Early childhood IgE reactivity to pathogenesis-related class 10 proteins predicts allergic rhinitis in adolescence

Marit Westman, MD,[a,b] Christian Lupinek, MD,[c] Jean Bousquet, MD, PhD,[d] Niklas Andersson, MSc,[e] Sandra Pahr, MSc,[c] Alexandra Baar, PhD,[c] Anna Bergström, PhD,[e] Mats Holmström, MD, PhD,[a,b] Pär Stjärne, MD, PhD,[a,b] Karin C. Lødrup Carlsen, MD, PhD,[f] Kaj-Håkon Carlsen, MD, PhD,[f] Josep M. Antó, MD, PhD,[g,h,i,j] Rudolf Valenta, MD, PhD,[c] Marianne van Hage, MD, PhD,[k] and Magnus Wickman, MD, PhD,[e,l] on behalf of the Mechanisms for the Development of Allergies (MeDALL) consortium *Stockholm, Sweden, Vienna, Austria, Villejuif, France, Oslo, Norway, and Barcelona, Spain*

Background: Component-resolved diagnosis might improve the prediction of future allergy in young children.

Objective: We sought to investigate the association between IgE reactivity to the pathogenesis-related class 10 (PR-10) protein family and allergic rhinitis to birch pollen (AR_{bp}) from early childhood up to age 16 years.

Method: Questionnaire data and sera obtained at 4, 8, and 16 years of age from the Barn/Children Allergi/Allergy Milieu Stockholm Epidemiologic (BAMSE) study birth cohort were used. Sera from 764 children were analyzed for IgE reactivity to 9 PR-10 allergen proteins at the 3 time points by using an allergen chip based on ISAC technology. AR_{bp} was defined as upper airway symptoms during birch pollen exposure.

Results: IgE reactivity to Bet v 1 was found in 12%, 17%, and 25% of children at 4, 8, and 16 years of age. IgE reactivity of PR-10 proteins showed a hierarchic intrarelationship: Bet v 1 > Mal d 1 > Cor a 1.04 > Ara h 8 > Pru p 1 > Aln g 1 > Api g 1 > Act d 8 > Gly m 4. There was an increased risk of incidence and persistence of AR_{bp} up to age 16 years with increasing levels of Bet v 1–specific IgE or increasing numbers of IgE-reactive PR-10 proteins at 4 years. Children with severe AR_{bp} at age 16 years had higher levels of Bet v 1–specific IgE at age 4 years compared with children with mild symptoms.

Conclusion: AR_{bp} at age 16 years can be predicted by analysis of IgE reactivity to PR-10 proteins in early childhood. (J Allergy Clin Immunol 2014;■■■:■■■-■■■.)

Key words: *Allergen components, allergic rhinitis, oral allergy syndrome, BAMSE, birch pollen, cohort, cross-reactivity, IgE, MeDALL, microarray*

Allergic rhinitis (AR), the most common chronic disease in childhood, has a substantial effect on quality of life.[1] One of the major challenges that remain to be addressed is the prediction of onset, persistence, and severity of allergic diseases across the life cycle. Long-term birth cohort studies are essential to understanding the life course and childhood predictors of allergy and the complex interplay between genes and the environment.[2] Respiratory allergy to birch and other Fagales pollens, such as hazel and alder, is frequent in the Northern Hemisphere.[3] Allergen molecules from Fagales pollen (ie, Bet v 1 from birch pollen, Cor a 1.01 from hazel pollen, and Aln g 1 from alder), as well as some proteins in fruits, vegetables, and nuts, such as apple (Mal d 1), peach (Pru p 1), kiwi (Act d 8), celery (Api g 1), soy (Gly m 4), hazelnut (Cor a 1.04), and peanut (Ara h 8), all belong to the pathogenesis-related class 10 (PR-10) protein family and share common epitopes.[4-6] Therefore IgE antibodies to such allergen molecules might cross-react.[7]

The introduction of component-resolved diagnosis based on molecular allergens has increased the accuracy of allergy diagnosis and prognosis, particularly for peanut and hazelnut allergy.[8,9]

From [a]the Department of Clinical Science, Intervention and Technology, Division of Ear, Nose and Throat Diseases, and [e]the Institute of Environmental Medicine, Karolinska Institutet, Stockholm; [b]the Department of Ear, Nose and Throat Diseases, Karolinska University Hospital, Stockholm; [c]the Division of Immunopathology, Department of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna; [d]University Hospital of Montpellier, Hôpital Arnaud de Villeneuve, Montpellier, INSERM 1018, Villejuif; [f]the Department of Pediatrics, University of Oslo, Oslo University Hospital; [g]the Centre for Research in Environmental Epidemiology (CREAL), [h]IMIM (Hospital del Mar Research Institute), [i]Universitat Pompeu Fabra (UPF), and [j]CIBER Epidemiología y Salud Pública (CIBERESP), Barcelona; [k]the Clinical Immunology and Allergy Unit, Department of Medicine Solna, Karolinska Institutet and University Hospital, Stockholm; and [l]Sachs' Children's Hospital, Södersjukhuset, Stockholm.

Supported by the Swedish Asthma and Allergy Research Foundation; the Frimurare Barnhuset Foundation; the Acta Oto-Laryngologica Foundation; Stockholm County Council; the Swedish Research Council of Health, Working Life and Welfare; the Swedish Research Council, Swedish Heart-Lung Foundation; the Swedish Cancer and Allergy Foundation; the European Commission's Seventh Framework 29 Program MeDALL under grant agreement no. 261357, and in part by grant F4605 of the Austrian Science Fund (FWF).

Disclosure of potential conflict of interest: C. Lupinek has received payment for delivering lectures from Thermo Fisher. J. Bousquet has received consultancy fees from Actelion, Almirall, Meda, Merck, MSD, Novartis, Sanofi-Aventis, Takeda, Teva, Uriach, AstraZeneca, Chiesi, GlaxoSmithKline, OM Pharma, Schering Plough, and Stallergènes and received support for travel to meetings for this study or other purposes from Actelion, Almirall, Meda, Merck, MSD, Novartis, Sanofi-Aventis, Takeda, Teva, and Uriach. P. Stjärne's institution has received funding, and has received or has grants pending from the ALF. K. C. Lødrup Carlsen's institution has received funding from the EU-MeDALL (grant no. 261357). K.-H. Carlsen has received compensation for board membership from Meda and Boehringer Ingelheim, as well as consultancy fees from Novartis, and payment for delivering lectures from Takeda, Novartis, and Boehringer Ingelheim, and he has received compensation for travel and other meeting-related expenses from Sandoz. J. M. Antó's institution has received funding from the European Commission (grant no. 261357). R. Valenta's institution has received funding from the EU (grant no. 261357), he has received consultancy fees, and he has received or has grants pending from Biomay AG, Thermo Fisher Scientific, and Fresenius Medical Care. M. van Hage has received consultancy fees from Hycor Biomedical, as well as payment for delivering lectures from Thermo Fisher Scientific, Novartis, and ALK-Abelló. M. Wickman's institution has received funding from the European Union (grant no. 261357); he has received consultancy fees, payment for delivering lectures, and has received or has grants pending from Thermo Fisher Scientific; and he has also received consultancy fees from Mictrotest DX. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication June 25, 2014; revised September 25, 2014; accepted for publication October 20, 2014.

Corresponding author: Marit Westman, MD, Institute of Environmental Medicine, Karolinska Institutet, PO Box 210, SE 171 77 Stockholm, Sweden. E-mail: marit.westman@ki.se.

0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2014.10.042

Abbreviations used
AR: Allergic rhinitis
AR_{bp}: Allergic rhinitis to birch pollen
BAMSE: Barn/Children Allergi/Allergy Milieu Stockholm Epidemiologic
ISU-E: ISAC standardized units for IgE detection
MeDALL: Mechanisms for the Development of Allergies
OAS: Oral allergy syndrome
OR: Odds ratio
PR-10: Pathogenesis-related class 10

Furthermore, component-resolved IgE testing has also improved our knowledge regarding the progression of sensitization and development of symptoms[10] and selection of immunotherapy.[11]

Molecular multiplex platforms, such as ISAC,[12] are promising tools for obtaining an overview of the sensitization profile to a number of allergen sources, enabling discrimination between genuine versus cross-reactive sensitization.

PR-10 component-resolved diagnosis has been used in various populations of allergic patients,[13,14] but no study has thus far assessed the predictive value of IgE testing to PR-10 proteins in a population-based child cohort.

The aim of this study was to investigate whether IgE reactivity to allergenic molecules of the PR-10 protein family in childhood was associated with the occurrence, incidence, and persistence of AR to birch pollen up to 16 years of age. Secondary aims were to assess the association between IgE reactivity to PR-10 proteins and the severity of AR to birch pollen and occurrence of oral allergy syndrome (OAS) at age 16 years.

METHODS

Barn/Children Allergi/Allergy Milieu Stockholm Epidemiologic study cohort

Within the framework of Mechanisms for the Development of Allergies (MeDALL), a European Union–funded project (http://medall-fp7.eu/),[15] we analyzed data from the population-based birth cohort Barn/Children Allergi/Allergy Milieu Stockholm Epidemiologic (BAMSE) study of 4089 children born in the mid-1990s in Sweden. The cohort has been described in detail elsewhere.[16] In brief, baseline data were obtained shortly after birth and repeatedly thereafter up to age 16 years by using parental questionnaires on symptoms of allergy-related disorders (see Fig E1 in this article's Online Repository at www.jacionline.org). At age 16 years, the children answered questionnaires as well. The response rate at the latest follow-up at age 16 years was 78% from baseline. Families who completed the questionnaire for their children at 4, 8, and 16 years of age were invited for blood sampling. The number of children with blood samples obtained at all 3 time points was 1699. In a previous study background characteristics between the 1699 children and the BAMSE cohort were assessed, and no significant differences were found.[17] Permission for the study was obtained from the Regional Ethical Review board at Karolinska Institutet, Stockholm, Sweden, throughout the study years, and parents provided informed consent for each follow-up.

