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Brief Communication

Skin Testing With Peach Peel Extract *Versus* Serum IgE to Pru p 3 as a Stronger Predictor of Peach-Induced Anaphylaxis

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There are no financial or other issues that might lead to conflict of interest.

ABSTRACT

The most important peach fruit allergen is Pru p 3, followed by Pru p 1, Pru p 4, and Pru p 7. We aimed to assess their role in subjects with peach fruit-induced allergy (anaphylaxis and OAS) and compare skin prick tests (SPT) vs. specific immunoglobulin E (sIgE) for predicting anaphylaxis. We also selected a control group. SPT included prevalent inhalant and plant food allergens plus peach peel extract. The sIgE to Pru p 1, Pru p 3, Pru p 4, and Pru p 7 were quantified. Compared with controls (n = 42), cases (n = 41) were younger ($P = 0.003$), more frequently female ($P < 0.05$) and had higher SPT positivity to peach peel (44% vs. 2.4%, $P < 0.0001$). There were significant differences in sensitization to several pollens: *Olea europaea*, *Artemisia vulgaris*, *Prunus persica*, *Platanus acerifolia* (all $P < 0.001$); and fruits: apple ($P < 0.04$), peanut ($P < 0.002$), tomato ($P < 0.005$), and melon ($P < 0.05$). Pru p 3 sIgE was detected in 61% of all cases (85% anaphylaxis and 38% OAS; $P < 0.01$ each) and 5% of controls ($P < 0.001$). Pru p 4 sIgE was present in 19% of cases and 7% of controls. The sIgE to Pru p 1 and Pru p 7 were not found. The odds ratio to predict anaphylaxis for peach peel SPT was 113 (confidence interval [CI], 20–613; $P < 0.0001$); for sIgE to Pru p 3, 22 (CI, 5.3–93; $P < 0.0001$); and for SPT positivity to selected plant food allergens, 5 (CI, 1–19; $P < 0.05$). In our study group, SPT with peel peach extract was a better predictor of anaphylaxis than Pru p 3 sIgE or other variables considered. The role of sIgE to Pru p 1, Pru p 4, and Pru p 7 seemed negligible.

Keywords: Peach; fruit; pollen; food hypersensitivity; allergy; anaphylaxis; skin tests; immunoglobulin E

INTRODUCTION

The high global consumption of peach fruit is made possible by large expanses of peach orchards around the world.[1] In many regions, peach is also a leading cause of food allergy.[2] After being reported in Mediterranean countries,[2,3] studies from other European countries,[4] and elsewhere have found similar results. Peach allergy can arise from primary sensitization[2,3] or secondary sensitization[5,6] to other plant allergens.

Pru p 3, a 10-kDa non-specific lipid transfer protein 1, is the most common allergen involved in peach fruit allergy,[2-4] and the best marker of severity in patients sensitized to it.[2,7] However, it is not the only allergen associated with allergic reactions.[5,6] Like Bet v 1 from birch pollen, Pru p 1 is an 18-kDa pathogenesis-related protein and causes allergic reactions in individuals suffering from peach allergy.[8] Pru p 2 is a 25 to 28-kDa thaumatin-like protein proposed as important in the Mediterranean area,[9] while Pru p 4 is a 14-kDa protein in the profilins family associated with oral allergy syndrome (OAS).[10] Pru p 7 is a gibberellin-regulated protein (7 kDa), associated with anaphylaxis in people negative to Pru p 3.[11] Other allergens like Pru p 9 are involved in respiratory allergy.[12]

We studied patients who developed allergic reactions to peach fruit, with a particular focus on peach-induced anaphylaxis, in a region of high peach fruit exposure. We aimed to assess the role of the molecular components Pru p 1, Pru p 3, Pru p 4, and Pru p 7, and compare skin prick test (SPT) with peach peel extract vs. serum specific immunoglobulin E (sIgE) for predicting anaphylaxis.

MATERIALS AND METHODS

Study subjects

This study took place in Valle de Ricote: Hoya del Campo, San Jose Artesano, Ricote and Abaran villages (Murcia, South-East Spain), a region with extensive peach orchards and a population with high exposure to peach tree fruit and pollen.[12,13]

Subjects aged ≥ 20 years with repeated episodes of peach allergy were included. OAS was defined as previously described.[2,3] When symptoms extended to 2 or more organs, we considered the reaction anaphylaxis.[14] Criteria for diagnosing rhinitis, asthma and conjunctivitis were made as previously described.[15]

Controls aged ≥ 20 years from the same population were included. They had good tolerance to peach fruit but could be sensitized to the environmental allergens of the area. These were randomly selected from a large database of 1,000 subjects already available from a large population study. Controls were randomly taken 1 out of 24 tolerant subjects with a total number equivalent to that of the positive cases.

