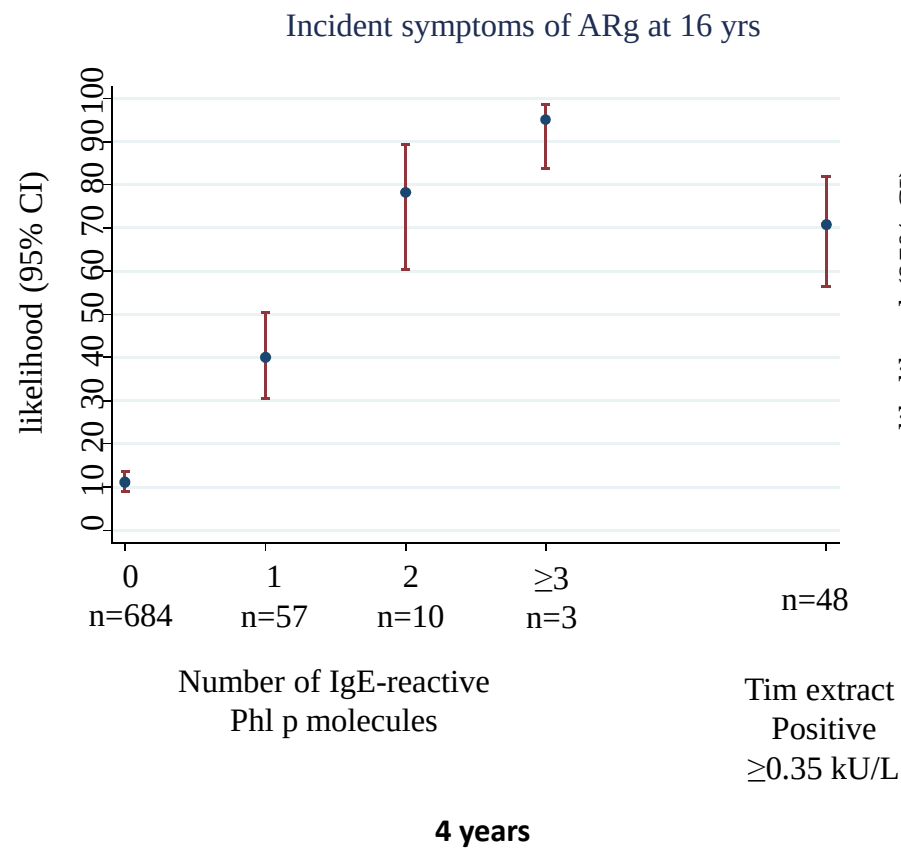
N=763

⊙ IgE-reactivity to any of Phl p 1/4/5

⊙ IgE-reactivity to timothy extract

⊙ Symptoms of AR at exposure to grass pollen





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

Symptoms of ARg									
	4 years			8 years			16 years		
	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	n.a	8/34	23.5	11.8-41.5	7/30	23.3	11.0-42.8
Phl p 5	0/2	0.0	n.a	0/3	0.0	n.a.	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

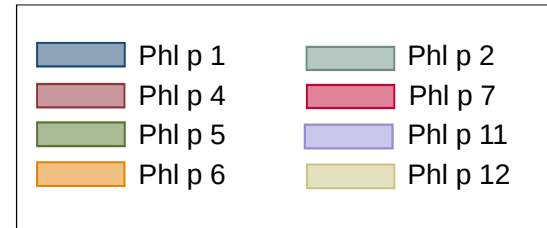
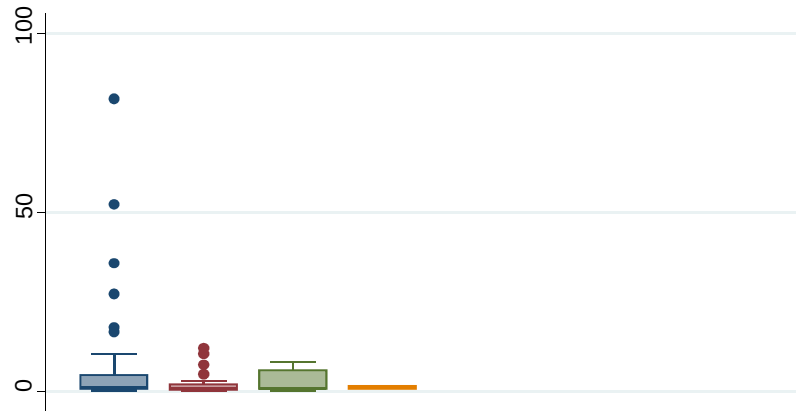
n/N=number of children with symptoms/number of children with sensitization to the allergen molecule/s at the particular time point.