Study population

Eight hundred of the 1699 children with available sera from all 3 time points were randomly selected for microarray testing. Children with results from the microarray testing and complete information from the parental questionnaires on AR symptoms when exposed to birch pollen at 4, 8, and 16 years of age were included in the study (n = 764, see Fig E1).

Definition of symptoms

Allergic rhinitis to birch pollen (AR_{bp}) was defined as sneezing; runny, itchy, or blocked nose; and itchy eyes when exposed to birch pollen.[18,19] Birch pollen–related asthma was defined as respiratory symptoms (difficult breathing, tightness of chest, and wheezy or raspy breathing) or bothersome cough when exposed to birch pollen.

Incident AR_{bp} at 8 and 16 years of age refers to children with AR_{bp} at the respective time points but with no previously reported AR_{bp}. Children who reported AR_{bp} at an earlier age were excluded from the analysis. Persistent AR_{bp} among 4-year-olds was defined as AR_{bp} at 4, 8, and 16 years of age, and that among 8-year-olds was defined as AR_{bp} at 8 and 16 years of age but no AR_{bp} at age 4 years. Severity of AR_{bp} at age 16 years was classified according to Allergic Rhinitis and its Impact on Asthma into mild (no effect on daily activities or sleep) and moderate/severe (effect on daily activities, sleep, or both).[20]

OAS was defined as itch in the mouth, throat, or ears and/or swollen feeling in the mouth or throat after consumption of PR-10 allergen–containing plant food.[21]

Information on outcome (AR_{bp} and OAS) was obtained from the parental questionnaires, except for severity of disease, which was based on children's answers.

Specific IgE reactivity

The serum samples were analyzed for IgE reactivity to microarrayed allergen molecules by using the MeDALL chip, which is based on the ISAC microarray platform (Phadia Multiplexing; Thermo Fisher Scientific, Uppsala, Sweden) but differs from the commercially available ISAC regarding outlay and the number of allergens. The technical details and features of the MeDALL chip together with the cutoff of 0.3 ISAC standardized units for IgE detection (ISU-E) or greater are described in detail by Lupinek et al.[22] IgE reactivity profiles and levels were measured for PR-10 proteins (Bet v 1, Mal d 1, Aln g 1, Cor a 1.04, Ara h 8, Pru p 1, Api g 1, Act d 8, and Gly m 4). A level of 0.3 ISU-E or greater was considered positive. Briefly, aliquots of 35 μL of serum were incubated on the microarray, and after 120 minutes of incubation at room temperature, slides were washed, and fluorescence-labeled anti-IgE antibodies (Thermo Fisher) were added and incubated for 30 minutes. Chips were then washed, dried, and analyzed with a Laser Scan Confocal microarray reader (LuxScan 10K/A; Capital-Bio, Beijing, China). The results were evaluated by using Phadia Microarray Image Analysis (MIA) software and are reported in ISU-E.[22]

Statistical analyses

The distributions of selected baseline characteristics for the study population and the original cohort were compared by using the *t* test with finite population correction. For significant results, a sensitivity analysis was performed. IgE reactivity levels are presented as box plots with median levels and 25th and 75th percentiles. Comparison of specific IgE levels between time points or between those with mild or moderate/severe symptoms was performed with quantile regression. Correlations between levels of Bet v 1–specific IgE and numbers of IgE-reactive components were assessed with the Spearman correlation test. Fitted predicted probability curves were plotted based on a logistic regression model to assess the probability of AR_{bp} in relation to Bet v 1–specific IgE levels. Fitted predicted probability curves were also performed for comparison between Bet v 1–specific IgE levels (ISAC) and birch-specific IgE levels (ImmunoCAP) and between the study population and the original cohort. Similarly, probability curves for OAS from apple, hazelnut, peanut, kiwi, and soy in relation to specific IgE levels of the corresponding PR-10 proteins were plotted. Children with IgE reactivity to other allergen components (eg, Ara h 2 and Cor a 9) known to produce severe reactions were excluded from the analyses in this context.

The number of recognized PR-10 proteins was categorized into 3 groups at 4 and 8 years of age (1, Bet v 1 only; 2, Bet v 1 and up to the median number of other recognized PR-10 proteins; and 3, Bet v 1 and greater than the median number of other PR-10 proteins). The association of IgE reactivity according to these categories in relation to AR_{bp} up to age 16 years was calculated with generalized estimating equations. As a complement, absolute risks of AR_{bp} at 8 or 16 years of age was calculated.

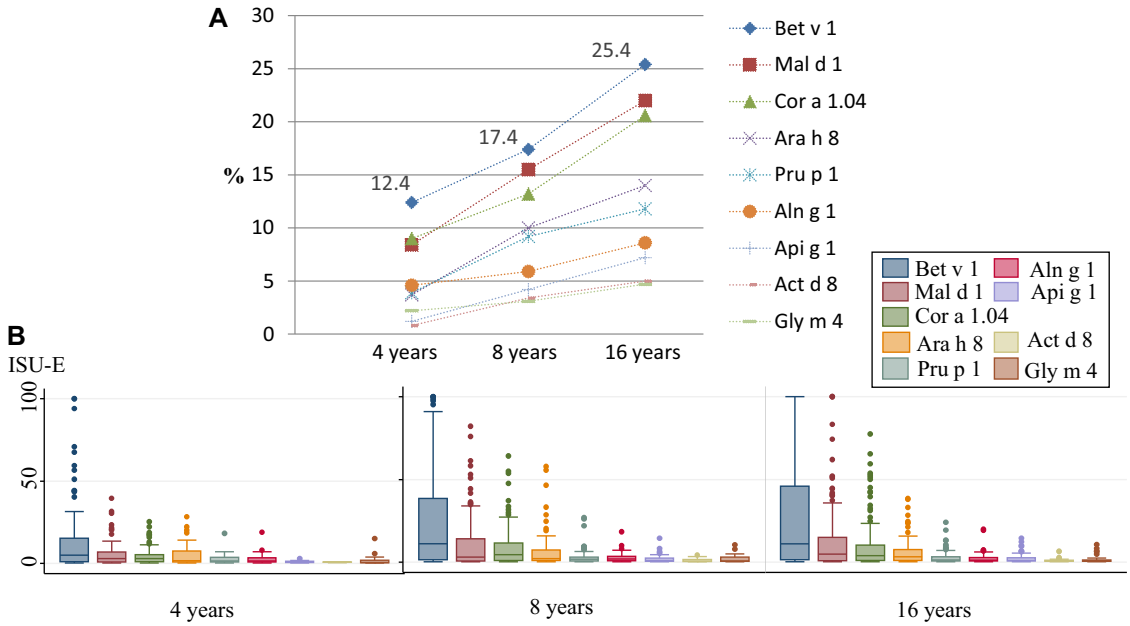


FIG 1. A, Prevalence of IgE reactivity to PR-10 proteins in the population (≥0.3 ISU-E). **B,** Specific IgE levels to the different PR-10 proteins among sensitized children (≥0.3 ISU-E), where *boxes* show the median levels and 25th and 75th percentiles at 4, 8, and 16 years of age, respectively.

P values of less than .05 were considered as statistically significant. A detailed description of the statistical analyses is presented in the Methods section in this article's Online Repository at www.jacionline.org. All analyses were conducted with STATA Statistical Software, version 13.1 (StataCorp, College Station, Tex). For drawing of proportional Venn diagrams, eulerAPE was used (http://www.eulerdiagrams.org/eulerAPE).

RESULTS

Study population versus study base

When comparing baseline characteristics at birth between the study population and the BAMSE cohort, no significant differences were found, except for low socioeconomic status and birth month (see Table E1 in this article's Online Repository at www.jacionline.org). However, these differences were small and shown by means of sensitivity analysis not to influence the results (data not shown). Moreover, the probability of AR_{bp} at 4, 8, or 16 years of age in relation to IgE levels to birch did not differ between the study population and the original cohort (see Fig E2 in this article's Online Repository at www.jacionline.org).

IgE reactivity to PR-10 proteins

At 4, 8, and 16 years of age, 12.4%, 17.4%, and 25.4% of the children had IgE reactivity to Bet v 1, respectively. The presence of IgE reactivity to the other PR-10 proteins was seen in the following order at 8 and 16 years of age: Bet v 1 > Mal d 1 > Cor a 1.04 > Ara h 8 > Pru p 1 > Aln g 1 > Api g 1 > Act d 8 > Gly m 4 (Fig 1). Among children with IgE reactivity to Bet v 1, the proportion of IgE reactivity to any of the other 8 remaining PR-10 proteins was 75.8%, 82.0%, and 83.0% at 4, 8, and 16 years of age, respectively. IgE levels at the 3 time points were highest for Bet v 1 compared with the other PR-10 proteins (Fig 1). Between 4 and 8 years of age, the median IgE level for Bet v 1 doubled (5.1 ISU to 11.3 ISU-E, $P = .055$), but from 8 to 16 years of age, the corresponding median level remained unchanged (11.1 ISU-E, 4 vs 16 years; $P = .080$).

The hierarchy of IgE reactivity to the different PR-10 proteins was further assessed (Fig 2). Among the 4-, 8-, and 16-year-olds with IgE reactivity to Bet v 1, between 65% and 79% also exhibited IgE reactivity to Mal d 1, but only 18% to 19% exhibited IgE reactivity to Gly m 4. On the other hand, among children with IgE reactivity to Gly m 4, 100% had IgE reactivity to Bet v 1, Mal d 1, and Cor a 1.04. A close correlation (Rho = 0.87-0.90) between IgE levels to Bet v 1 and the number of other IgE-reactive PR-10 proteins was noted (see Fig E3 in this article's Online Repository at www.jacionline.org).