SPT

Participants underwent SPT for a large panel of inhalant and plant food allergens (**Supplementary Data S1**) provided by Inmunotek® (Madrid, Spain). Peach peel was extracted, and results evaluated as previously described.[12,16] The optimal concentration for Pru p 3 in the peach extract for skin testing was determined by titration with the following concentrations of native Pru p 3: 40, 20, 15, 10, and 1 μg/mL^{-1} (provided by Dr. Díaz Perales, Polytechnic

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University, Madrid, Spain) in a group of 10 subjects with SPT positive to peach peel extract. Positive SPT was considered when the wheal area was ≥3 mm, providing that the diluent skin response was negative.

sIgE

The sIgE to Pru p 3, Pru p 4, Pru p 1, and Pru p 7 was measured by ImmunoCAP® (Thermo Fisher Scientific, Wahtham, MA, USA). Values > 0.35 kU_A/L were considered positive.

Data analysis

We compared cases of allergy to peach fruit (anaphylaxis and OAS) with controls and also anaphylaxis vs. OAS.

Quantitative variables were described using means, medians, standard deviations (SDs), and confidence intervals (CIs). Qualitative variables, expressed as relative frequencies and percentages, were analyzed using the chi-squared statistic or Fisher's exact test when frequencies numbered 5 or less (**Supplementary Data S2**).

We also performed multivariable logistic regression using the Stepwise method for 2 different outcomes: peach-peel SPT and Pru p 3 sIgE for inducing anaphylaxis. Both models included all the study variables in the analysis. The level of significance was 0.05 and we used the SAS v 9.4 Software (SAS Institute, Cary, NC, USA).

Ethics statement

The study was approved by our Institutional Ethics Committee: Comité de ética de la investigación. Hospital General Universitario Gregorio Marañón (approval number: 41/18) and Comisión de ética investigación del Hospital Universitario Infanta Leonor y Hospital Virgen de la Torre (approval number: 100/20). All patients signed a written informed consent prior to the inclusion in the study.

RESULTS

Study subjects

We included 41 cases and 42 controls, the former were younger (median age, 35.5 years [SD, 12] vs. 44.6 years [SD, 15]; $P = 0.003$) and more frequently female (71% vs. 50%; $P < 0.05$).

The optimal concentration for Pru p 3 in the peach extract for skin testing was 20 μg/mL, which was used for our experiments.

Among the cases, 20 had anaphylaxis (**Table 1**) and 21 OAS (**Table 2**). Nearly half (44%) had a positive response to peach peel extract. Anaphylaxis was considered to be of grade 1 severity in 8 participants, grade 2 in 6, and grade 3 in 6.[14]

Only 1 in the control group was positive to peach peel extract on SPT, indicating a specificity of 97.6%. This was control 20 who had also positive SPT to apple, peanut, and walnut (**Supplementary Tables S1** and **S2**).

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Table 1. Cases of anaphylaxis induced by peach fruit

