

Peach allergy in spanish children: tolerance to the pulp and molecular sensitization profile

Teresa Boyano-Martínez, María Pedrosa, Teresa Belver, Santiago Quirce & Carmen García-Ara

Department of Allergy, Hospital La Paz Health Research Institute (IdiPaz), Madrid, Spain

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Correspondence

Teresa Boyano-Martínez, Department of Allergy, Hospital La Paz Health Research Institute (IdiPaz), C/ Ginzo de Limia 55, 9° C, 28034 Madrid, Spain.
Tel.: +34 917304920
Fax: +34 917277033
E-mail: med012396@saludalia.com

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Abstract

Background: Peach allergy is the main cause of vegetable food allergy in the Mediterranean area. Pru p 3 is the major allergen, and it is mainly found in the peel.

Objective: We sought to calculate the frequency of tolerance to peach pulp in Spanish children suffering from allergic reactions after eating or having contact with peach and to analyze the sensitization pattern to peach allergens.

Methods: Fifty-seven children (32 boys; median age, 7.4 yr) were included in the study. A systematized questionnaire on allergic reactions to peach was administered. Prick tests with peach peel and pulp, peach lipid transfer protein (LTP), and profilin were performed. Serum-specific IgE to peach, rPru p 1, rPru p 3, and rPru p 4 was determined by ImmunoCAP. Oral food challenges (OFCs) with peach pulp were performed on all but one child.

Results: Eighty-eight percent of the children had positive prick tests with peach peel, 35% with peach pulp, 88% with peach LTP, and 9% with profilin. Serum-specific IgE to peach was demonstrated in 100% of the patients; rPru p 3-specific IgE in 96%; rPru p 1-specific IgE in 11%; and rPru p 4-specific IgE in 10%. Oral food challenge was negative in 52 (93%) children.

Conclusions: More than 90% of the study population tolerated peeled peach. Pru p 3 was the major allergen. Pru p 1 and 4 showed low prevalence.

Originally from China, the peach belongs to the *Rosaceae* family. It is cultivated in temperate climates, especially in countries from Southern Europe. The prevalence of sensitization to peach is not known, although it is estimated to be high, especially among patients allergic to pollen. More than 25% of pollinic patients in central Spain have shown sensitization to peach (1), and more than 50% of birch pollen-allergic patients in Central Europe report symptoms after ingestion of this fruit (2). Two syndromes have been well characterized in countries of Northern and Central Europe and in Southern Europe, respectively (3–5). The first is observed in birch-allergic patients who present oral allergy syndrome (OAS) upon fruit ingestion, in which the primary sensitizer is Bet v 1, the major birch pollen allergen that belongs to the Pathogenesis-related protein 10 family (PR-10), and secondarily become sensitized to other Bet v 1-homologues such as Mal d 1 (PR-10 from apple) or Pru p 1 (PR-10 from peach). The syndrome in Southern Europe is found in patients without pollinosis, who are sensitized to Pru p 3 (a member of the non-specific lipid transfer proteins family, nsLTP), whose clinical presentation may vary from OAS to systemic, sometimes severe reactions.

Four allergens have been identified in peach. Pru p 3 was recognized as a major allergen in 1999 (6,7). As a member of the nsLTP family, Pru p 3 is highly resistant to extreme pH and temperature conditions and to pepsin digestion because of the stability the four disulfide bonds confer to this structure. Pru p 1 belongs to PR-10 family, and Pru p 4 is a profilin; both are labile molecules that lose their structure under high temperatures. Pru p 2 is one of the large thaumatin-like proteins (TLP) that have 16 cysteine residues that form 8 disulfide bonds that confer high stability to the molecule (8).

Clinical experience demonstrates that peach peel is more allergenic than pulp, because some patients report to spontaneously tolerate peeled but not the whole fruit, and this fact has been confirmed in clinical routines in our department. The peel allergenicity is mainly due to the higher concentration of Pru p 3, the major allergen, in peel than in pulp, as has been demonstrated in some clinical and laboratory studies (9–14).

The aims of this study were 2-fold: (i) to ascertain the frequency of tolerance to peach pulp in children with a previous history of allergic reactions after ingestion or contact

with the fruit; and (ii) to analyze the sensitization pattern of peach allergens Pru p 1, Pru p 3, and Pru p 4 in a birch-free area population.