IgE reactivity to PR-10 proteins in relation to symptoms

The prevalence of birch-related airway symptoms in Bet v 1–sensitized subjects is shown in Fig 3. At 4, 8, and 16 years of age, 2.5%, 10.6%, and 17.8% of the subjects had AR_{bp}. At age 4 years, only 15% (n = 14) of Bet v 1–specific IgE–positive children had AR_{bp} (Fig 3). At 8 and 16 years of age, the corresponding proportions were 50% (n = 66) and 54% (n = 104). The median ISU-E levels in asymptomatic children were significantly lower in all age groups compared with those in symptomatic children (3.2 vs 19.9 at age 4 years, 1.8 vs 28.3 at age 8 years, and 2.3 vs 32.7 at age 16 years; Fig 3).

The probability of reporting AR_{bp} in relation to Bet v 1–specific IgE levels at the different time points was assessed cross-sectionally. At 4, 8, and 16 years of age, the cross-sectional probability to report AR_{bp} increased with increasing levels of Bet v 1–specific IgE (see Fig E4 in this article's Online Repository at www.jacionline.org) and with the number of IgE-reactive PR-10 proteins (see Table E2 in this article's Online Repository at www.jacionline.org). As a comparison, probability curves for AR_{bp} in relation to IgE to birch was performed. The probability

4 years	n	Bet v 1 %	Mal d 1 %	Cor a 1.04 %	Ara h 8 %	Pru p 1 %	Aln g 1 %	Api g 1 %	Act d 8 %	Gly m 4 %
Bet v 1	95	100	65.3	70.5	29.5	31.6	36.8	8.4	6.3	17.9
Mal d 1	64	96.9	100	89.1	42.2	46.9	53.1	12.5	9.4	26.6
Cor a 1.04	69	97.1	82.6	100	40.6	42.0	49.3	11.6	8.7	24.6
Ara h 8	28	100	96.4	100	100	75.0	85.7	25.0	21.4	57.1
Pru p 1	30	100	100	96.7	70.0	100	80.0	23.3	20.0	53.3
Aln g 1	35	100	97.1	97.1	68.6	68.6	100	20.0	17.1	45.7
Api g 1	9	88.9	88.9	88.9	77.8	77.8	77.8	100	22.2	77.8
Act d 8	6	100	100	100	100	100	100	33.3	100	66.7
Gly m 4	17	100	100	100	94.1	94.1	94.1	41.2	23.5	100
8 years										
Bet v 1	133	100	77.4	75.2	57.1	51.1	33.1	22.6	17.3	18.1
Mal d 1	118	87.3	100	80.5	63.6	57.6	37.3	25.4	19.5	20.3
Cor a 1.04	101	99.0	94.1	100	74.3	66.3	43.6	29.7	22.8	23.8
Ara h 8	76	100	98.7	98.7	100	82.9	54.0	38.2	30.3	31.6
Pru p 1	70	97.1	97.1	95.7	90.0	100	57.1	38.6	31.4	31.4
Aln g 1	45	97.8	97.8	97.7	91.1	88.9	100	40.0	40.0	37.8
Api g 1	32	93.8	93.8	93.8	90.6	84.4	56.3	100	53.1	46.9
Act d 8	26	88.5	88.5	88.5	88.5	84.6	69.2	65.4	100	38.5
Gly m 4	24	100	100	100	100	91.7	70.8	62.5	41.7	100
16 years										
Bet v 1	194	100	78.9	78.4	54.6	46.4	34.0	26.8	19.1	18.6
Mal d 1	168	91.1	100	86.3	63.1	53.6	39.3	31.0	22.0	21.4
Cor a 1.04	157	96.8	92.4	100	68.2	56.7	42.0	33.1	23.6	22.9
Ara h 8	107	99.1	99.1	100	100	77.6	54.2	47.7	34.6	33.6
Pru p 1	90	100	100	98.9	92.2	100	64.4	53.3	41.1	36.7
Aln g 1	66	100	100	100	87.9	87.9	100	54.6	48.5	43.9
Api g 1	55	94.6	94.6	94.6	92.7	87.3	65.5	100	52.7	45.5
Act d 8	38	97.4	97.4	97.4	97.4	97.4	84.2	76.3	100	55.3
Gly m 4	36	100	100	100	100	91.7	80.6	69.4	58.3	100

FIG 2. Number of children with IgE-reactivity to the different PR-10 proteins *(left column)* and percentages with additional IgE-reactivity to other PR-10 proteins. *Green,* High degree of IgE cross reactivity; *Red,* low degree of IgE cross reactivity.

of AR_{bp} was higher at a Bet v 1–specific IgE level of 50 ISU-E compared with a birch-specific IgE level of 50 kU/L at 4 and 8 years of age (see Fig E2).

Trajectories of PR-10 protein IgE, sensitization, and symptoms

Onset, persistence, and severity of AR_{bp} were studied longitudinally. The probability to report incident symptoms of AR_{bp} at age 8 years increased with increasing levels of Bet v 1–specific IgE at 4 years, as did the probability to report incident symptoms at age 16 years with increasing Bet v 1–specific IgE levels at age 8 years (Fig 4).

The odds ratios (ORs) for incident AR_{bp} in relation to the number of IgE-reactive PR-10 proteins is displayed in Table I, and absolute risks are shown in Table E3 in this article's Online Repository at www.jacionline.org. Among asymptomatic children with IgE reactivity to Bet v 1 only at age 4 years, the overall OR for having AR_{bp} at 8 or 16 years of age was 7.1 (95% CI, 3.3-15.3). The OR increased if 1 to 2 (26.2; 95% CI, 13.1-52.3) or 3 or more (45.1; 95% CI, 21.3-95.5) IgE reactivities beside Bet v 1 were present (Table I). Between 8 and 16 years of age, this pattern was less clear (Table I and see Table E3).

No child with AR_{bp} at age 4 years had IgE reactivity to Bet v 1 alone (see Table E4 in this article's Online Repository at www.jacionline.org). The absolute risk of persistence of AR_{bp} was 67% in subjects with IgE reactivity to Bet v 1 in combination with 1 to 3 other PR-10 proteins (see Table E4). If IgE reactivity was present to 4 or more PR-10 proteins at age 4 years, the corresponding risk for persistence of AR_{bp} reached 100%. At 8 years of age, only 3 children with AR_{bp} had IgE reactivity to Bet v 1 alone, and all of them had persistent symptoms up to age 16 years (see Table E4). Analysis of the other PR-10 proteins for persistence of symptoms from 8 to 16 years of age did not provide much added value (see Table E5 in this article's Online Repository at www.jacionline.org).

Among the 30% of 16-year-old children who were classified as having moderate/severe AR_{bp}, Bet v 1–specific IgE levels at age 4 years were higher than those in children with mild AR_{bp} (25th percentile: 1.5 vs 8.1 ISU-E, $P = .001$; median: 4.4 vs 15.1 ISU-E, $P = .090$; Fig 5).

Among children with AR_{bp} and IgE reactivity to Bet v 1 at age 16 years, 63% reported symptoms of OAS to any of the food items apple, hazelnut, peanut, peach, kiwi, or soy. Apple was the most frequently reported food item (47%), followed by peach (29%) and hazelnut (27%). The probability of reporting symptoms of OAS after ingestion of any of the food items apple, hazelnut, peanut, peach, kiwi, or soy increased with increasing Bet v 1–specific IgE levels (see Fig E5 in this article's Online Repository at www.jacionline.org). A similar or even higher probability was seen for OAS after ingestion of a specific food item in relation to the IgE level of the corresponding PR-10 component (see Fig E5).

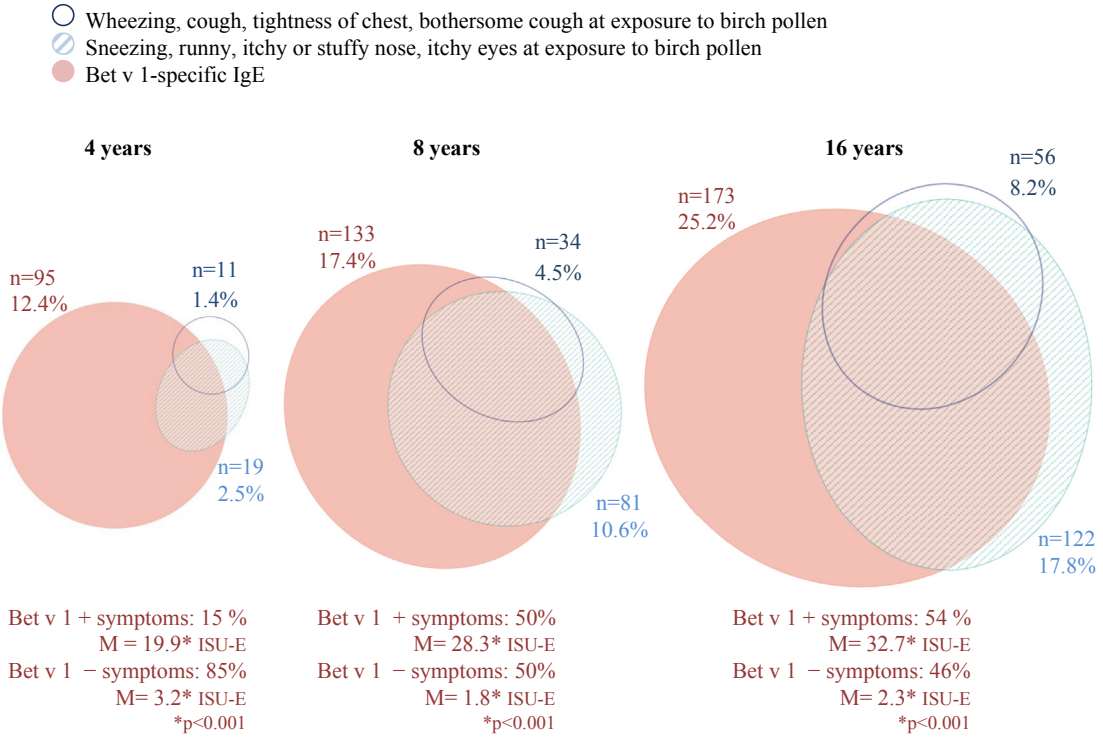


FIG 3. Proportional Venn diagram of numbers of children who reported symptoms after exposure to birch pollen from the *upper* and *lower* airways, respectively, and IgE reactivity to Bet v 1 at 4 (n = 764), 8 (n = 763), and 16 (n = 686) years of age.

