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Fennel Allergy Is a Lipid-Transfer Protein (LTP)-Related Food Hypersensitivity Associated with Peach Allergy

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ABSTRACT: Fennel allergy has been rarely reported, and the association with peach allergy has never been described. Our aim was to (i) study the correlation between symptom severity of peach and fennel and (ii) identify fennel allergens and the role of rPru p 3 antibodies in severe reactions to fennel. In 148 patients with peach allergy, we investigated 58 patients with symptoms and IgE antibodies positive to fennel. IgE to rPru p 1, 3, and 4 and rBet v 1, 2, and 4 were measured by immunoblotting, and the N-terminal amino acid sequences and relevant allergens were determined. We found significant association between severe reactions to fennel and peach ($p = 0.0009$). A major allergen was ∼9 kDa lipid-transfer protein (LTP), cross-reactive with Pru p 3, a 15 kDa protein identified as a pathogenesis-related protein 1 of the Bet v 1 family. In conclusion, peach and fennel severe allergic symptoms are significantly related, and LTP is a major fennel allergen. Fennel should be included in the LTP syndrome.

KEYWORDS: *fennel allergy, Foeniculum vulgare, lipid-transfer protein, peach allergy, Pru p 3*

INTRODUCTION

Fennel (*Foeniculum vulgare*) is a member of the Apiaceae (formerly called Umbrelliferae) family, a large group of plants encompassing approximately 300 genera and more than 3000 species. These species include some important allergenic plants, such as carrot and celery. Fennel is native to southern Europe, where it has been used for centuries as a spice and for medicinal purposes[1,2] and more recently consumed as a fresh vegetable. In contrast, in northern Europe, fennel seeds are consumed in bread and sausages. Perhaps because of its infrequent consumption, fennel allergy has been rarely studied. The few publications that have addressed fennel allergy have focused on the relationship to birch and mugwort pollen allergy in the so-called birch−weed or fruit−spice syndrome.[3−5] In fact, fennel allergens are not included in the International Union of Immunological Societies (IUIS) database. The only previous paper regarding fennel allergy is that by Jensen-Jarolim et al.,[6] who studied six fennel-allergic patients. These patients were suffering from mugwort and birch hay fever, and IgE from these patients reacted with 14 and 17 kDa allergens likely to be birch-related and with 50−70 kDa mugwort-related allergens, thus demonstrating the immunologic basis of the clinical association between fennel and birch or mugwort pollen allergies. Similarly, in the other Apiaceae plant foods, represented by celery and carrot, the major allergens Api g 1 and Dau c 1 are Bet v 1 homologues and Api g 4 and Dau c 4 are Bet v 2 homologues.[7] We recently observed an unexpectedly high rate of fennel allergy in a group of severe peach-allergic patients,[8] some of whom also suffered from birch and/or mugwort pollinosis. Fennel-induced symptoms ranged from mild oral allergy syndrome (OAS) to urticaria and anaphylaxis, suggesting a role of allergens unrelated to birch. In our study, peach-allergic patients were subdivided into those with mild symptoms and those with severe symptoms, thus defining a relationship between peach symptom severity and sensitization to rPru p 3 allergen. Because we detected a high number of individuals with fennel allergic symptoms within the group of peach-allergic patients, we aimed to investigate the clinical relationship between peach and fennel symptom severity, the fennel allergens involved, and the correlation between fennel symptom severity and positive responses to one or more fennel allergens.

MATERIALS AND METHODS

Chemicals. All reagents used for protein buffer extraction were from Carlo Erba (Milan, Italy), and those reagents used for sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) were from Bio-Rad Laboratories (Richmond, CA). For blotting, we used a nitrocellulose membrane by Amersham Biosciences (GE Healthcare, U.K.), and ^{125}I-labeled anti-human IgE antibodies were provided by Radim (Rome, Italy).

