

Anti-rPru p 3 IgE Levels Are Inversely Related to the Age at Onset of Peach-Induced Severe Symptoms Reported by Peach-Allergic Adults

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Key Words

Pru p 3 · Pru p 1 · Pru p 4 · Peach · Lipid transfer protein · Bet v 1 · Food allergy · Recombinant allergen · Oral allergy syndrome · Age

Abstract

Sensitisation to peach lipid transfer protein (LTP; Pru p 3) is significantly associated with severe allergic symptoms in adults, but little is known about the age at onset of peach allergy. We investigated a possible correlation between specific IgE levels to Pru p 3 and the age at onset of peach allergy. One hundred and forty-eight patients allergic to peach were divided into 6 classes according to the age at onset. Sera were analyzed for IgE antibodies to peach, rPru p 3, rPru p 1, rPru p 4, rBet v 1, rBet v 2, total IgE titre, and tryptase; all collected data were statistically analysed. A significant inverse correlation was found between the age at onset of peach allergy and anti-rPru p 3 IgE levels at diagnosis ($p < 0.0005$; Spearman's $\rho = -0.3833$). In contrast, the age at onset was directly correlated with both anti-rPru p 1 IgE levels ($p = 0.0001$; Spearman's $\rho = 0.3197$) and anti-rBet v 1 IgE levels ($p = 0.0006$; Spearman's $\rho = 0.2914$) at diagnosis. No correla-

tions were detected between the reported age at onset and anti-peach, anti-rPru p 4, anti-rBet v 2 IgE and total IgE values and serum tryptase levels. At diagnosis, when peach allergy starts at a younger age, it is likely associated with Pru p 3 sensitisation, and the younger the onset, the higher the IgE titres. When peach allergy starts at an older age, it is more likely the result of cross-reactivity to Bet v1.

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Introduction

Peach is the most frequent cause of food allergy in adults living in the Mediterranean area [1] where it also represents the main cause of food-induced anaphylaxis [2]; however, its relevance in children is ill defined. In peach-allergic adults, severe symptoms are significantly associated with sensitisation to peach lipid transfer protein (LTP; Pru p 3), while sensitisation to both the peach Bet v 1 homologue (Pru p 1) and peach profilin (Pru p 4) is mostly associated with mild local symptoms (OAS, oral allergy syndrome) [3]. This probably depends on the physical and chemical characteristics of the allergens: Pru

p 3 is strongly heat and pepsin resistant, while Pru p 1 and Pru p 4 are easily destroyed by heat and gastrointestinal digestion. However, in a recent study, we found a significant correlation between symptom severity and anti-rPru p 3 IgE levels only in Pru p 3 positive/Pru p 1 and Pru p 4 negative patients [4], suggesting that the mutual influences within a complex immunological network, together with the biochemical properties of allergens, influence the grade of symptom severity to plant foods in multi-sensitised patients. The role of Pru p 3 IgE in children has been scarcely investigated, although recently, Novembre et al. [5] found a 50% prevalence of systemic reactions to peach without any association between symptom severity and anti-rPru p 3 sensitisation. In order to investigate the prognostic value of anti-rPru p 3 IgE levels in the first years of life, we looked for a possible correlation between the age at onset of peach allergy and the levels of IgE specific to different peach allergens at diagnosis in 148 well-characterized peach-allergic patients [4].

Methods

Patients

The study was carried out on 148 subjects (41 males, 107 females; median age 37 years, range 13–62) with peach allergy of different degrees of severity confirming the diagnosis by open challenge performed distant from the birch pollen season. The Ethics Committee approved the study (ClinicalTrials.gov, protocol ID NCT00715156). Seventy-six patients presented only local symptoms (OAS) and 72 had systemic symptoms as well. The age at onset of peach allergy was thoroughly investigated in all patients, checking as best as possible all available recordings and interviewing both patients and their relatives about the first allergic reaction [4]. All patients showed positive skin prick tests and specific anti-peach IgE. In order to minimize potential errors in the age at onset of peach-induced symptoms, ages at onset were divided into 6 classes, (0–9, 10–19, 20–29, 30–39, 40–49, and 50+).

In vitro Tests

The different sensitisation profiles to the peach allergens Pru p 1, Pru p 3 and Pru p 4 and to the birch allergens Bet v 1 and Bet v 2 were detected by ImmunoCAP system 1000 (Phadia, now Thermo Fisher Scientific, Uppsala, Sweden); IgE levels were expressed as kUA/l and considered positive when exceeding 0.10 kUA/l. Tryptase was measured by an immunofluorimetric assay (ImmunoCAP 100; Thermo Fisher Scientific). Values <5 µg/l were considered normal.