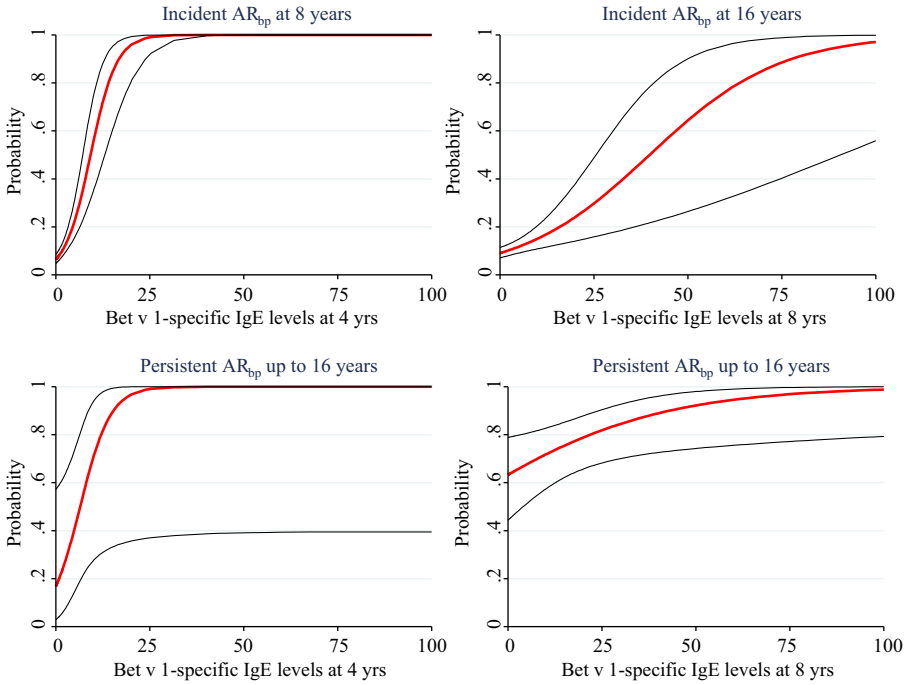


FIG 4. Probabilities for incident or persistent AR_{bp} from 4 to 16 years of age in relation to Bet v 1–specific IgE levels (ISU-E) at 4 and 8 years of age, respectively.

DISCUSSION

To our knowledge, this is the first study to investigate IgE reactivity to PR-10 allergen components as a possible predictor for AR_{bp} during childhood to adolescence. We found that the risk of later onset or persistence of symptoms of AR_{bp} increased with increasing levels of Bet v 1–specific IgE or increasing numbers of recognized PR-10 proteins at 4 years. High levels of IgE to Bet v 1 at age 4 years were associated with severe AR_{bp} at age 16 years.

TABLE I. Proportions and ORs (generalized estimating equations) for onset of AR_{bp} at 8 and 16 years of age in relation to the number of IgE-reactive PR-10 proteins at 4 and 8 years of age, respectively

		AR_{bp} at age 8 y				AR_{bp} at age 16 y				Overall	
		No.	Percent	OR	95% CI	No.	Percent	OR	95% CI	OR	95% CI
No. of PR-10 at age 4 y (n = 740)†											
0	659	23	3.5	Reference	Reference	51	7.7	Reference	Reference	Reference	Reference
Bet v 1 only	23	4	17.4	5.8	1.8-18.5	8	34.8	8.2	3.5-19.4	7.1§	3.3-15.3
Bet v 1 and 1-2*	29	19	65.5	52.5	22.0-125.6	3	10.3	12.7	5.8-27.8	26.2§	13.1-52.3
Bet v 1 and ≥3	29	19	65.5	52.5	22.0-125.6	5	17.2	43.2	15.9-116.8	45.1§	21.3-95.5
No. of PR-10 at age 8 y (n = 656)‡	No.										
0	590	NA	NA	NA	NA	36	6.1	Reference	Reference	NA	NA
Bet v 1 only	21					9	42.9	11.5	4.6-29.2		
Bet v 1 and 1-2*	23					9	39.1	9.9	4.0-24.4		
Bet v 1 and ≥3	22					11	50.0	15.4	6.2-37.9		

*Median number of components or less.
†No symptoms of AR_{bp} reported at age 4 years.
‡No symptoms of AR_{bp} reported at age 4 or 8 years.
§*P* for trend < .001.

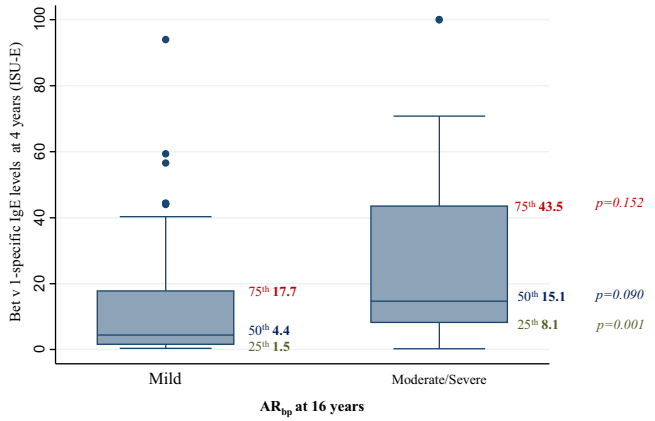


FIG 5. Bet v 1–specific IgE levels (ISU-E) at age 4 years among children with mild AR_{bp} compared with those with moderate/severe AR_{bp} at 16 years of age, with *box plots* showing median levels and 25th and 75th percentiles.

Furthermore, the likelihood of reported OAS increased by increasing ISU-E levels to the corresponding PR-10 protein.

Bet v 1 was the most prevalent sensitizing PR-10 protein at 4, 8, and 16 years of age, and median levels of Bet v 1–specific IgE were higher than for the other PR-10 proteins. This was consistent with results from earlier studies indicating that in a birch-endemic region, Bet v 1 is the driving allergen in sensitization to the other PR-10 proteins.[23,24]

The IgE reactivity to PR-10 proteins showed a hierarchic intrarelationship as follows: Bet v 1 > Mal d 1 > Cor a 1.04 > Ara h 8 > Pru p 1 > Aln g 1 > Api g 1 > Act d 8 > Gly m 4, where IgE reactivity to the more prevalent components was almost always present when IgE reactivity to the less common components were seen. The result might reflect routes and amounts of allergen exposure, different allergenicity, and/or the degree of homology between Bet v 1 and the other PR-10 protein molecules.[25] This pattern might look different in a region with less dominant Bet v 1 exposure.[26]

A large proportion of children with IgE reactivity to Bet v 1 did not report symptoms. The presence of IgE sensitization without clinical symptoms is well known.[10,19,27] IgE levels were higher among children with symptoms than among asymptomatic children, a finding in accordance with previous studies.[27] There were also children who reported symptoms but did not have IgE reactivity. This has also been reported and might be due to nonallergic rhinitis or infectious rhinitis misinterpreted as allergic symptoms[18,28] or local AR.[29]

At 4 or 8 years of age, the possibility to predict onset of AR_{bp} up to 16 years of age increased with increasing Bet v 1–specific IgE levels. Several studies have shown that sensitization often precedes rhinitis symptoms.[10,19,30] In our study we saw that levels of Bet v 1–specific IgE correlated very well with numbers of other IgE-reactive PR-10 proteins at all 3 time points. Consequently and in line with our hypothesis, the more PR-10 proteins recognized by IgE, the higher the probability to report symptoms to birch pollen. When analyzing the data longitudinally, we saw a tendency of higher risk for incident symptoms from 4 to 8 or 16 years of age, as well as persistence of symptoms from age 4 up to 16 years with increasing numbers of PR-10 proteins, although interpretation should be carried out with caution because of low numbers of children in these analyses. Yet one has to bear in mind that PR-10 cross-reactivity will increase with Bet v 1–specific IgE levels and that levels and affinities of Bet v 1–specific IgE are the driving forces in this process.

The probability of having persistence of symptoms from 8 to 16 years of age was high already at low IgE levels to Bet v 1 at the age of 8 years. Analysis of Bet v 1–specific IgE levels and IgE reactivity to other PR-10 proteins did not seem to provide much added value in this context. Thus it appears as if the 8-year-old children, who were sensitized already at age 4 years, were no longer in their early phase of disease. In addition, those who were sensitized and had symptoms at this age had a persistence of disease,[10] which is important information when intervention treatment, such as allergen-specific immunotherapy, is considered. In fact, it has been reported that allergen-specific immunotherapy prevents the progression of AR to asthma,[31] and based on our data, it is quite tempting to consider specific immunotherapy as a preventive intervention in the early phase of allergic sensitization when there is still plasticity of the IgE response.