Study Population. A total of 148 peach-allergic patients in whom peach, birch, and specific recombinant (rPru p 1, 3, and 4 and rBet v 1, 2, and 4) IgE levels were measured[8] were also evaluated for hypersensitivity to other allergenic plant foods. The patients with a history of allergic reactions to fennel and a prick−prick positivity for fresh fennel[9] were recruited for further evaluations. Patients with a

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Table 1. Demographic Data, Symptoms (Classified According to Oral Allergy Syndrome (OAS) Grade[12]) to Fennel and Peach, and Specific IgE Levels to Fennel, Peach, rPru p 1, 3, and 4, and rBet v 1, 2, and 4 by ImmunoCAP

patients			peach		fennel		recombinant allergen IgE values (kU_A/L)					
no.	sex	age	OAS	CAP (kU_A/L)	OAS	CAP (kU_A/L)	Pru p 1	Pru p 3	Pru p 4	Bet v 1	Bet v 2	Bet v 4
1	female	31	I	44.50	I	18.80	74.40	0.21	12.10	100.00	14.60	40.70
2	female	39	I	28.80	I	8.08	56.00	3.36	10.80	100.00	8.45	0.00
3	male	24	I	20.00	I	5.94	0.00	24.40	4.16	0.00	4.48	0.00
4	female	40	I	8.32	III	3.41	0.00	5.86	0.00	0.00	0.00	0.00
5	female	34	I	7.03	I	2.49	1.18	7.37	0.00	3.56	0.00	0.00
6	male	45	I	5.32	III	2.43	25.60	0.00	0.00	42.00	0.00	0.00
7	female	38	I	5.25	I	1.66	0.00	5.30	0.00	0.00	0.00	0.00
8	female	25	I	0.14	I	1.59	10.80	0.14	4.28	35.30	4.23	0.00
9	female	70	I	11.80	I	0.80	16.00	0.00	0.16	23.20	0.00	0.00
10	female	42	I	0.96	I	0.79	0.00	0.00	0.85	0.00	1.21	0.00
11	female	39	I	5.35	I	0.73	15.90	0.00	0.00	22.80	0.00	0.00
12	male	23	I	53.50	I	22.00	26.30	51.80	11.00	30.30	13.30	0.00
13	female	38	III	91.50	IV	59.10	55.20	100.00	32.10	100.00	54.20	96.90
14	male	39	III	3.20	IV	0.46	0.00	4.76	0.00	0.00	0.00	0.00
15	male	56	III	7.45	I	5.63	12.90	0.12	10.60	80.60	14.00	0.00
16	female	34	II	7.79	I	5.37	0.00	10.70	0.00	0.00	0.00	0.00
17	female	28	II	16.20	I	2.07	0.00	15.60	0.00	0.00	0.00	0.00
18	female	38	III	3.76	III	0.52	3.32	2.39	0.00	8.59	0.00	0.00
19	female	51	III	2.88	I	1.43	8.77	0.39	0.89	20.90	0.60	0.00
20	female	40	III	6.74	III	1.34	0.00	10.20	0.00	0.00	0.00	0.00
21	female	53	II	5.33	II	0.76	16.80	0.00	0.00	24.70	0.00	0.00
22	male	38	IV	2.80	III	0.56	0.00	4.13	0.00	0.00	0.00	0.00
23	female	45	III	13.20	III	1.85	31.00	0.00	0.00	45.00	0.00	0.00
24	male	24	III	53.20	III	17.60	0.00	42.00	0.00	0.00	0.00	0.00
25	female	30	II	49.50	IV	25.30	24.10	36.70	0.00	48.40	0.00	0.00
26	male	27	I	6.44	III	0.75	5.24	0.00	0.69	18.40	0.64	1.54
27	male	28	I	11.70	I	3.14	1.60	13.90	0.00	0.00	0.00	0.00
28	female	46	I	4.50	I	0.33	4.59	0.00	0.25	12.20	0.19	0.00
29	female	27	I	1.58	I	1.26	0.00	0.00	1.77	0.98	2.10	0.00
30	female	27	I	5.32	I	3.17	0.00	5.85	0.00	0.00	0.00	0.00
31	female	66	I	6.08	I	1.42	7.10	0.00	3.72	12.10	4.64	2.47
32	female	31	I	11.30	I	4.11	0.00	0.12	11.80	0.00	15.30	0.00
33	female	44	I	2.26	I	0.00	0.00	2.44	0.00	0.00	0.00	0.00
34	female	42	I	4.17	III	0.82	8.10	0.00	0.00	18.40	0.00	0.00
35	female	38	I	6.50	I	6.99	13.00	0.00	1.81	17.80	2.98	4.27
36	female	48	I	5.86	III	0.00	9.30	0.00	0.00	16.50	0.00	0.00
37	female	48	I	2.99	I	0.00	6.56	0.00	0.00	9.41	0.00	0.00
38	female	55	I	1.58	I	0.00	4.69	0.00	0.00	15.90	0.00	0.00
39	female	31	I	8.01	I	0.00	0.00	10.10	0.00	0.00	0.00	0.00
40	female	49	I	6.00	I	0.00	10.40	0.00	0.00	17.90	0.00	0.00
41	female	44	I	1.72	III	0.13	2.12	0.00	0.00	12.40	0.00	0.00
42	female	26	III	1.29	III	0.34	2.85	0.00	0.47	10.20	0.33	0.00
43	female	35	IV	6.54	IV	0.37	13.10	0.00	0.00	28.20	0.00	0.00
44	female	13	III	2.70	III	0.17	0.00	2.69	0.00	0.00	0.00	0.00
45	female	29	III	3.68	I	10.90	0.00	6.94	14.50	0.00	19.80	12.90
46	female	40	III	1.55	I	0.23	5.66	0.00	0.30	30.40	0.00	0.00
47	female	29	II	5.41	III	1.85	0.00	5.48	0.00	0.36	0.00	0.00
48	male	36	III	43.30	III	3.87	29.90	30.20	0.11	80.40	0.00	0.00
49	female	46	IV	2.75	I	0.00	1.65	1.32	0.00	10.90	0.00	0.00
50	female	29	II	2.36	III	0.00	0.27	2.85	0.00	1.75	0.00	0.00
51	male	60	III	0.62	III	0.00	2.50	0.00	0.00	14.90	0.00	0.00
52	female	27	III	5.05	I	0.00	0.00	6.51	0.00	0.00	0.00	0.00
53	female	56	I	3.07	I	0.62	3.27	0.00	0.00	10.40	0.00	0.00
54	female	59	I	4.09	I	0.57	11.80	0.00	0.00	37.20	0.00	0.43
55	female	40	I	2.68	I	0.41	0.59	2.95	0.50	4.55	0.32	0.00
56	male	30	I	3.05	I	0.38	9.99	1.91	1.25	32.60	1.16	0.00
57	male	63	I	2.78	II	0.19	0.00	1.11	0.00	0.00	0.00	0.00
58	male	21	I	0.64	I	0.14	9.80	0.00	0.00	53.40	0.00	0.00