Case	Age (yr)	Sex	Symptoms	SPT peach peel	Plant foods with symptoms
1	22	Male	Urticaria, systemic pruritus, and difficulty breathing with peanut and peach	+	2
2	44	Male	Systemic pruritus, lip angioedema and difficulty breathing with peach, peanut, and almond	+	3
3	28	Female	Lip angioedema, oral pruritus and difficulty breathing with peach, cherry, prune, and mango	+	4
4	26	Female	Nausea, vomiting, and systemic pruritus with peach and pomegranate	+	2
5	39	Female	Facial angioedema, Dyspnea, systemic pruritus, and abdominal pain with peach and apple. Rhinitis and difficulty breathing around peach tree orchards	+	2
6	34	Female	Generalized angioedema, systemic pruritus, and difficulty breathing with walnut. Gastrointestinal pain and eye angioedema with peach, nectarine, and apricot	+	4
7	48	Male	Systemic pruritus, nausea, vomiting, dizziness, and hypotension with banana and peach	+	2
8	62	Female	Dyspnea, facial angioedema and systemic pruritus with peach, sunflower seed and strawberry	–	3
9	25	Female	Dyspnea, generalized urticaria and difficulty breathing with peach, peanut, almond, walnut and sunflower seed	+	5
10	44	Female	Systemic pruritus, facial angioedema, and dyspnea with apricot and peanut	+	2
11	33	Female	Dyspnea, systemic pruritus and angioedema and hypotension with banana, peach, peanut and walnut	+	4
12	66	Female	Systemic pruritus and difficulty breathing with peanut, peach and apple	+	3
13	23	Female	Hypotension, generalized urticaria, and systemic pruritus with walnut and peach	+	2
14	42	Male	Oral pruritus and difficulty breathing with walnut, apple, and peach	+	3
15	37	Male	Difficulty swallowing, dysphonia, and systemic pruritus with peach, peanut, sunflower seed, almond, and pistachio	+	5
16	47	Female	Nausea, vomiting, and systemic pruritus with peach. Lip angioedema with walnut	–	2
17	25	Female	Generalized urticaria with facial angioedema and difficulty breathing with apple and peach	–	2
18	35	Female	Systemic pruritus, facial angioedema, and difficulty breathing with peanut. Oral and ear pruritus with peach	+	2
19	42	Female	Systemic pruritus, difficulty breathing, and abdominal pain with peach, peanut, and apple	+	3
20	35	Male	Facial angioedema with pruritus and abdominal pain with peach and peanut	–	2

SPT, skin prick test.

Table 2. Cases of peach fruit-induced oral allergy syndrome

Case	Age (yr)	Sex	Symptoms	SPT peach peel	Plant foods with symptoms
21	26	Male	Facial erythema and tongue pruritus with peach, apple, pineapple, and melon	–	4
22	27	Female	Oral pruritus with peach, banana, almond, and peanut	–	4
23	28	Female	Tongue and pharyngeal pruritus with peach, melon, watermelon, and banana	–	4
24	41	Female	Tongue pruritus and lip pruritus and angioedema with peach, and peanut	–	2
25	43	Female	Oral pruritus with peach, melon, and peanut. Rhynorrhea and nose pruritus with sunflower seed	+	4
26	24	Female	Oral pruritus with peach and peanut	+	2
27	32	Female	Oral pruritus and facial erythema with peach, apricot, apple, and pear Difficulty swallowing with pineapple	–	5
28	37	Female	Oral and lip angioedema with peach	–	1
29	39	Female	Tongue angioedema and oral pruritus with walnut and peach	–	2
30	34	Male	Oral pruritus with peach	–	1
31	29	Female	Oral and lip angioedema with peach	–	1
32	48	Male	Tongue pruritus and angioedema with tomato, melon, peach, and apricot	–	4
33	32	Female	Pharyngeal pruritus with peach	–	1
34	49	Female	Pharyngeal pruritus and difficulty swallowing with peach and apricot	–	2
35	24	Female	Lip angioedema and tongue pruritus with melon and peach	–	2
36	33	Female	Pharyngeal pruritus with peach	–	1
37	20	Male	Lip angioedema and tongue pruritus with pineapple, melon, and peach	–	3
38	33	Female	Oral pruritus with peach	–	1
39	22	Female	Pharyngeal pruritus with peanut, walnut, peach, banana, and kiwi	–	5
40	36	Male	Tongue pruritus with peach	–	1
41	62	Male	Oral pruritus and lip angioedema with peach	–	1

SPT, skin prick test.

SPT to pollens

Although the prevalence was high with all the pollens tested in cases, the strongest association was for *Olea europaea*, *Artemisia vulgaris*, *Prunus persica*, and *Platanus acerifolia* ($P < 0.001$, **Fig. 1**). When we considered the percentage of subjects sensitized to pollens, we