Methods

Subject population

A prospective cross-sectional study was performed. Spanish children with a previous history suggestive of immediate hypersensitivity reactions clearly related to intake or contact with peach were included. An allergist administered a questionnaire specifically designed for this purpose. The questionnaire included data regarding the offending food (fresh or processed fruit with or without peel), route of exposure (oral or cutaneous), the number of reactions, the time interval between intake and symptoms, clinical characteristics of the most important reaction (if more than one), treatment required and place where it was administered, and the evolution of the reaction. Tolerance to other fruits from the *Rosaceae* family was also assessed.

Laboratory tests

Prick tests

Skin prick tests were performed with commercial extracts of peach peel (5 mg/ml) and pulp (5 mg/ml) (Leti, Madrid, Spain), natural purified peach LTP (0.1 mg/ml; Bial Aristegui, Bilbao, Spain), and the more prevalent pollens (*Lolium perenne*, *Olea europaea*, *Cupressus arizonica*) (ALK-Abelló, Madrid, Spain) in the study area, Madrid (Spain). In a subgroup of patients, a skin prick test was also performed with natural purified date palm profilin (Pho d 2; 50 µg/ml; ALK-Abelló). Sodium chloride 0.9% and histamine hydrochloride were used as negative and positive controls. The responses were read 15 min after puncture, and results were expressed as the mean wheal diameter in millimeters. A diameter ≥ 3 mm was considered as a positive test.

Fluorescence enzyme immunoassays

Serum-specific IgE antibody (sIgE) levels to peach and to recombinant allergens Pru p 1 and Pru p 3 were determined, and in a subgroup of patients, recombinant Pru p 4-sIgE was also analyzed. All determinations were performed using the ImmunoCAP System® (Phadia Diagnostics, Uppsala, Sweden). Titers higher than 0.34 kU/l were considered positive.

Peach pulp challenge tests

Oral food challenges (OFC) with peach pulp were performed on all patients, unless contraindicated due to severe previous reactions or a concomitant condition that involved a risk for children. All parents signed a written informed consent form that was previously approved by our Institutional Ethics Committee.

Two different types of OFC were programmed depending on the reaction that children reported. Patients who reported objective signs, such as generalized urticaria and/or angioedema, underwent open OFC. Those patients who mainly complained about subjective symptoms underwent

double-blind, placebo-controlled food challenge (DBPCFC). The use of antihistamines or antileukotrienes was not allowed during the previous week. Oral corticosteroids were forbidden during the 2 weeks prior to the challenge.

Fresh fruit pulp was used for the OFC. The fruit was peeled and then rinsed under a cold-water spurt to clear any possible trace of peel down that may have adhered to the pulp. Open OFC was performed with 1/8, 1/4, and 1/2 of a medium-sized peach in 60-min intervals. DBPCFC was performed on two consecutive days: one with placebo and the other with peach. The same amount, number of doses, and intervals as used in open OFC were used. Patients were randomized to receive placebo or peach on the first day. Fruit was masked with vanilla custard, milk, sugar, and food coloring. Sensitization to any of those ingredients was excluded in all patients. The same products and proportions were used as placebo. Children with a negative outcome in the DBPCFC underwent an open OFC on another day to avoid possible false negatives as result of eventual denaturation of labile allergens such as Pru p 1 or Pru p 4 in the masking process. Food challenges were supervised by an allergist and were stopped when characteristic, immediate objective hypersensitivity signs appeared or when all doses had been administered.

Statistical analysis

An analysis of normality (Kolmogorov-Smirnov test) indicated a normal distribution for the variable age and a non-normal distribution for total IgE and sIgE. The correlation coefficient was calculated using the Spearman method for non-parametric data.

Results

Fifty-seven children (32 boys, 56%) were included over an 18-month period. At the time of the study, the mean age was 7.4 yr (range 2–17 yr). Nineteen (33%) children had atopic dermatitis, 14 (25%) had asthma, and 26 (46%) had seasonal allergic rhinoconjunctivitis. Twenty-four (92%) of 26 pollinic children were sensitized to *Lolium perenne*, 15 (58%) to *Olea europaea*, and 13 (50%) to *Cupressus arizonica*. Thirty-six (63%) children had other food allergies (other fruits, 25; nuts and peanut, 17; legumes, 5; egg, 13; fish, 3; cow's milk, 2; and seafood, 1). Thirty-two (56%) reported allergic reactions upon ingestion of other edible vegetables, including other fruits, nuts, and peanuts or legumes.