Statistical Analysis

All collected data were validated and then submitted to descriptive statistical analyses. Categorical/nominal variables were evaluated by frequency tables, while the shape of distribution of the continuous variable was checked by both visual inspection of the graph and by the Shapiro-Wilk test. Pearson's χ^2 test or Fisher's

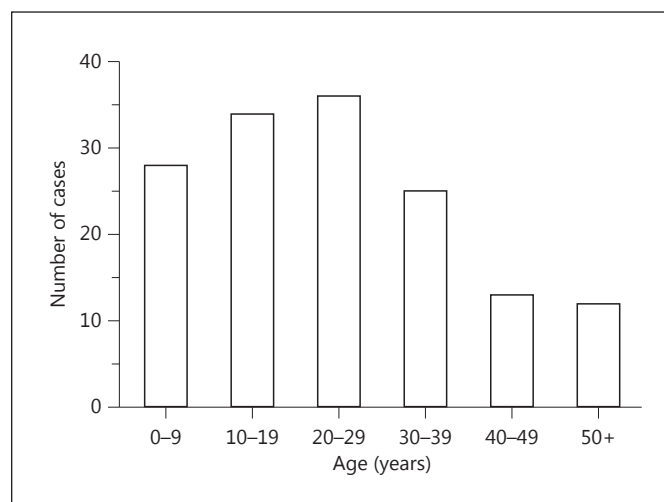


Fig. 1. Reported age at onset of peach allergy in the study population subdivided in age classes.

exact test (if needed) was used for the comparison of categorical variables, whereas comparisons of continuous variables were carried out by Student's *t* test or the Mann-Whitney *U* test. Continuous correlations were analysed with Pearson's *r* or Spearman's ρ , depending on distributions.

The Kruskal-Wallis test, followed by the test for pair-wise comparison according to Conover [6], was used to check the between-group differences of continuous variables among more than two groups.

Significance was assumed for $p < 0.05$, adjusting by Bonferroni for possible multiple comparisons. Statistical analysis was done using Stata/SE 12.1 (The Stata Corp., College Station, Tex., USA).

Results

Patients

The final dataset included 141 patients for whom the age at onset of peach allergy was reliably ascertained. The average reported age at onset of peach allergy was 22 ± 13 years. Almost 50% of patients started having peach allergy between 10 and 29 years of age (fig. 1). A total of 48 patients (34%; 9 males, 39 females) started having peach allergy at 2–15 years of age: 42 of them (87.5%) showed specific IgE to rPru p 3, 26/42 (62%) to rPru p 3 only, 13/42 (31%) to both rPru p 3 and rPru p 1, and 3 (7%) to both rPru p 3 and rPru p 4. Four out of 6 rPru p 3 negative peach-allergic patients were positive only to rPru p 1, and 2 patients to both rPru p 1 and rPru p 4.

Analysing the 6 age classes, a significant inverse correlation was found between the reported age at onset of

Table 1. Median values of anti-rPru p 3, anti-rPru p 1 and anti-rBet v 1 IgE levels and average age in the 6 classes of reported age at onset of the peach allergy symptoms

Age class	Average age, years	Anti-rPru p 3 IgE levels kUA/l	Anti-rPru p 1 IgE levels kUA/l	Anti-rBet v 1 IgE levels kUA/l
1 (0–9 years)	5.2	4.79	<0.10	<0.10
2 (10–19 years)	14.5	3.13	<0.10	<0.10
3 (20–29 years)	22.9	2.07	<0.10	<0.10
4 (30–39 years)	32.2	<0.10	4.74	14.9
5 (40–49 years)	43.0	0.12	11.3	19.8
6 (50+ years)	52.6	0.05	6.53	16.8
Total		1.99	<0.10	0.20

peach allergy and the anti-rPru p 3 IgE levels at diagnosis ($p < 0.0005$; Spearman's $\rho = -0.3833$), i.e. the lower the age at onset, the higher the anti-rPru p 3 IgE level at diagnosis. In contrast, the age at onset of peach allergy was directly correlated with both anti-rPru p 1 IgE levels ($p = 0.0001$; Spearman's $\rho = 0.3197$) and anti-rBet v 1 IgE levels ($p = 0.0006$; Spearman's $\rho = 0.2914$) at diagnosis. No correlations were detected between the reported age at onset and anti-peach ($p = 0.1800$), anti-rPru p 4 ($p = 0.9358$) and anti-rBet v 2 ($p = 0.9537$) IgE values. Further, the age at onset did not correlate with total IgE levels ($p = 0.4873$), the serum tryptase levels ($p = 0.2075$) at diagnosis and the number of other plant foods causing allergic symptoms ($p = 0.0939$).