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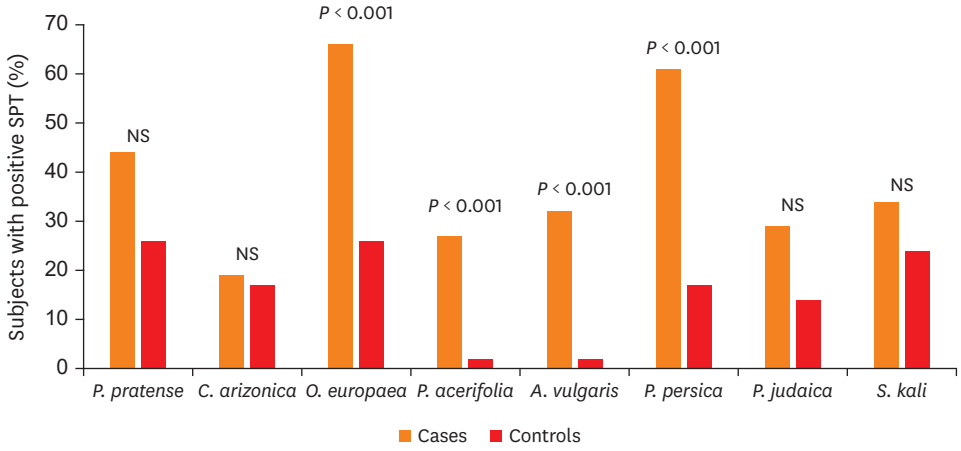

Fig. 1. Percentage of subjects with positive SPT to pollens in cases vs. controls.
NS, not significant; SPT, skin prick test.

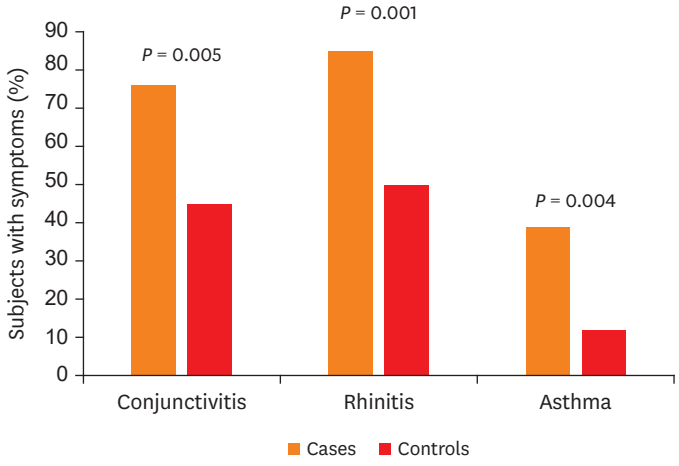

Fig. 2. Concurrent allergic diseases in cases vs. controls.

observed a strong statistical difference between cases and controls: 83% vs. 45% respectively ($P < 0.0001$) (**Supplementary Table S3**).

Concurrent allergic diseases

Differences were found in cases compared with controls in conjunctivitis (76% vs. 45%, $P = 0.005$), rhinitis (85% vs. 50%, $P = 0.001$) and asthma (39% vs. 12%, $P = 0.004$) (**Fig. 2**).

SPT to plant food allergens

Differences in positivity between cases and controls were found for apple (15% vs. 2%, $P = 0.04$), peanut (27% vs. 2%, $P = 0.002$), tomato (17% vs. 0%, $P < 0.005$) and melon (15% vs. 2.4%, $P = 0.04$). SPT to plant food allergens was also higher in the cases compared with the controls (36% vs. 9.5%; $P = 0.007$). **Supplementary Table S4** presents SPT to plant food allergens in cases.

sIgE to Pru p 3, Pru p 4, Pru p 1, and Pru p 7

The proportion of positive sIgE to Pru p 3 was detected in 61% of cases vs. 5% of controls ($P < 0.001$), indicating a sensitivity of 61% and specificity of 95% for this marker. There was no

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correlation between sIgE to Pru p 3 and anaphylaxis severity. The sIgE to Pru p 3 and Pru p 4 were 19% and 7%, respectively, with no significant differences between the groups. This result provided a very low sensitivity, but a good specificity (93%). For Pru p 1 and Pru p 7, we did not observe any positive result (**Supplementary Table S5**).

Comparison of anaphylaxis vs. OAS

When comparing anaphylaxis vs. OAS, no differences were observed in age or sex (**Table 3**). Eighty percent of cases with anaphylaxis had a positive SPT to peach, compared with only 9.5% of cases with OAS ($P < 0.001$). Sensitization to pollen tended to be higher in anaphylaxis cases, except for *Cupressus arizonica*, but these differences were not significant. No differences were observed for conjunctivitis, rhinitis, or asthma. Sensitization to plant food allergens was also more likely in anaphylaxis cases, with significant differences for apple (25% vs. 4%, $P < 0.05$) and peanut (40% vs. 14%, $P < 0.05$) (**Table 3**). In consonance with these observations, 50% of anaphylaxis cases vs. 24% of OAS were sensitized to plant food allergens ($P = 0.03$).