At 16 years of age, the probability of reporting symptoms of OAS after exposure to apple, peach, hazelnut, peanut, and soy

also increased with increasing IgE reactivity to the corresponding PR-10 protein. This is consistent with what is seen for other types of food allergies.[32,33] These results most likely reflect the probability of reporting OAS symptoms for the corresponding PR-10 protein because we excluded children with IgE to allergen components known to cause severe reactions, such as storage and lipid transfer proteins in the analysis. Among children with AR_{bp} and IgE reactivity to Bet v 1 at 16 years of age, the most commonly reported food item was apple, which is consistent with studies mostly among adults.[34,35] However, the proportion of children reporting OAS symptoms from apple (47%), as well as from hazelnut, peanut, peach, kiwi, and soy, was lower than previously reported. These studies were conducted among adults and at allergy clinics, which differs from the current study, which was conducted among adolescents in a population-based setting.

The strengths of this study are the large sample size of children with results from IgE testing in a population-based design and the high follow-up rate (ie, 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 PR-10 proteins, requiring only a small serum volume, which would have been impossible with traditional diagnostic tests. Therefore the MeDALL chip is well suited for the analysis of sera in birth cohorts and children, where only small volumes of serum are available.

However, there are some limitations. The study population was a sample of the original cohort, but a comparison of baseline characteristics, as well as cross-sectional probability curves for AR_{bp} in relation to levels of specific IgE to birch, between the study population and the original cohort showed no major differences.

The definition of AR is based on questionnaires only, and reporting of symptoms might 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 response.[18] In our study a similar proportion (74% to 81%) of children who reported symptoms of AR at exposure to birch pollen had IgE reactivity to Bet v 1. The questionnaires were answered without parents or children knowing about the IgE reactivity, and thus any misclassification would be nondifferential.

In conclusion, in this birth cohort of well-characterized children, we show that the risk of onset and persistence of AR_{bp} up to 16 years of age increased with Bet v 1–specific IgE levels, as well as the number of IgE-reactive PR-10 proteins in early childhood. Thus analysis of early IgE reactivity to PR-10 allergen components might be a useful tool in predicting the course of AR_{bp} up to 16 years of age.

We thank all the families who participated and the staff working with the BAMSE project. We also thank Renata Kiss, Department of Pathophysiology and Allergy Research, Medical University of Vienna, Austria, for technical assistance regarding chip measurements.

Clinical implications: Analysis of IgE reactivity to PR-10 allergen molecules in childhood might be a useful tool for predicting the onset and persistence of AR_{bp} up to 16 years of age.

REFERENCES

1. Meltzer EO, Blaiss MS, Derebery MJ, Mahr TA, Gordon BR, Sheth KK, et al. Burden of allergic rhinitis: results from the Pediatric Allergies in America survey. J Allergy Clin Immunol 2009;124(suppl):S43-70.
2. Bousquet J, Gern JE, Martinez FD, Anto JM, Johnson CC, Holt PG, et al. Birth cohorts in asthma and allergic diseases: report of a NIAID/NHLBI/MeDALL joint workshop. J Allergy Clin Immunol 2014;133:1535-46.
3. D'Amato G, Cecchi L, Bonini S, Nunes C, Annesi-Maesano I, Behrendt H, et al. Allergenic pollen and pollen allergy in Europe. Allergy 2007;62:976-90.
4. Andersen MB, Hall S, Dragsted LO. Identification of European allergy patterns to the allergen families PR-10, LTP, and profilin from Rosaceae fruits. Clin Rev Allergy Immunol 2011;41:4-19.
5. Breiteneder H, Ebner C. Molecular and biochemical classification of plant-derived food allergens. J Allergy Clin Immunol 2000;106:27-36.
6. Breiteneder H, Pettenburger K, Bito A, Valenta R, Kraft D, Rumpold H, et al. The gene coding for the major birch pollen allergen Betv1, is highly homologous to a pea disease resistance response gene. EMBO J 1989;8:1935-8.
7. Ebner C, Hirschwehr R, Bauer L, Breiteneder H, Valenta R, Ebner H, et al. Identification of allergens in fruits and vegetables: IgE cross-reactivities with the important birch pollen allergens Bet v 1 and Bet v 2 (birch profilin). J Allergy Clin Immunol 1995;95:962-9.
8. Asarnoj A, Moverare R, Ostblom E, Poorafshar M, Lilja G, Hedlin G, et al. IgE to peanut allergen components: relation to peanut symptoms and pollen sensitization in 8-year-olds. Allergy 2010;65:1189-95.
9. Masthoff LJ, Mattsson L, Zuidmeer-Jongejan L, Lidholm J, Andersson K, Akkerdaas JH, et al. Sensitization to Cor a 9 and Cor a 14 is highly specific for a hazelnut allergy with objective symptoms in Dutch children and adults. J Allergy Clin Immunol 2013;132:393-9.
10. Hatzler L, Panetta V, Lau S, Wagner P, Bergmann RL, Illi S, et al. Molecular spreading and predictive value of preclinical IgE response to *Phleum pratense* in children with hay fever. J Allergy Clin Immunol 2012;130:894-901.e5.
11. Stringari G, Tripodi S, Caffarelli C, Dondi A, Asero R, Di Rienzo Businco A, et al. The effect of component-resolved diagnosis on specific immunotherapy prescription in children with hay fever. J Allergy Clin Immunol 2014;134:75-81.
12. Canonica GW, Ansotegui IJ, Pawankar R, Schmid-Grendelmeier P, van Hage M, Baena-Cagnani CE, et al. A WAO-ARIA-GA(2)LEN consensus document on molecular-based allergy diagnostics. World Allergy Org J 2013;6:17.
13. Panzner P, Vachova M, Vitovcova P, Brodska P, Vlas T. A comprehensive analysis of middle-European molecular sensitization profiles to pollen allergens. Int Arch Allergy Immunol 2014;164:74-82.
14. Vieira T, Cunha L, Neves E, Falcao H. Diagnostic usefulness of component-resolved diagnosis by skin prick tests and specific IgE to single allergen components in children with allergy to fruits and vegetables. Allergol Immunopathol (Madr) 2014;42:127-35.
15. Bousquet J, Anto J, Auffray C, Akdis M, Cambon-Thomsen A, Keil T, et al. MeDALL (Mechanisms of the Development of ALLergy): an integrated approach from phenotypes to systems medicine. Allergy 2011;66:596-604.
16. Wickman M, Kull I, Pershagen G, Nordvall SL. The BAMSE project: presentation of a prospective longitudinal birth cohort study. Pediatr Allergy Immunol 2002;13(suppl 15):11-3.
17. Wickman M, Asarnoj A, Tillander H, Andersson N, Bergstrom A, Kull I, et al. Childhood-to-adolescence evolution of IgE antibodies to pollens and plant foods in the BAMSE cohort. J Allergy Clin Immunol 2014;133:580-2.
18. Kilpelainen M, Terho EO, Helenius H, Koskenvuo M. Validation of a new questionnaire on asthma, allergic rhinitis, and conjunctivitis in young adults. Allergy 2001;56:377-84.
19. Westman M, Stjarne P, Asarnoj A, Kull I, van Hage M, Wickman M, et al. Natural course and comorbidities of allergic and nonallergic rhinitis in children. J Allergy Clin Immunol 2012;192:403-8.
20. Bousquet J, Van Cauwenberge P, Khaltaev N. Allergic rhinitis and its impact on asthma. J Allergy Clin Immunol 2001;108(suppl):S147-334.
21. Ortolani C, Ispano M, Pastorello E, Bigi A, Ansaloni R. The oral allergy syndrome. Ann Allergy 1988;61:47-52.
22. Lupinek C, Wollmann E, Baar A, Banerjee S, Breiteneder H, Broecker BM, et al. Advances in allergen-microarray technology for diagnosis and monitoring of allergy: the MeDALL allergen-chip. Methods 2014;66:106-19.
23. Kazemi-Shirazi L, Pauli G, Purohit A, Spitzauer S, Froschl R, Hoffmann-Sommergruber K, et al. Quantitative IgE inhibition experiments with purified recombinant allergens indicate pollen-derived allergens as the sensitizing agents responsible for many forms of plant food allergy. J Allergy Clin Immunol 2000;105:116-25.
24. Valenta R, Kraft D. Type 1 allergic reactions to plant-derived food: a consequence of primary sensitization to pollen allergens. J Allergy Clin Immunol 1996;97:893-5.
25. Valenta R, Steinberger P, Duchene M, Kraft D. Immunological and structural similarities among allergens: prerequisite for a specific and component-based therapy of allergy. Immunol Cell Biol 1996;74:187-94.
26. Scala E, Alessandri C, Bernardi ML, Ferrara R, Palazzo P, Pomponi D, et al. Cross-sectional survey on immunoglobulin E reactivity in 23,077 subjects using an

allergenic molecule-based microarray detection system. Clin Exp Allergy 2010;40:911-21.
27. Bousquet J, Anto JM, Bachert C, Bousquet PJ, Colombo P, Crameri R, et al. Factors responsible for differences between asymptomatic subjects and patients presenting an IgE sensitization to allergens. A GA2LEN project. Allergy 2006;61:671-80.
28. Bousquet J, Fokkens W, Burney P, Durham SR, Bachert C, Akdis CA, et al. Important research questions in allergy and related diseases: nonallergic rhinitis: a GA2LEN paper. Allergy 2008;63:842-53.
29. Rondon C, Canto G, Blanca M. Local allergic rhinitis: a new entity, characterization and further studies. Curr Opin Allergy Clin Immunol 2010;10:1-7.
30. Kellberger J, Dressel H, Vogelberg C, Leupold W, Windstetter D, Weinmayr G, et al. Prediction of the incidence and persistence of allergic rhinitis in adolescence: a prospective cohort study. J Allergy Clin Immunol 2012;129:397-402, e1-3.
31. Jacobsen L, Niggemann B, Dreborg S, Ferdousi HA, Halken S, Host A, et al. Specific immunotherapy has long-term preventive effect of seasonal and perennial asthma: 10-year follow-up on the PAT study. Allergy 2007;62:943-8.
32. Maloney JM, Rudengren M, Ahlstedt S, Bock SA, Sampson HA. The use of serum-specific IgE measurements for the diagnosis of peanut, tree nut, and seed allergy. J Allergy Clin Immunol 2008;122:145-51.
33. Yunginger JW, Ahlstedt S, Eggleston PA, Homburger HA, Nelson HS, Ownby DR, et al. Quantitative IgE antibody assays in allergic diseases. J Allergy Clin Immunol 2000;105:1077-84.
34. Geroldinger-Simic M, Zelniker T, Aberer W, Ebner C, Egger C, Greiderer A, et al. Birch pollen-related food allergy: clinical aspects and the role of allergen-specific IgE and IgG4 antibodies. J Allergy Clin Immunol 2011;127:616-22.e1.
35. Rashid RS, Smith KA, Nambiar KZ, Frew AJ, Tarzi MD. Pollen-food syndrome is related to Bet v 1/PR-10 protein sensitisation, but not all patients have spring rhinitis. Allergy 2011;66:1391-2.