history of mild OAS were tested using an open food challenge (OFC). For the patients who reported severe reactions, the documentation of the patients was accurately reviewed.[10] Fennel-induced symptoms were classified into four possible grades of severity:[11] grade I, only OAS symptoms (mild OAS); grade II, OAS and urticaria/angioedema; grade III, OAS with gastrointestinal symptoms and asthma; and grade IV, life-threatening symptoms, such as glottis edema, hypotension, and shock (severe symptoms included grades II, III, and IV). The Ethics Committee approved the study (ClinicalTrials.gov, protocol ID NCT00715156). Blood was drawn from all of the enrolled patients, and serum was stored at −20 °C.

***In Vivo* Tests.** *Fennel OFC.* OFC was performed with fresh fennel in a graduated manner at a time when birch pollen levels were low. Patients were instructed to chew a small piece (0.5 g) of fennel, keep it in the oral cavity for 1 min, and then spit it out. Patients in whom no symptoms occurred then chewed and swallowed a second piece. Subsequent doses (also swallowed) were doubled until symptoms appeared or the entire dose of fennel was ingested (80 g). The additional doses were delivered at 15 min intervals. The test was stopped and considered positive when symptoms appeared. Patients with a clear-cut history of anaphylaxis or documented severe systemic reaction to fennel were not challenged to avoid provoking severe reactions. The objective symptoms included urticaria, edema, oral mucosal lesions, asthma, nasal secretion, and sneezing; a complaint of itching of the oral mucosa without any further objective evidence was recorded as a subjective symptom.