Table 3. Comparison of cases of Anaphylaxis vs. OAS to peach

Variables	Anaphylaxis (n = 20)	Oral allergy syndrome (n = 21)	*P* value
Age (yr)	38 ± 12	33 ± 12	NS
Sex (female/total)	48	52	NS
SPT + to peach peel	80	9.5	**< 0.001**
Positive SPT to pollen			
Phleum pratense	55	33	NS
Cupressus arizonica	15	24	NS
Olea europaea	70	62	NS
Platanus acerifolia	35	19	NS
Artemisia vulgaris	40	19	NS
Prunus persica	70	52	NS
Parietaria judaica	35	24	NS
Salsola kali	35	33	NS
NP	90	76	NS
Clinical entities			
Conjunctivitis	85	66	NS
Rhinitis	90	81	NS
Asthma	45	33	NS
Positive SPT to plant foods			
Apple	25	4	**< 0.05**
Banana	5	0	-
Peanut	40	14	**< 0.05**
Almond	10	14	NS
Walnut	20	5	NS
Tomato	25	9	NS
Kiwi	5	9	NS
Melon	15	14	NS
Pineapple	0	0	NS
N+	50	24	**0.03**
Positive serum specific IgE to peach components			
Pru p 3	85	38	**< 0.001**
Pru p 4	20	19	NS
Pru p 1	0	0	NS
Pru p 7	0	0	NS

Data are shown as median ± standard deviation or number (%).
NS, not significant; SPT, skin prick test; NP, percentage of cases with positive SPT to pollen; N+, percentage of cases with positive SPT to plant foods; IgE, immunoglobulin E. Bold numbers: statistically significant.

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Differences were observed in sensitivity in Pru p 3 sIgE, which were found in 85% of cases with anaphylaxis vs. 38% with OAS ($P < 0.001$). Pru p 4 sIgE was detected equally in both subgroups (20% vs. 19%, respectively) (**Table 3**).

Logistic regression analysis showed a highly significant association between a positive SPT to peach peel and anaphylaxis: odds ratio (OR), 113 (CI, 20–613; $P < 0.0001$). The predictive value of Pru p 3 sIgE antibodies for this outcome was substantially lower (OR, 22; CI, 5.3–93; $P < 0.0001$), as was sensitization to other plant food allergens (OR, 5; CI, 1–19; $P < 0.05$). The OR of other variables included in the analysis and described in **Supplementary Data S1** or **S2** did not show statistical significance.

Sensitivity and negative predictive value of peach peel SPT and Pru p 3 sIgE for anaphylaxis were similar (80% vs. 85% and 93.7% vs. 94.5%, respectively) whereas specificity and positive predictive value were higher for peach peel SPT (95.2% vs. 82.5% and 84.2% vs. 60.7%, respectively) (**Supplementary Tables S6**).

DISCUSSION

It is well known that allergy to fruits, including peach, is variable in different world regions and that the context in which sensitization develops is related to the intake of other fruit and plant food allergens as well as pollen inhalation.[3,4,14]

We tested the classic pollen and plant food allergens prevalent in the population. We included peach peel extract, as a source of Pru p 3 at 20 μg/mL, the optimal concentration for SPT. For the other allergens like Pru p 1 and Pru p 4, SPT with peach peel extract has proven inadequate due to their thermolability.[3,17,19] Peach peel extract has also been shown to contain peamaclein, also known as Pru p 7,[19] although we did not estimate the presence in our extract.

For verifying the contribution of the peach allergens, we used commercially available assay for Pru p 1, Pru p 3, Pru p 4, and Pru p 7. Although Pru p 3 is a major inducer of peach allergy, the precise extent of its contribution depends on the areas where the studies are taken. Initially reported in Mediterranean countries,[2,3] the leading role has also been reported in Central Europe[4] and elsewhere.[5,6,11] Regarding Pru p 9, a recently recognized allergen,[12,13] it is not present in fruits and therefore does not contribute to sensitization from peach intake.[12]

Cases were selected based on repeated episodes of allergy to peach fruit. In our study, 44% of cases were SPT positive to peach peel, but when we stratified participants in 2 groups according to clinical entity, the sensitivity was 80% in anaphylaxis and 9.5% in OAS. Thus, SPT sensitivity depends on the clinical manifestations. The fact that this value is under 100% in most studies suggests that other peach allergens may play a role in sensitization.[18,19]