METHODS

Statistical analyses

To analyze whether there were any differences in important background characteristics between the study population (the 764 children included in this study) and the study base (the original BAMSE cohort), we used the *t* test with finite population correction. When using finite population correction, one is able to account for the fact that the sample (n = 764) is not picked from an infinite population (n = 4089), which means that the variance gets smaller, and consequently, the CIs get smaller.

In Fig 1 the prevalence of IgE reactivity to PR-10 proteins at 4, 8, and 16 years of age is expressed as a percentage of the total number of available observations. Levels of the different PR-10 proteins are presented as box plots with medians and 25th and 75th percentiles. Quantile regression was used to compare the median IgE levels of the different PR-10 proteins. This method was also used to compare the median IgE levels of Bet v 1 between time points. Because the prevalence and IgE levels of the PR-10 proteins seemed to follow a certain order, we wanted to further analyze the hierarchy within the PR-10 protein group, which is presented in Fig 2. Among children with IgE reactivity to a certain PR-10 protein, we calculated the proportion of children with IgE reactivity to each of the other PR-10 proteins. Because the highest prevalence and IgE level was seen for Bet v 1 and there seemed to be a certain hierarchy within the PR-10 protein group, we wanted to assess whether IgE levels of Bet v 1 were correlated with the number of IgE reactivities to other PR-10 proteins. For the analysis of correlation, we used Spearman rho.

The next step was to analyze IgE reactivity in relation to symptoms of AR_{bp}. A Venn diagram was performed to see the proportions of symptoms to birch pollen from the upper and lower airways, respectively, and IgE reactivity to Bet v 1 in relation to each other. Levels of specific IgE to Bet v 1 among asymptomatic children compared with those in children with symptoms were again analyzed with quantile regression. The cross-sectional probability to report symptoms of AR_{bp} in relation to Bet v 1–specific IgE levels was assessed with fitted predicted probability curves, which are based on a logistic regression model. The same method was used to analyze the cross-sectional probability to report OAS from apple, hazelnut, peanut, kiwi, and soy in relation to specific IgE levels of the corresponding PR-10 protein. Children with IgE reactivity to other than PR-10 allergen components known to produce severe reactions were excluded from these analyses. For Mal d 1– and Pru p 1–specific IgE, children with concomitant IgE to Pru p 3 were excluded. For Act d 8–specific IgE, children with IgE to Act d 1/Act d 5 were excluded. For Ara h 8–specific IgE, children with IgE to Ara h 2/Ara h 6 were excluded. For Gly m 4–specific IgE, children with IgE to Gly m 5/Gly m 6 were excluded. For Cor a 1.04–specific IgE, children with IgE to Cor a 9 were excluded. To test whether we had to perform a logarithmic transformation before analysis of specific IgE levels, we tested the log-linear relationship. The different IgE levels showed a log-linear relationship to the respective outcome and were thus not logarithmically transformed.

Finally, we wanted to analyze whether the onset, persistence, or severity of AR_{bp} could be predicted by either levels of specific IgE to Bet v 1 or numbers of IgE reactivites to other PR-10 proteins. For analysis of probability of onset or persistence of symptoms in relation to levels of specific IgE to Bet v 1, the same method of fitted predicted probability curves as used for the cross-sectional analysis was performed. For probability of onset of AR_{bp}, the analysis was performed among children without symptoms of AR_{bp} at baseline, and for persistence of symptoms, the analysis was performed among children with AR_{bp} at baseline.

For assessing the possible association between onset or persistence of AR_{bp} in relation to the number of recognized PR-10 proteins, we divided the number of recognized PR-10 proteins into 3 categories; Bet v 1 only, Bet v 1 and up to the median number of other recognized PR-10 proteins, and Bet v 1 and a number of other recognized PR-10 proteins above the median. The association between these categories of numbers of recognized PR-10 proteins at baseline and onset of AR_{bp} at ages 8 and 16 years was calculated with generalized estimating equations. As a complement, absolute risks of AR_{bp} at 8 or 16 years of age were calculated. The absolute risk was calculated as the number of children with the exposure (specific IgE ≥0.3 ISU-E) and the outcome (AR_{bp}) at 8 or 16 years of age, respectively, as divided by the total number of children with the exposure at baseline. Ninety-five percent CIs were calculated with the binomial test of statistical significance. *P* values of less than .05 were considered statistically significant.

The severity of AR_{bp} at 16 years of age in relation to Bet v 1–specific IgE levels at age 4 years are presented as box plots with median levels and 25th and 75th percentiles. Specific IgE levels at the 50th percentile (median), as well as the 25th and 75th percentiles, were compared between patients with mild and those with moderate/severe AR_{bp} by using quantile regression.

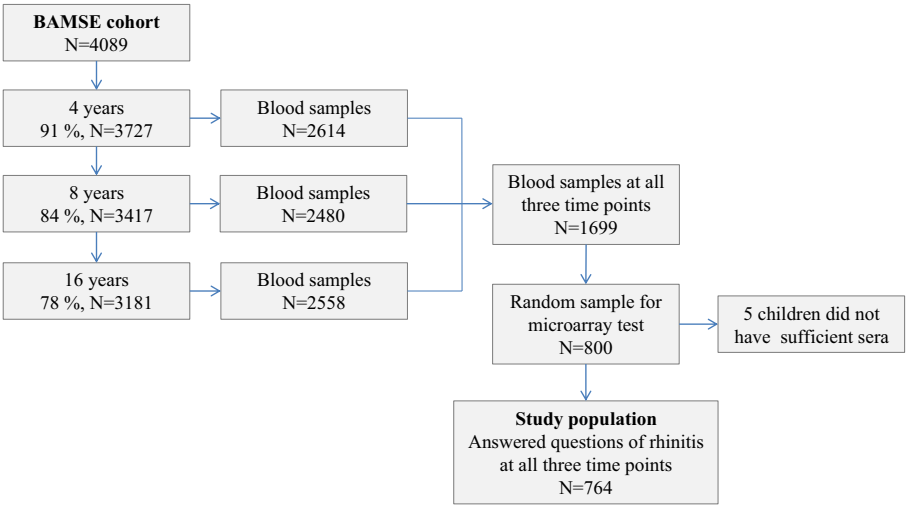


FIG E1. Flow chart of the study.