***In Vitro* Test.** *Serum-Specific IgE Determination.* The sera of all patients were tested for specific IgE toward fennel, peach, rPru p 1, 3, and 4, and rBet v 1, 2, and 4 by the ImmunoCAP System (Thermo Fisher Scientific, Milan, Italy), according to the instructions of the manufacturer. The results were expressed as kU_A/L. IgE values were considered positive when a value greater than 0.10 kU_A/L was obtained.

Immunodetection of Fennel Allergens. Fresh fennel and peach extracts were prepared according to the Bjorksten method.[12] After centrifugation, the supernatants were dialyzed against phosphate-buffered saline (0.1 M PBS at pH 7.4) for 48 h at 4 °C. The protein contents, determined by the colorimetric Lowry method,[13] for peach and fennel extracts were 5.47 and 6.77 mg/mL, respectively. SDS−PAGE was performed according to Neville and Glossmann.[14] The proteins were separated in a discontinuous gel with a 6% stacking gel and a 7.5−20% separation gel at 6 mA for 16 h in a Bio-Rad Protein II xi vertical electrophoresis cell (Bio-Rad Laboratories, Richmond, CA), as previously reported.[15] After electrophoresis, each fraction was electrotransferred to a nitrocellulose membrane (pore size of 0.45 μm; Amersham Biosciences, GE Healthcare, U.K.) using a Trans-Blot cell (Bio-Rad Laboratories, Richmond, CA) at 0.45 A and 100 V for 4 h at 4 °C. After washing and blocking with PBS (pH 7.4 ± 0.2) and 0.5% Tween 20, the nitrocellulose membrane was cut into strips and incubated with the sera of each patient and control subject diluted 1:5 in PBS (pH 7.4 ± 0.2) and 0.1% Tween 20.[16] In particular, sera from the first 25 enrolled patients were used to perform IgE immunoblotting (in Table 1, corresponding to patients 1−25). The specific IgE-binding proteins were detected by incubation with ^{125}I-labeled anti-human IgE antibodies (Radim, Rome, Italy) diluted 1:2 in the same buffer used for diluted sera and exposure to autoradiographic film (Hyperfilm, Amersham) at −70 °C for 4−7 days.

Protein Identification. Bands corresponding to the IgE binding fennel proteins were excised from SDS−PAGE gel, passively eluted, and microsequenced on a Procise 492 protein sequencer (Applied Biosystems, Foster City, CA) as described by Pessione et al.[17] The amino acid sequences were searched using the BLASTP software (http://www.expasy.ch/tools/blast) against both Uniprot KB and NCBInr.2011.01.09 databases.

Band 2 from Figure 2 was also characterized by liquid chromatography−tandem mass spectrometry (LC−MS/MS). For LC−MS/MS, the selected band was excised from the SDS−PAGE gel, destained, reduced, alkylated, and digested with trypsin as described elsewhere.[18] The peptide mixture was analyzed by means of LC−MS/MS using Agilent Technologies 1100 series nano-high-performance liquid chromatography (HPLC), coupled to an Agilent XCT Plus ion trap fitted with a nano-electrospray nebulizer. The chromatographic separations were run on a 150 × 0.075 mm C18 nanocolumn Zorbax 300SB (Agilent) using a linear 5−70% gradient of 0.1% formic acid in acetonitrile within 45 min, with a flow of 0.3 μL/min. The injection volume was 1 μL. The MS parameters were a capillary voltage of 1600 V and a fragmentation voltage of 1.3 V. The data analysis for LC/MSD Trap version 5.2 software (Agilent) was used to elaborate the LC and MS/MS data. The BachTag Web engine at Protein Prospector (http://prospector.ucsf.edu/prospector/mshome.htm) was used to identify the protein against the NCBInr.2011.01.09 database and selecting the Apiaceae family. The parameters used for the search were *S*-carbamidomethyl derivate on cysteine at a fixed modification, oxidation on methionine at a variable modification, and two missed cleavage sites for trypsin digestion. The peptide mass tolerance was set to 0.6 Da, and the fragment mass tolerance was set to 0.8 Da. The homology search was allowed as a "single base change". Protein hits were validated if the protein scores were above the MS batch default and the best expected value ($p < 0.05$) and considering at least two unique peptide sequences.