We observed significant differences in the sensitization to *O. europaea*, *A. vulgaris*, *P. persica*, and *P. acerifolia* pollens in cases vs. controls. The high association observed for *P. persica* and *O. europaea* could be explained by the presence of a glucan endo-1,3-beta-glucosidase-like protein in peach tree pollen also present in *O. europaea* pollen (Ole e 4) and a *P. persica* pollen polygalacturonase, related to Ole e 14.[12] The high response to *P. acerifolia* and *A. vulgaris* can be explained by Pru p 3 cross-reactivity with Pla a 3, and Art v 3.[20,21]

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As for skin sensitization to different plant food allergens in our cases, a significant difference was observed with apple and peanut, 2 fruits with equivalent lipid transfer proteins (LTPs; Mal d 3 and Ara h 9).[22,23] Other sources of LTPs are almond and tomato.[24] Sensitization to melon could be explained by the presence of profilin, also existing in peach fruit.[12,25]

Our study group included a similar number of cases with anaphylaxis and OAS. The high proportion of subjects sensitized to peach in the anaphylaxis subgroup contrasted with the low number in the OAS subgroup. A plausible reason may be that labile allergens were not represented in the allergenic extract, possibly leading to an underrepresentation of cases with OAS, where LTP values are lower than with anaphylaxis.

Although it has been reported that pollen allergy is associated with OAS,[14,17] in our study the number of positive cases to pollens was higher than that reported in the control group, but similar between the anaphylaxis and OAS subgroups. As for sensitization to plant food allergens, the anaphylaxis subgroup showed a much higher sensitization than the OAS subgroup to apple and peanut. This general pattern held for sensitization to other plant food allergens, but these data were difficult to interpret due to the underrepresentation of thermolabile allergens in fruit allergen extracts.[18,19]

Pastorello *et al.*[10] reported 65.8% positivity to Pru p 1 in OAS vs. 31.9% in anaphylaxis, and 40.8% positivity to Pru p 4 in OAS vs. 18.1% in anaphylaxis. In northern Italy, another group reported sensitization to Pru p 1 of 42.8% and to Pru p 4 of 12.7%, while in southern Italy no positives cases were found.[26] In contrast to other studies carried out in Spain, we did not find serum specific IgE antibodies to PR-10 allergens quantified with Pru p 1.[27] In line with other reports, data in our study group showed that 19% of cases were sensitized to Pru p 4 with no differences between anaphylaxis and OAS subgroups.[26,27] Pru p 7 may have a role, particularly in cases of peach anaphylaxis that are negative to Pru p 3.[28] However, in our study no association was found with cases positive to cypress pollen.[29]

Although our cases were not challenged with peach fruit, the consistent and repeated history of allergic reactions and positive skin tests strongly supported the diagnosis of peach allergy. We did not include cases with urticaria or other clinical entities because of the very low prevalence in the population evaluated. Another issue worth considering was the wide confident interval of the OR for peach peel SPT that weakened the conclusion of our study. This may have been attributed to the sample size and distribution of subjects.

Our data provided evidence for the role of skin testing with peach peel extract and commercial assays with available molecular components of peach fruit for evaluating people sensitized and with allergy to peach fruit. Results indicated that a positive skin test for peach peel extract was the strongest predictor of anaphylaxis, yielding an OR 5 times higher than sIgE antibodies to Pru p 3 and 22 times greater than a positive skin test to plant food allergens. Ongoing studies are investigating the role of other peach fruit allergens, particularly those involved in OAS.

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SUPPLEMENTARY MATERIALS

Supplementary Data S1
The allergens tested

Click here to view

Supplementary Data S2
Variables

Click here to view

Supplementary Table S1
Skin prick test results to inhalant allergens and concurrent allergic diseases in the control group

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Supplementary Table S2
Skin prick tests to plant foods in the control group

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Supplementary Table S3
Skin prick test results to inhalant allergens and concurrent allergic diseases in cases

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Supplementary Table S4
Skin prick tests to other plant foods in cases of peach allergy

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Supplementary Table S5
Serum specific immunoglobulin E (kU_A/L) to peach components in cases

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Supplementary Table S6
Sensitivity, specificity as well as positive and negative predictive values for peach peel skin prick test and serum specific IgE to Pru p 3 for anaphylaxis

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