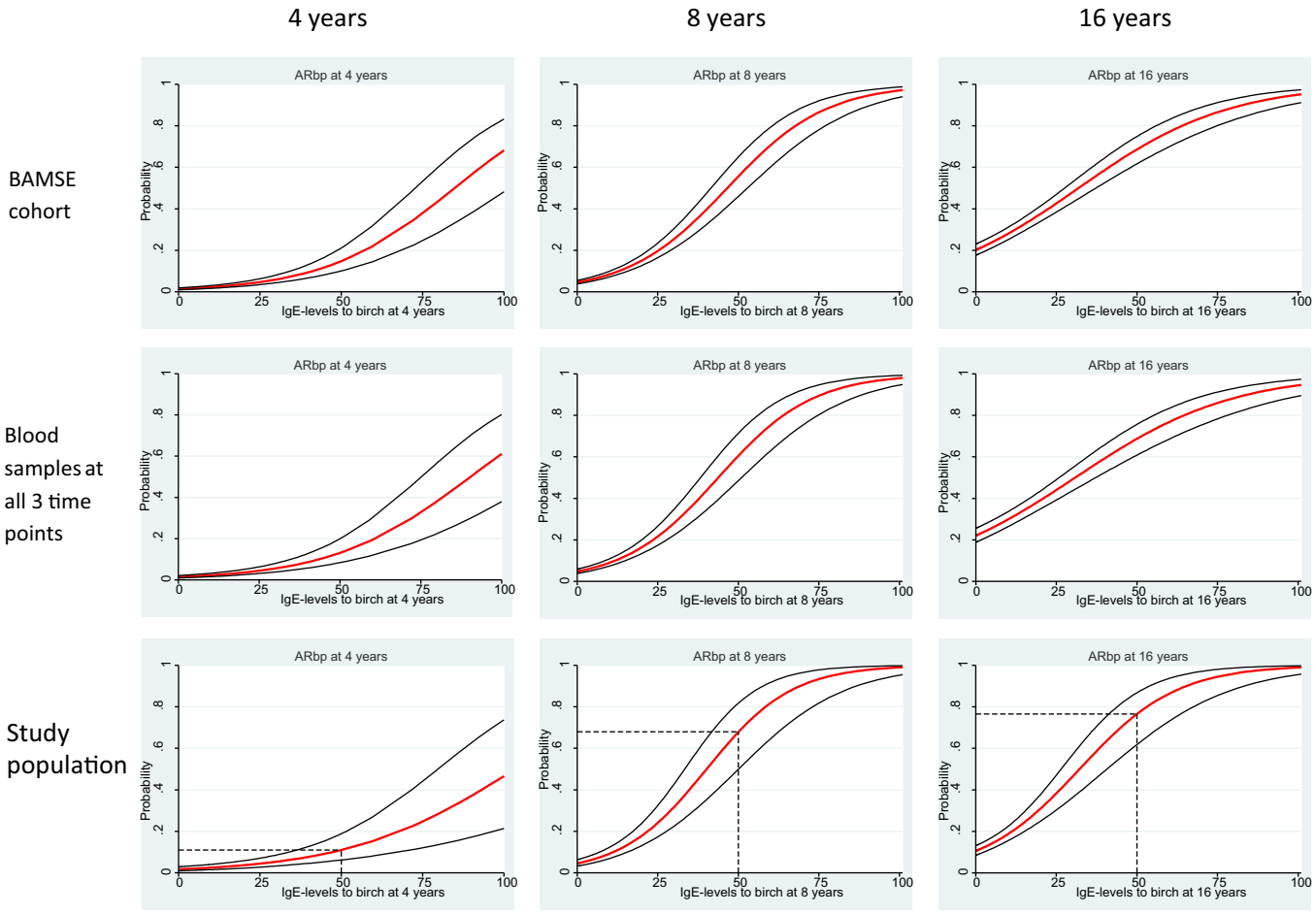


FIG E2. Cross-sectional probabilities to report AR_{bp} at 4, 8, and 16 years of age in relation to levels of specific IgE to birch (measured with ImmunoCAP) among all children in the BAMSE cohort, the 1699 children with blood samples from all 3 time points, and the study population.

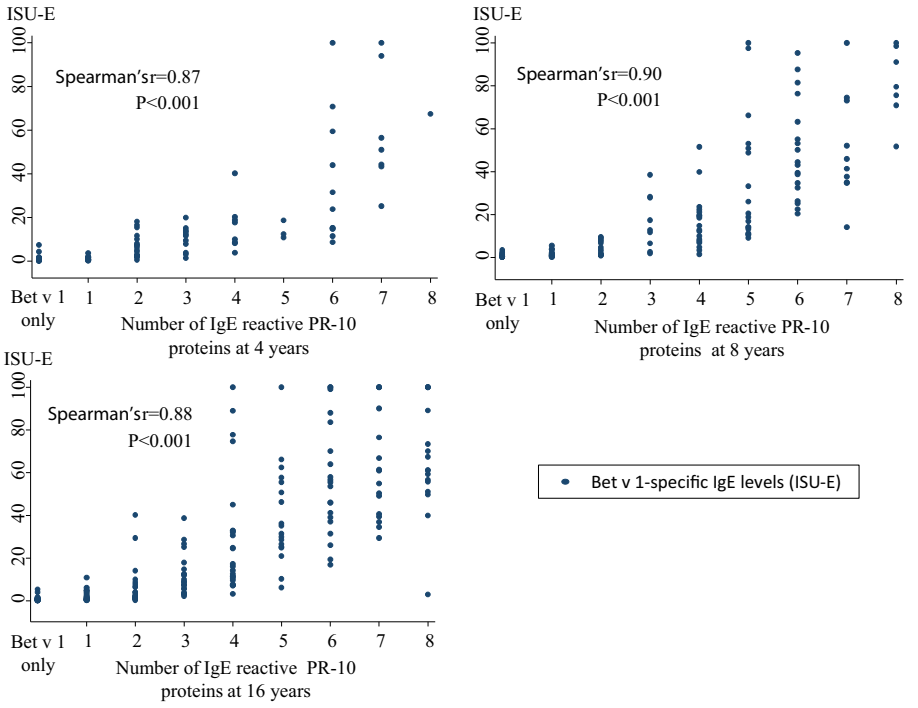


FIG E3. Correlation between Bet v 1–specific IgE levels (ISU-E) and numbers of other IgE-reactive PR-10 proteins.

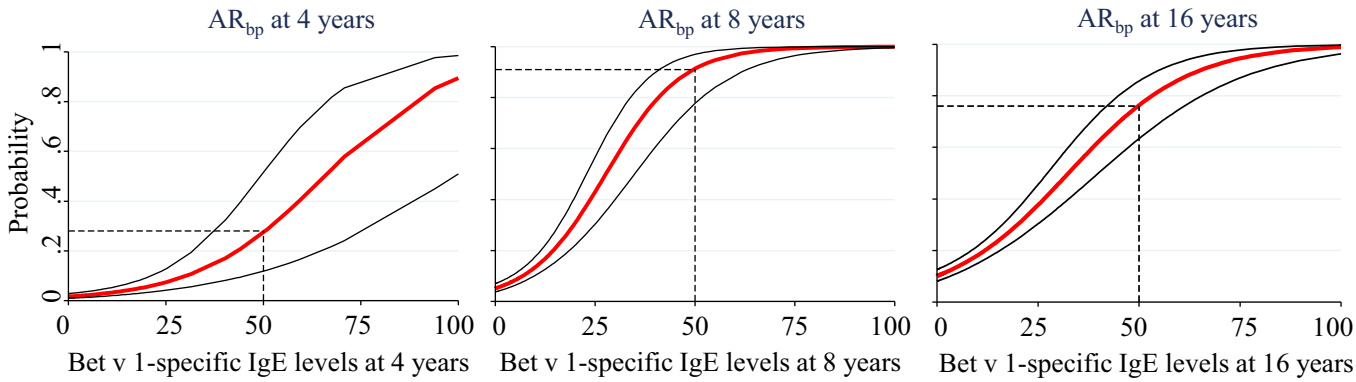
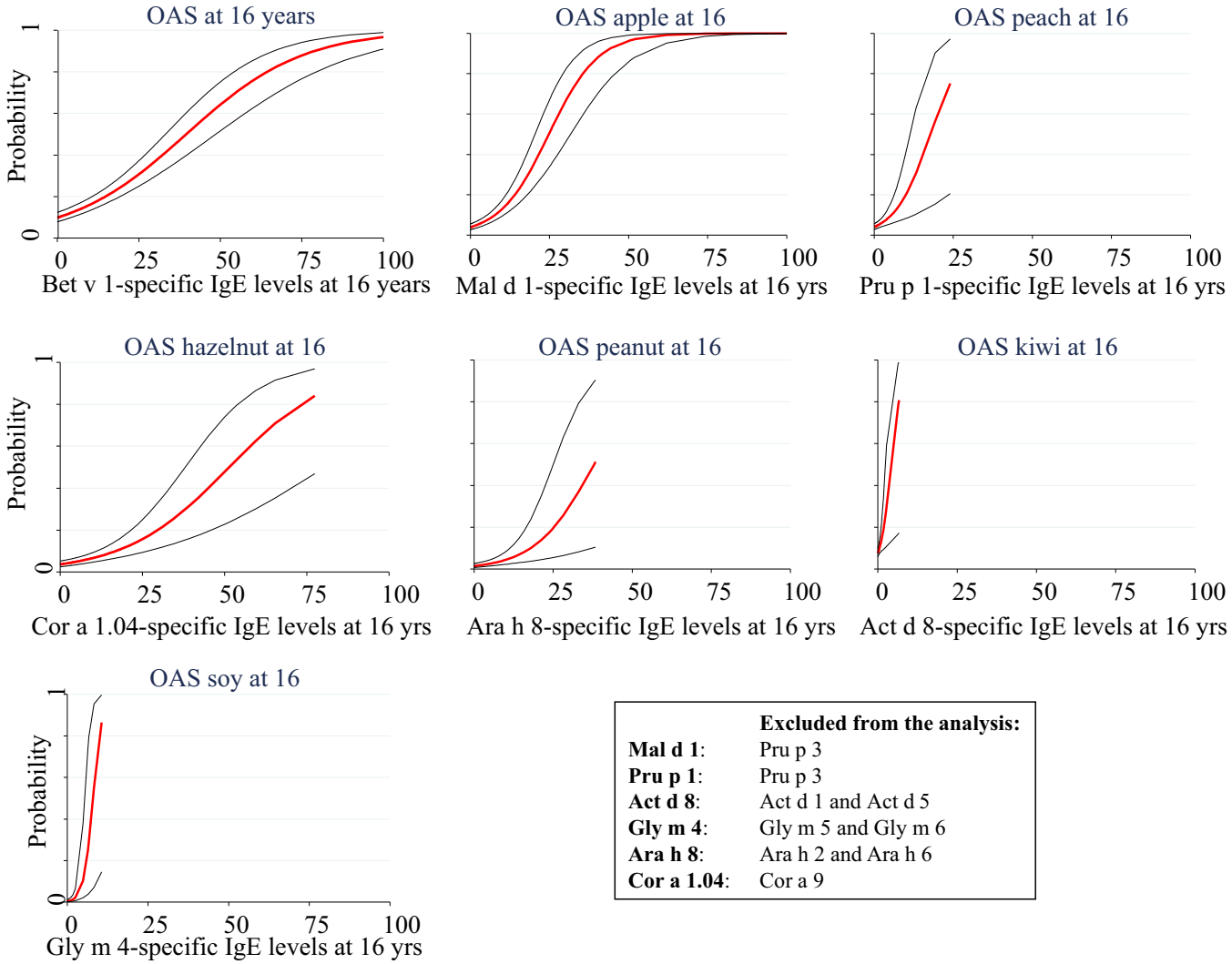


FIG E4. Cross-sectional probabilities to report AR$_{bp}$ in relation to Bet v 1–specific IgE levels (ISU-E) at 4, 8, and 16 years of age.



	Excluded from the analysis:
Mal d 1:	Pru p 3
Pru p 1:	Pru p 3
Act d 8:	Act d 1 and Act d 5
Gly m 4:	Gly m 5 and Gly m 6
Ara h 8:	Ara h 2 and Ara h 6
Cor a 1.04:	Cor a 9

FIG E5. Cross-sectional probabilities to report symptoms of OAS to any food item in relation to Bet v 1–specific IgE levels or specific food items in relation to levels of IgE specific for the corresponding PR-10 protein at 16 years of age.