The median anti-rPru p 3, anti-rPru p 1 and anti-rBet v 1 IgE levels at diagnosis and the average ages of patients in the 6 age-at-onset classes are shown in table 1. In the first 3 classes, anti-rPru p 3 IgE levels were significantly higher than in patients who developed peach allergy at older ages, thus confirming the inverse relationship between age at onset and anti-rPru p 3 IgE levels at diagnosis. In contrast, the median anti-rPru p 1 and anti-rBet v 1 IgE levels at diagnosis were higher among patients of the last 3 classes of age at onset, suggesting that anti-rPru p 1 and anti-rBet v 1 IgE levels show a tendency to increase with age at onset of peach allergy (fig. 2). The Kruskal-Wallis test confirmed an overall difference between classes of age for anti-rPru p 3 ($p = 0.0037$; fig. 2a), anti-rPru p 1 ($p = 0.0445$; fig. 2b) and anti-rBet v 1 ($p = 0.0428$; fig. 2c) IgE levels.

Pair-wise comparisons, adjusted by Bonferroni, showed that anti-rPru p 3 IgE levels in patients of the first class of age were significantly higher than in the last 3

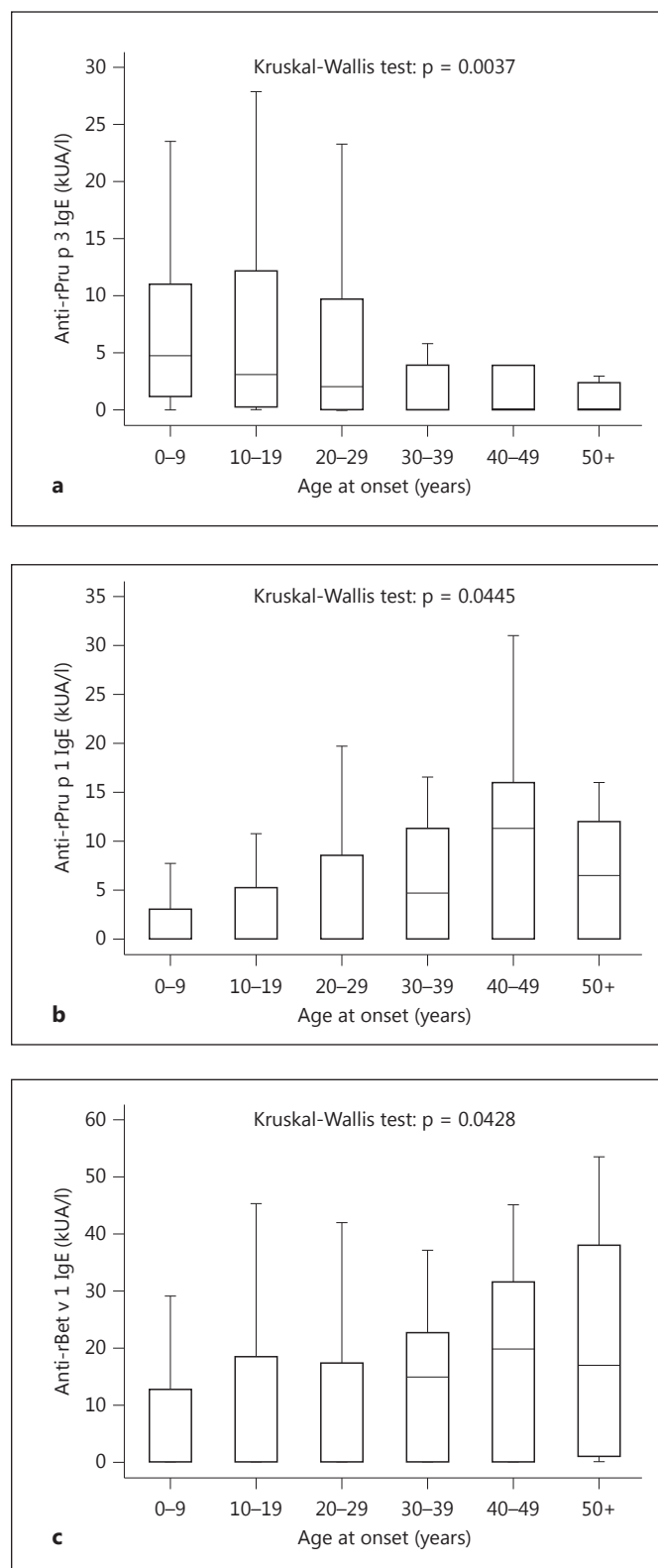


Fig. 2. Boxplot graphs reporting anti-rPru p 3 (a), anti-rPru p 1 (b) and anti-rBet v 1 (c) IgE levels at diagnosis for the 6 classes of reported age at onset of the peach allergy symptoms.