Immunoblotting Inhibition with Peach Extract. An immunoblotting inhibition experiment was performed to evaluate the cross-reactivity between fennel and peach extract (peach extract undiluted, 1:2 or 1:4) using a pool serum from patients 2, 5, 14, and 15 (Table 1 and Figure 2) selected on the basis of their fennel allergenic pattern. The pool serum was pre-incubated for 1 h on a shaker with the peach extract,[19] and then IgE immunoblotting was performed as described above.

Statistical Analysis. After validation, conventional descriptive analysis was performed on all of the data. Comparisons between categorical variables were carried out by Pearson's χ^2 or Fisher's exact test; comparisons between continuous variables were performed using the Mann−Whitney U test (for between patient analysis) or the Wilcoxon signed-rank test (for within subject analysis).

The association of symptom severity between peach and fennel allergy was evaluated using the Mantel−Haenszel odds ratio; moreover, the general linear model with logit link function was used to evaluate the association of symptom severity (treated as a binary variable) with IgE levels. Correlations among continuous variables were analyzed by Spearman's rank test. The Bonferroni method was used to adjust the level of significance for multiple comparisons.

RESULTS

Patients. A total of 58 patients of the 148 (39.2%) peach-allergic subjects were selected for the present study. These subjects included 44 females and 14 males, with a median age of 38 years (range of 13−70); no differences in age ($p = 0.4523$) or OAS severity ($p > 0.9999$) were found with respect to gender. OAS severity was also not affected by the age of the patients ($p = 0.2891$). The demographic data, the symptoms to fennel and peach, and serum-specific IgE levels to fennel, peach, rPru p1, 3, and 4, and rBet v 1, 2, and 4 are provided in Table 1.

Fennel OFC. Fennel allergy was confirmed by OFC with fresh fennel in 16 of 58 patients. A total of 17 patients with a history of fennel-induced OAS refused to undergo the challenge because of concerns regarding previous symptoms. The other 25 patients were not challenged because of a documented history of severe reactions to fennel. It is interesting to observe that none of the 16 challenged patients was able to proceed beyond 7.5 g of fennel cumulative dose without provoking symptoms. In one patient (number 12), the oral mucosa became severely oedematous at such a rapid rate that epinephrine had to be administered. In three cases, severe challenge symptoms were observed, one case of intense rhinorrea and dyspnoea, one case of erythema, and one case

of wheals and vesicles in the oral mucosa. The symptoms appeared within 3 min at doses ranging from 0.5 to 4 g.

Relationship between Peach and Fennel Symptom Severity. As seen from Table 2, the majority of patients with mild peach OAS also demonstrated mild symptoms to fennel (27/34). Only 7 of 34 patients with mild OAS to peach demonstrated severe symptoms to fennel. In contrast, the majority of patients (16/24) with severe reactions to peach demonstrated severe symptoms to fennel. On the basis of these data, we found a significant correlation between the severity of the allergic reaction to peach and to fennel ($p = 0.0009$).

Table 2. Distribution of Patients with Mild and Severe Symptoms to Peach in Patients Allergic to Fennel[a]

groups	patients with mild symptoms to fennel	patients with severe symptoms to fennel	total
patients with mild symptoms to peach	27 (79.4%)	7 (20.6%)	34
patients with severe symptoms to peach	8 (33.3%)	16 (66.7%)	24
total	35 (60.3%)	23 (39.6%)	58

[a]There is a significant correlation between peach and fennel symptom severity ($p = 0.0009$).

Relationship between Anti-peach- and Anti-fennel-Specific IgE Antibodies. A significant correlation was found between anti-fennel and anti-peach IgE levels ($p < 0.000\,05$); however, anti-fennel IgE levels were lower than anti-peach IgE titers in 55/58 cases (94.8%; $p < 0.000\,05$). Furthermore, significant correlations were also found between fennel IgE levels and rPru p 3 ($p = 0.0002$), Pru p 4 ($p = 0.0002$), rBet v 2 ($p = 0.0002$), and rBet v 4 ($p = 0.0140$) IgE values (Table 3).