TABLE E1. Background characteristics of participants in the BAMSE birth cohort, Stockholm, Sweden

	Study population (n = 764)			BAMSE cohort (n = 4089)			
	No.	Percent	95% CI	No.	Percent	95% CI	*P* value
Sex							
Male	383	50.1	46.6-52.8	2065	50.5	49.0-52.0	.821
Heredity for AR							
Yes	286	37.6	34.1-41.0	1397	34.5	33.1-36.0	.055
Low socioeconomic status							
Yes	109	14.3	12.0-17.0	695	17.1	15.9-18.2	**.016**
Birth month							
December-February	139	18.2	15.6-21.1	722	17.7	16.5-18.8	.672
March-May	240	31.4	28.2-34.8	1201	29.4	28.0-30.8	.177
June-August	223	29.2	26.1-32.5	1190	29.1	27.7-30.5	.952
September-November	162	21.2	18.4-24.3	976	23.9	22.6-25.2	**.046**
Mother's age							
<26 y	57	7.5	5.6-9.3	319	7.8	7.0-8.6	.692
Parent born outside Scandinavia							
Yes	142	18.6	15.8-21.4	707	20.8	19.4-22.2	.076
Older siblings							
Yes	382	50.0	45.4-53.6	1980	48.4	46.9-50.0	.336
Breast-feeding exclusively ≥4 mo							
Yes	595	78.9	76.0-81.8	3116	79.5	78.2-80.8	.655
Furred animals at home							
Yes	120	15.7	13.1-18.3	629	15.4	14.3-16.5	.783
Mother smoking							
Yes	95	12.4	10.1-14.8	563	13.8	12.7-14.8	.212
Smell of mildew in home							
Yes	55	7.2	5.4-8.8	324	7.9	7.1-8.8	.393
Moisture damage in home							
Yes	150	19.6	16.8-22.5	812	19.9	18.6-21.1	.861

Boldface indicates statistical significance.

TABLE E2. Cross-sectional proportions of AR_{bp} among children with IgE reactivity to Bet v 1 only or in combination with the other PR-10 proteins, as well as for certain numbers of other PR-10 proteins, at 4, 8, and 16 years of age (n = 764)

	AR at age 4 y		AR at age 8 y		AR at age 16 y	
	No./†	Percent	No./†	Percent	No./†	Percent
Bet v 1 only	0/23	0	3/24	12.5	5/33	15.2
Bet v 1 + any PR-10	14/72	19.4	63/109	57.8	99/161	61.5
Bet v 1 + Mal d 1	14/62	22.6	61/103	59.2	95/153	62.1
Bet v 1 + Cor a 1.04	13/67	19.4	62/100	62.0	94/152	61.8
Bet v 1 + Ara h 8	6/28	21.4	55/76	72.4	76/106	71.7
Bet v 1 + Pru p 1	11/30	36.7	50/68	73.5	72/90	80.0
Bet v 1 + Aln g 1	9/35	25.7	32/44	72.7	50/66	75.8
Bet v 1 + Api g 1	2/8	25.0	25/30	83.3	40/52	76.9
Bet v 1 + Act d 8	4/6	66.7	21/23	91.3	29/37	78.4
Bet v 1 + Gly m 4	3/17	17.7	19/24	79.2	29/36	80.6
No. of PR-10 proteins						
0	5/664	0.8	15/608	2.5	27/546	5.0
Bet v 1 only	0/23	0	3/24	12.5	5/33	15.2
Bet v 1 and median*	6/42	14.3	22/56	39.3	42/90	46.7
Bet v 1 and above median	8/30	26.7	41/53	77.4	57/71	80.3

*Median number of components: age 4 years, ≤3; 8 and 16 years of age, ≤4.
†Number of children with IgE reactivity to the particular PR-10 protein analyzed.

TABLE E3. Absolute risks for onset of AR_{bp} at 8 or 16 years of age in relation to IgE reactivity to PR-10 proteins among asymptomatic children at 4 and 8 years of age, respectively

		Incident AR_{bp} at age 8 or 16 y			No onset of symptoms at age 8 or 16 y	
		No.	**Percent**	**95% CI**	**No.**	**Percent**
4 y (n = 740)*	No.	n = 86			n = 608	
No IgE reactivity	659	74	11.2		585	88.8
Bet v 1 only	23	12	52.2	30.6-73.2	11	47.8
Bet v 1 + any PR-10 protein	58	46	79.3	66.6-88.8	12	20.7
Bet v 1 + Mal d 1	48	40	83.3	69.8-92.5	8	16.7
Bet v 1 + Cor a 1.04	54	42	77.8	64.4-88.0	12	22.2
Bet v 1 + Ara h 8	22	19	86.4	65.1-97.1	3	13.6
Bet v 1 + Pru p 1	19	16	84.2	60.4-96.6	3	15.8
Bet v 1 + Aln g 1	26	22	84.6	65.1-95.6	4	15.4
Bet v 1 + Api g 1	6	6	100	54.1-100	0	0
Bet v 1 + Act d 8	2	2	100	15.8-100	0	0
Bet v 1 + Gly m 4	14	13	92.9	66.1-99.8	1	7.1
		Incident AR_{bp} at age 16 y				
8 y (n = 656)†	No.	n = 65			n = 591	
No IgE reactivity	590	36	6.1	4.3-8.3	554	93.9
Bet v 1 only	21	9	42.9	21.8-66.0	12	57.1
Bet v 1 + any PR-10 protein	45	20	44.4	29.6-60.0	25	55.6
Bet v 1 + Mal d 1	41	18	43.9	28.4-60.3	23	56.1
Bet v 1 + Cor a 1.04	37	18	48.6	31.9-65.6	19	51.4
Bet v 1 + Ara h 8	20	10	50.0	27.2-72.8	10	50.0
Bet v 1 + Pru p 1	17	9	52.9	27.8-77.0	8	47.1
Bet v 1 + Aln g 1	12	7	58.3	27.7-84.8	5	41.7
Bet v 1 + Api g 1	5	3	60.0	14.7-94.7	2	40.0
Bet v 1 + Act d 8	2	2	100	15.8-100	0	0
Bet v 1 + Gly m 4	5	2	40.0	5.3-85.3	3	60.0

*No symptoms of AR_{bp} reported at age 4 years.
†No symptoms of AR_{bp} reported at age 4 or 8 years.

TABLE E4. Absolute risks for persistence of AR_{bp} up to age 16 years in relation to the number of IgE-reactive PR-10 proteins at 4 and 8 years of age, respectively

		Transient		Persistent		
	No.	No.	Percent	No.	Percent	95% CI
No. of PR-10 proteins at age 4 y (n = 19)†						
0	5	5	100	0	0	0-52.2
Bet v 1 only	0	—	—	—	—	
Bet v 1 and 1-3*	6	2	33.3	4	66.7	22.3-95.7
Bet v 1 and ≥4	8	0	0	8	100	63.1-100
No. of PR-10 proteins at age 8 y (n = 66)‡						
0	13	7	53.9	6	46.2	19.2-74.9
Bet v 1 only	3	0	0	3	100	29.2-100
Bet v 1 and 1-4*	17	4	23.5	13	76.5	50.1-93.2
Bet v 1 and ≥5	33	3	9.1	30	90.9	75.7-98.1

*Median number of IgE-reactive proteins or less.
†AR_{bp} at age 4 years.
‡AR_{bp} at age 8 years but not age 4 years.

TABLE E5. Absolute risks for persistence of AR_{bp} up to age 16 years in relation to IgE reactivity to PR-10 proteins at 4 and 8 years of age, respectively

		Transient (n = 7 [36.8%])		Persistent* (n = 12 [63.2%])	
4 y (n = 19)†	**No.**	**No.**	**Percent**	**No.**	**Percent**
No IgE reactivity	5	5	100	0	0
Bet v 1 only	0	—	—	—	—
Bet v 1 + any PR-10 protein	14	2	14.3	12	85.7
Bet v 1 + Mal d 1	14	2	14.3	12	85.7
Bet v 1 + Cor a 1.04	13	2	15.4	11	84.6
Bet v 1 + Ara h 8	6	0	0	6	100
Bet v 1 + Pru p 1	11	1	9.1	10	90.9
Bet v 1 + Aln g 1	9	0	0	9	100
Bet v 1 + Api g 1	2	0	0	2	100
Bet v 1 + Act d 8	4	0	0	4	100
Bet v 1 + Gly m 4	3	0	0	3	100

		Transient (n = 14 [21.2%])		Persistent (n = 52 [78.8%])	
8 y (n = 66)‡	**No.**	**No.**	**Percent**	**No.**	**Percent**
No IgE reactivity	13	7	53.9	6	46.2
Bet v 1 only	3	0	0	3	100
Bet v 1 + any PR-10 protein	50	7	14.0	43	86.0
Bet v 1 + Mal d 1	48	5	10.4	43	89.6
Bet v 1 + Cor a 1.04	49	6	12.2	43	87.8
Bet v 1 + Ara h 8	44	5	11.4	39	88.6
Bet v 1 + Pru p 1	39	4	10.3	35	89.7
Bet v 1 + Aln g 1	28	3	10.7	25	89.3
Bet v 1 + Api g 1	18	1	5.6	17	94.4
Bet v 1 + Act d 8	14	1	7.1	13	92.9
Bet v 1 + Gly m 4	15	1	6.7	14	93.3

*Reported symptoms to birch pollen at both 8 and 16 years of age. Only 1 child reported symptoms at age 8 years but not at age 16 years and 1 child at age 16 years but not at age 8 years.

†AR_{bp} at age 4 years.

‡AR_{bp} at age 8 years but not at age 4 years.