classes; in the second class, levels were significantly higher than in classes 4 and 6. Finally, in class 3, levels were significantly higher than in class 4 (table 2a). Anti-rPru p 1 IgE levels in the first class of age were significantly lower than those observed in classes 4 and 5 (table 2b). Also, anti-rBet v 1 levels resulted significantly lower in the patients of the first class than in those of classes 4, 5 and 6 (table 2c).

Discussion

This study showed that in Mediterranean countries, severe peach allergy, associated with Pru p 3 sensitisation, occurs at a young age, whereas peach allergy associated with sensitisation to Pru p 1, the allergen homologous to the major birch pollen allergen Bet v 1, appears in adults. We found that the age at onset of peach allergy is inversely related to anti-rPru p 3 IgE levels at diagnosis and directly related to anti-rPru p 1 and anti-rBet v 1 IgE levels. The statistical analyses confirmed a highly significant difference between reported classes of age at onset of peach allergy for anti-rPru p 3 ($p = 0.037$), anti-rPru p 1 ($p = 0.0445$) and anti-rBet v 1 ($p = 0.0428$) IgE levels (table 2) at diagnosis, however, with different trends (fig. 2). In particular, anti-rPru p 3 IgE levels were significantly higher in peach allergy patients at a younger age (first class of age at onset) than at older ages (last 3 classes of age at onset). These observations clearly show that the younger the age when the symptoms start, the higher the anti-rPru p 3 IgE levels in adult age. In a recent paediatric study [5], anti-rPru p 3 IgE levels were not associated with systemic symptoms and did not correlate with the severity of the reaction; however, a long-term follow-up of these young patients was missing and might confirm the association we have found.

On the contrary, anti-rPru p 1 and anti-rBet v 1 IgE levels were higher in patients developing peach allergy at an older age. The different anti-rPru p 1 and anti-rPru p 3 IgE levels as a function of age might depend on the different routes of sensitisation: Pru p 3 most probably causes sensitisation via the gastrointestinal tract and Bet v 1/Pru p 1 via the respiratory tract. The immature gastrointestinal mucosa in the younger patients might favour Pru p 3 sensitisation, whereas in our geographic area, birch pollen sensitisation (and subsequent cross-reactivity to plant-derived foods) occurs at an older age than sensitisation to other airborne allergens [7]. This can explain why anti-Pru p 1 IgE antibodies, strictly associated with anti-Bet v 1 IgE, are higher in those patients.

Table 2. Pair-wise comparisons by Dunn's test (following Kruskal-Wallis) for anti-rPru p 3 ($p = 0.0037$), anti-rPru p 1 ($p = 0.0445$) and anti-rBet v 1 IgE levels at diagnosis in the different age classes ($p = 0.0428$)

a Anti-rPru p 3

Age class, years	n	Average rank	Different ($p < 0.05$) from age class
1 (0–9)	28	91.84	4-5-6
2 (10–19)	34	85.94	4-6
3 (20–29)	36	76.19	4
4 (30–39)	25	52.62	1-2-3
5 (40–49)	13	61.92	1
6 (50+)	12	55.75	1-2

b Anti-rPru p 1

Age class, years	n	Average rank	Different ($p < 0.05$) from age class
1 (0–9)	28	61.09	4-5
2 (10–19)	34	69.82	
3 (20–29)	36	68.93	
4 (30–39)	25	87.52	1
5 (40–49)	13	94.38	1
6 (50+)	12	87.08	

c Anti-rBet v 1

Age class, years	n	Average rank	Different ($p < 0.05$) from age class
1 (0–9)	28	62.95	4-5-6
2 (10–19)	34	70.56	
3 (20–29)	36	66.17	
4 (30–39)	25	86.92	1
5 (40–49)	13	91.27	1
6 (50+)	12	93.58	1

Previous studies found that birch pollen-specific IgE is directly related to the probability of developing an OAS due to cross-reactivity with plant-derived foods [8, 9].