*subgroup of children with polysensitization, not mutually exclusive

		ARg 16 years		
		n	%	95% CI
4 years	N			
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
8 years	N			
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	n.a.
Polysensitization				
≥2 Phl p molecules	55	28	50.9	37.5-64.2

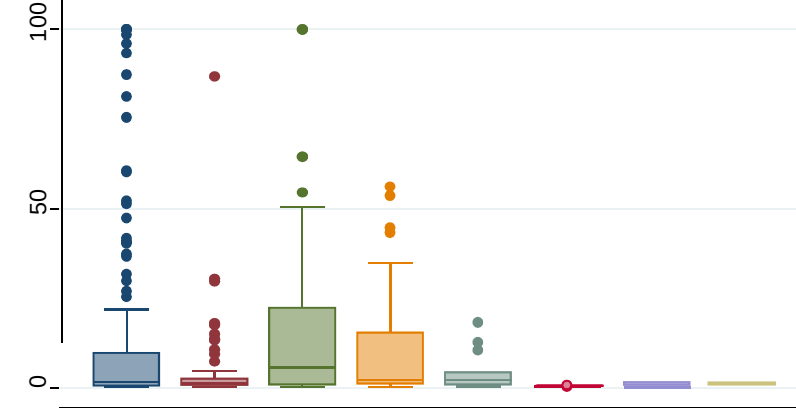
			ARg 16 years		
			n	%	95%CI
4 years	N				
Phl p 1/4/5:	Timothy extract:				
-	-	676	68	10.1	8.0-12.3
+	-	30	7	23.3	11.0-42.8
-	+	8	5	62.5	20.8-91.3
+	+	40	29	72.5	56.1-84.5
8 years	N				
Phl p 1/4/5:	Timothy extract:				
-	-	528	24	4.5	3.1-6.7
+	-	95	16	16.8	10.5-25.9
-	+	2	0	n.a.	n.a.
+	+	78	39	50.0	38.8-61.2

ISU-E



A)

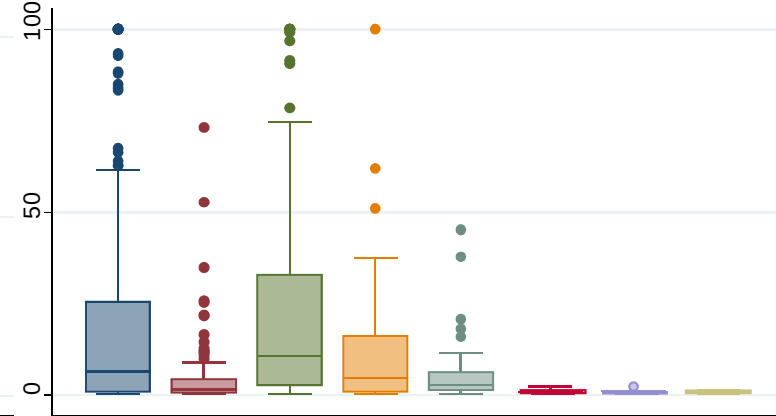
ISU-E



B)

Group	n	M	Range
Phl p 1	174	1.6	0.3-100
Phl p 4	99	1.1	0.3-86.8
Phl p 5	49	5.6	0.3-100
Phl p 6	21	2.1	0.3-56.0
Phl p 2	13	2.1	0.5-18.2
Phl p 7	5	0.5	0.3-0.7
Phl p 11	2	0.8	0.4-1.2
Phl p 12	2	1.1	0.9-1.4

ISU-E



C)

Group	n	M	Range
Phl p 1	238	6.4	0.3-100
Phl p 4	145	1.7	0.3-73.2
Phl p 5	113	10.8	0.3-100
Phl p 6	79	4.7	0.4-100
Phl p 2	52	2.9	0.4-45.3
Phl p 7	9	1.0	0.3-2.4
Phl p 11	6	0.4	0.3-1.8
Phl p 12	5	0.9	0.3-1.6

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

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

Figure E1: Levels of specific IgE (median, M; range) at A) 4 years, B) 8 years and C) 16 years of age, among 763 children from the BAMSE birth cohort.

Figure E2: The number 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 (top), 8 (middle) and 16 (bottom) (left column), and the percentages of children with additional sensitization to the other CCD-containing molecules (top, from left to right).

Table E2: Proportion of children with current symptoms of AR_g among those not sensitized, monosensitized to Phl p 1, 4 or 5 or with polysensitization (N=763). As a comparison is shown the proportion of children with symptoms when sensitized or not to timothy extract (N=763) is shown. The total number of children with symptoms at 4 years were 9, at 8 years 55 and at 16 years 116.

Table E3: The absolute risk of having incident symptoms of AR_g at 16 years of age among children without sensitization to Phl p molecules, with monosensitization to Phl p 1, 4 or 5, or with polysensitization (≥ 2 Phl p molecules), at 4 years (top) and 8 years (bottom). (Sensitization to timothy extract not taken into account). The groups are mutually exclusive.

Table E4: The absolute risk of having incident symptoms of AR_g at 16 years of age for the group of children without sensitization to Phl p 1/4/5 or timothy extract, for the group sensitized to Phl p 1/4/5 but not to timothy extract, for the group only sensitized to timothy extract and for the group with sensitization of Phl p 1/4/5 and timothy extract at 4 years (top) and 8 years (bottom). The groups are mutually exclusive. The confidence intervals are calculated as compared to no sensitization.