Questionnaire results

Data on allergic reactions to peach

Forty-six (81%) children had experienced reactions with fresh fruit (peach with peel, 24 children; without peel, 18; in four children this information was not recorded). Twenty-one (37%) children had reactions with processed foods: 13 with commercial peach juice; 9 with other foods such as marmalade, canned peach, and commercial fruit puree.

The number of reactions per child was variable: seventeen (30%) reported one reaction; 21 (37%) two reactions; and 15

(26%) three or more reactions. Four children could not specify this information.

Clinical symptoms experienced in the reactions are summarized in Table 1. The time from intake to development of symptoms was less than 15 min in 37 (65%) children; between 15 and 30 min in 4 (7%); and between 30 and 60 min in 2 (4%). The remaining 14 did not know this information.

The route of exposure of the most important reaction was oral in 47 (84%) children and cutaneous contact in 9 (16%). Eight children in the first group also reported reactions by contact. One patient could not specify this information.

Thirty-three (58%) children required treatment: antihistamines, 29 (51%); oral or parenteral corticosteroids, 16 (28%); adrenaline, 7 (12%); and short-acting beta-agonists, 3 (5%). Reactions in 22 (39%) children subsided spontaneously without treatment. In 2 cases, this information was not known.

Eleven (19%) children were treated at home. Twenty-one (37%) required assistance at the emergency department (seven at a general pediatrician's office and 14 at the hospital emergency room). In one case, this information was not recorded.

Most reactions resolved either spontaneously or with treatment within a few hours. Reactions in 20 (42%) children resolved in less than 1 h and 22 (47%) resolved in 1–6 h. In 5 (11%) cases, symptoms lasted more than 6 h. In 10 cases, this information was not known.

Tolerance to other fruits of the *Rosaceae* family

Table 2 shows data on tolerance to other frequently consumed foods of the *Rosaceae* family. Most of the patients in our study group tolerated these fruits. Apple was the fruit most frequently involved in reactions; nevertheless, some patients who reported previous reactions with apple or plum tolerated these fruits if they were peeled.

Table 1 Clinical symptoms experienced in the reactions

	N	%
Mucocutaneous	54	95
Urticaria	36	63
OAS	23	40
Exclusive OAS	13	23
Angioedema	21	37
Conjunctivitis	3	5
Vomiting	10	18
Respiratory symptoms	12	21
Cough	6	11
Bronchospasm	4	7
Rhinitis	4	7
Loss of voice	1	2
Uvula edema	1	2
One organ system affected	42	74
Two or more organ system affected	15	26

OAS, oral allergy syndrome.

Table 2 Tolerance to other vegetables from *Rosaceae* family

	N	Tolerance		No tolerance		No intake	
		N	%	N	%	N	%
Apple	55	47*	85	11*	20	0	0
Pear	52	49	94	2	4	1	2
Strawberry	52	46	88	2	4	4	8
Cherry	51	33	65	4	8	14	27
Plum	54	25*	46	3*	6	27	50
Almond	55	41	75	2	4	12	22

*Three patients allergic to apple peel and one patient allergic to plum peel tolerated the intake of peeled fruit.

Skin prick tests and serum-specific IgE determinations

Results of skin prick tests and sIgE levels are summarized in Table 3. The majority of patients showed sensitivity to peach peel extract (88%) and to peach LTP (88%), whereas peach pulp extract was positive in almost one-third of the population. Positivity of skin prick tests with profilin was 9%.

Serum-specific IgE to peach extract was demonstrated in all children, and rPru p 3-sIgE in all but two children (96%). The correlation between the levels of peach- and rPru p 3-sIgE was very high ($R^2 = 0.864$; $p < 0.001$) (Fig. 1). The proportion of children with positive sIgE to other peach allergens was much lower: rPru p 1 in 6 of 53 (11%) children and rPru p 4 in 4 of 39 (10%).