Another interesting point is the detection of high anti-rPru p 3 IgE levels in patients who stopped eating peach at a young age, after the first severe allergic reactions. Although serial measurements of IgE levels in single patients have not been carried out here, it seems that an apparent increase or persistence of high IgE levels (and hence, hypersensitivity) has occurred in the absence of contact with the offending food. This possibly happens via the ongoing ingestion of tolerated cross-reacting plant-derived foods, such as apple and Prunoideae, maize,

tree nuts, tomato, fennel, rice, green bean and others [10]. In another study, with the same study population, we have found that in LTP-allergic patients, the number of sensitising, cross-reacting foods was higher in rPru p 3 positive than in rPru p 3 negative patients being directly related to peach LTP IgE levels ($p < 0.00001$; Spearman's rank) [11]. In that study, we have also demonstrated that the baseline tryptase levels were related to anti-Pru p 3 IgE levels, thus indirectly confirming the hypothesis that the exposure to different LTP-containing foods may continu-

ously stimulate the immune system, provoking an increase in Pru p 3 IgE levels, in the absence of contact with peach. All these observations allow us to conclude that the cross-reactive nature of LTP may sustain the increase in LTP-specific IgE antibodies towards a big array of plant-derived foods and confirm previous similar data of one of the authors [12]. Alternatively, an ongoing stimulation of the immune system through the airways or the skin cannot be ruled out [13, 14].

References

- 1 Fernández-Rivas M, González-Mancebo E, Rodríguez-Pérez R, Benito C, Sánchez-Monge R, Salcedo G, et al: Clinically relevant peach allergy is related to peach lipid transfer protein, Pru p 3, in the Spanish population. *J Allergy Clin Immunol* 2003;112:789–795.
- 2 Asero R, Antonicelli L, Arena A, Bommarito L, Caruso B, et al: Causes of food-induced anaphylaxis in Italian adults: a multi-centre study. *Int Arch Allergy Immunol* 2009;150:271–277.
- 3 Gamboa PM, Cáceres O, Antepara I, Sánchez-Monge R, Ahrazem O, Salcedo G, Barber D, Lombardero M, Sanz ML: Two different profiles of peach allergy in the north of Spain. *Allergy* 2007;62:408–414.
- 4 Pastorello EA, Farioli L, Pravettoni V, Scibilia J, Mascheri A, et al: Pru p 3-sensitised Italian peach-allergic patients are less likely to develop severe symptoms when also presenting IgE antibodies to Pru p 1 and Pru p 4. *Int Arch Allergy Immunol* 2011;156:362–372.
- 5 Novembre E, Mori F, Contestabile S, Rossi ME, Pucci N: Correlation of anti-Pru p 3 IgE levels with severity of peach allergy reactions in children. *Ann Allergy Asthma Immunol* 2012;108:271–274.
- 6 Conover WJ: *Practical Nonparametric Statistics*, ed 3. New York, John Wiley and Sons, 1999.
- 7 Asero R, Antonicelli L, Arena A, Bommarito L, et al: EpidemAAITO: features of food allergy in Italian adults attending allergy clinics: a multi-centre study. *Clin Exp Allergy* 2009;39:547–555.
- 8 Asero R: Birch and ragweed pollinosis north of Milan: a model to investigate the effects of exposure to 'new' airborne allergens. *Allergy* 2002;57:1063–1066.
- 9 Asero R, Massironi F, Velati C: Detection of prognostic factors for oral allergy syndrome in patients with birch pollen hypersensitivity. *J Allergy Clin Immunol* 1996;97:611–616.
- 10 Geroldinger-Simic M, Zelniker T, Aberer W, Ebner C, Egger C, et al: Birch pollen-related food allergy: clinical aspects and the role of allergen-specific IgE and IgG4 antibodies. *J Allergy Clin Immunol* 2011;127:616–622.
- 11 Pastorello EA, Farioli L, Scibilia G, Pravettoni V, Mascheri A, Stafylaraki C, Nichelatti M, Asero R: Baseline tryptase levels are related to age, total IgE and anti-rPru p 3 IgE levels in peach allergic patients. *J Invest Allergol Clin Immunol* 2013, accepted for publication.
- 12 Asero R, Mistrello G, Roncarolo D, Amato S: Relationship between peach lipid transfer protein specific IgE levels and hypersensitivity to non-Rosaceae vegetable foods in patients allergic to lipid transfer protein. *Ann Allergy Asthma Immunol* 2004;92:268–272.
- 13 Asero R, Mistrello G, Roncarolo D, Amato S, Calderoni G, Barocci F, van Ree R: Immunological cross-reactivity between lipid transfer proteins from botanically unrelated plant-derived foods: a clinical study. *Allergy* 2002;57:900–906.
- 14 Borghesan F, Mistrello G, Roncarolo D, Amato S, Plebani M, Asero R: Respiratory allergy to lipid transfer protein. *Int Arch Allergy Immunol* 2008;147:161–165.