Challenge test results

OFCs with peach pulp were performed on 56 children. The challenge was not performed on one patient because he suffered from cardiopathy and had presented with anaphylactic reaction clearly related to the intake of peach on two occasions. Forty-three challenges were performed in an open

Table 3 Positive results of serum-specific IgE determinations, skin prick tests, and oral food challenge

	Positive			Median	P 25–75	Range
	N	N	%			
Specific IgE (kU/l)						
Peach	57	57	100	6.05	2.29–12.65	0.86–66.70
Pru p 3	57	55	96	5.24	2.71–13.40	0.91–82.10
Pru p 1	53	6	11	2.85	0.97–5.00	0.87–11.00
Pru p 4	39	4	10	2.50	1.32–7.50	1.09–9.00
Total serum IgE	56			174	53–665	6–4073
Skin prick tests (mm)						
Peach peel	57	50	88	4.50	4.00–7.00	3.00–11.50
Peach pulp	57	20	35	3.75	3.00–4.50	3.00–7.00
Peach LTP	50	44	88	5.50	3.50–8.50	3.00–13.50
Pho d 2	33	3	9	6.50	4.50–	5.00–11.00
Food challenge	56	4	7			

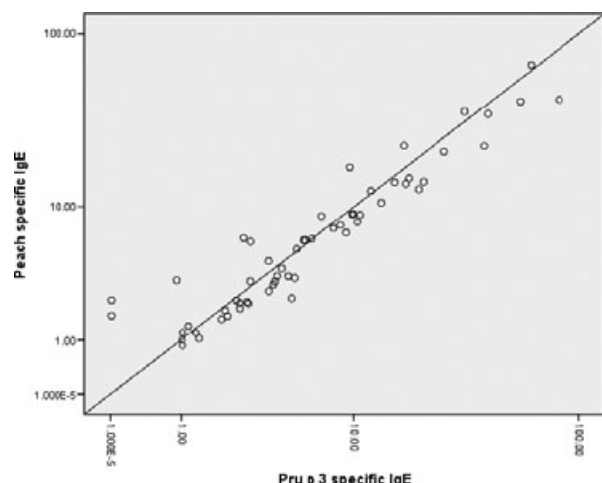


Figure 1 Correlation between levels of serum-specific peach-IgE and Pru p 3-IgE antibodies represented as logarithm of KU/l.

manner, and 13 were DBPCFC. The result was negative in 52 (93%) children, and positive in 4 (7%). The four patients who presented positive OFC underwent challenge in a blinded manner. Three patients presented with OAS, one with associated rhinoconjunctivitis, another with vomiting, and the other one with exclusive OAS. The fourth patient had facial angioedema and rhinoconjunctivitis. All children with positive challenge tests had positive sIgE for peach (range 6.33–8.10 kU/l) and rPru p 3 (range 2.71–10.4 kU/l). Moreover, the patient who presented isolated OAS had positive sIgE for rPru p 4 (3 kU/l) and reported the same symptoms after the intake of other fruits such as melon, watermelon, and pear.

Discussion

The first objective of this study was to assess tolerance to peach pulp in a population of Spanish children with a history of reactions after ingestion and/or contact with the fruit. In more than 90% of the patients, tolerance to pulp was confirmed, which indicates that the major allergen Pru p 3 may be exclusively localized in the peel or that the pulp may contain a minimal amount of Pru p 3, which is not enough to trigger symptoms. Cuesta-Herranz et al. (15) performed OFCs with peach peel and pulp separately and found that 68% of the studied population exclusively presented symptoms upon ingestion of the pulp. The discrepancy with our results may be due to two reasons: the first, the mean age of the studied population that was higher (mean age 20 ± 8 yr) in the group of Cuesta-Herranz et al., which determines a higher proportion of pollen-allergic patients, and thus, a higher prevalence of sensitization to panallergens such as profilins or PR-10 present in the pulp; the second reason may be that in our study we had the precaution of carefully clearing any possible trace of peel down that may have adhered to the pulp after peeling it off, as indicated in the Methods section. The ulterior rinse-off of the peach may explain why children who referred reactions upon ingestion of the peeled fruit tolerated it in the OFC. Comparison with other studies is not possible, as they were performed with the whole fruit (3,16,17).

Oral food challenge with the peeled fruit does not permit to exactly know the population of peach-allergic patients in our group. Nevertheless, OFC with the fruit peel was not performed because it exceeded the aim of the study. Previously cited studies have confirmed clinical allergy in 71–83% of patients with a history of allergic reactions to the fruit (3,16,17). It is possible that a similar or higher percentage of patients would have been confirmed to be allergic in our population if they had taken the whole fruit, because given the short age of our group, the time lapse between the reaction and the study was shorter, which makes the confirmation of clinical allergy more possible, as well as other characteristics, such as the high prevalence of sensitization to Pru p 3 or the clinical characteristics of the reactions.

Double-blind, placebo-controlled food challenges are the most suitable challenge tests in investigation studies or for evaluating subjective symptoms. Nevertheless, when the expected proportion of negative results is high, as was the case here, open OFCs are suitable because the bias of false-positive results is minimal and the procedure is time-saving (18). For this reason, we decided to perform open OFC on all cases except those children who presented with subjective symptoms in previous reactions, especially OAS.

The analysis of the allergen sensitization pattern showed that all children except for two had serum sIgE to rPru p 3; 6 to rPru p 1; and 4 to rPru p 4. These data confirm that in populations from Southern Europe, the major peach allergen is Pru p 3. The proportion of children sensitized to Pru p 3 was even higher than in other series (3), probably because we had fewer pollinic patients, in whom sensitization to profilin or Bet v 1-homologues is more common. On the other hand, the sensitization pattern considerably differed from that found in populations of Central Europe in which, as previously mentioned, Pru p 1 is the major allergen. Nevertheless, it has been recently shown that in patients from Austria (Central Europe), the major allergen in peach-allergic patients without sensitization to birch pollen is Pru p 3; and among those patients sensitized to birch pollen, the major allergen was found to be Pru p 1 (19). Pru p 4 has been shown to be a minor allergen in all the studied populations.

Some patients had positive skin prick test with peach pulp extract, although they have negative Pru p 1 and Pru p 4 sIgE in serum. This may be explained by the existence of other non-clinically relevant allergens or other allergens not studied in the present work such as Pru p 2 that may still reveal positive skin prick test, although the OFC is negative.

Knowledge of the localization of allergens in edible vegetables is of utmost importance to adequately guide patients in their dietary restrictions. For many years, it has been known that peach peel is more allergenic than pulp and that the major allergen was found in high amounts in the peel (10). Carnés et al. (13) analyzed the content of Pru p 3 in lyophilized extracts of red and yellow peach peel and pulp by means of *Enzyme-linked immunosorbent assay*. They used a polyclonal anti-Pru p 3 rabbit antibody and found that peel contains 7-fold more allergen than pulp. Some years later, Borges et al. (11), using the same method, demonstrated that

peel contained 2.5 times more Pru p 3 than pulp, which they referred to as fresh tissue. This proportion decreased to 1.6 when peach was previously rinsed with water. More recently, Cavatorta et al. (12), analyzed the localization of Pru p 3 in peach tissue by means of matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) and demonstrated that Pru p 3 is almost exclusively in the peel, although they cannot rule out its presence in the external part of the pulp, immediately under the peel. Along these lines, some other authors have demonstrated that Mal d 3 (apple nsLTP) is exclusively found in the peel (20). The main results of our study, that is, tolerance to peach pulp and Pru p 3 sensitization in more than 90% of the children, are in line with the almost exclusive localization of this allergen in the peel.

The four patients who presented with symptoms on OFC with peach pulp were sensitized to Pru p 3. We consider it unlikely that this allergen was responsible for the reaction. As previously mentioned, one patient who was also sensitized to rPru p 4 presented with OAS in OFC and reported the same symptoms upon ingestion of other fruits. Therefore, profilin is the most plausible allergen responsible for the reaction. In the other three children, the allergen responsible for the reaction may be Pru p 2, the recently described Pru p 7 (www.allergome.org) (21), or another unknown allergen.

On the other hand, it is worth noting that 8 patients with sensitization to Pru p 1 and/or Pru p 4 tolerated peeled fruit, although the pulp contains these allergens.

Food allergy affects the quality of life of patients and, in the case of children, of parents and caretakers because they are prompted to avoid foods usually consumed in their environment. They also must be aware of possible accidental-allergic reactions that in some cases may be life threatening. For this reason, it is important that dietary avoidance is well grounded on the basis of clinical allergy and not only on sensitization, which can be asymptomatic. In the case of peach allergy, this study indicates that the majority of children with a history of allergic reactions with the fruit can safely tolerate pulp if carefully peeled and rinsed with water. Nevertheless, given the possibility of reactions, we find necessary to perform OFC under medical supervised conditions before letting the patient take the peeled and rinsed fruit at home. Processed foods that contain peach, such as commercial juice, may contain Pru p 3 if the fruit has not been previously peeled, and it is not clear whether these patients can tolerate them.

Conflict of interest

The authors declare that they do not have any potential conflict of interest. The study was self-funded.

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