No significant correlations were found between anti-fennel and anti-rPru p 1 IgE levels or between the anti-fennel and anti-rBet v 1 IgE levels (Spearman's rank). Moreover, no significant correlations were detected between anti-rPru p 3 IgE levels and the severity of fennel-induced symptoms ($p = 0.221$) or between anti-rBet v 1 IgE levels and the severity of fennel reactions ($p = 0.894$). The severity of fennel symptoms was also independent from IgE levels to rPru p 1 ($p = 0.796$), rPru p 4 ($p = 0.444$), rBet v 2 ($p = 0.761$), and rBet v 4 ($p = 0.512$).

Fennel IgE Immunoblotting and Identification of Fennel Allergens. In the SDS−PAGE analysis of the fennel extract (Figure 1), there are numerous components with apparent molecular weight ranging from approximately 9 to 100 kDa. As seen from the IgE immunoblotting (Figure 2) of fennel extract, incubated with the sera of 25 patients, we found that the sera of 15/25 (60%) patients reacted toward an approximately 9 kDa band, the sera of 11/25 (44%) patients recognized a protein of approximately 15 kDa, and the sera of 24/25 (96%) patients reacted with bands in the range of 65−75 kDa.

Immunoblotting Inhibition. Pooled sera pre-incubation with peach extract at different dilutions completely inhibited IgE binding to fennel proteins (Figure 3). These results demonstrate a high cross-reactivity between the two food items.

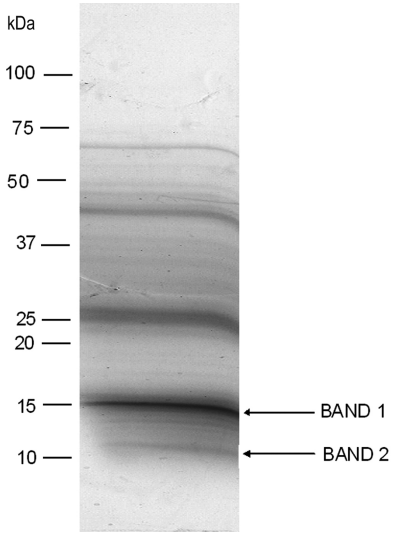

Figure 1. SDS−PAGE of fennel extract and protein bands that underwent N-terminal amino acid sequencing. Band 2 was further characterized by LC−MS/MS.

Amino Acid Sequences and Allergen Identification. The N-terminal amino acid sequences and the internal tryptic peptide amino acid sequences of the protein bands that we identified as fennel allergens through the SDS−PAGE and IgE immunoblotting experiments are provided in Table 4, with (1) band 1 corresponding to a pathogenesis-related protein 1 (PRP1) and showing a N-terminal amino acid sequence with 100% homology with the PRP1 from parsley (*Petroselinum crispum*) and (2) band 2, combining both the N-terminal amino acid sequencing and the LC−MS/MS analyses, showing a 89% homology with the celery non-specific lipid-transfer protein (nsLTP) (Q40795). The protein sequence data of the newly identified fennel LTP appears in the UniProt Knowledgebase under accession number B3EWP9.

In Table 5, you can see the percentage of identity and homology between fennel-identified allergens and their homologous proteins in peach, birch, and soy. In particular, we report the sequence alignments of some peach and fennel peptides identified by LC−MS/MS.

DISCUSSION

In this study, we found that a large number (58/148) of peach-allergic patients recruited in a previous study[8] were also allergic to fennel. We observed that many of these fennel-allergic patients (23/58; 39.65%) had either a history of documented fennel-induced systemic reactions or experienced severe OAS as the result of an OFC (4 of 16 challenged patients). The amount of fennel required to elicit OAS symptoms was very low (4 g maximum dose, 7.5 g cumulative dose), but at least in one case, the historically reported mild symptoms became

Table 3. Correlations between Fennel-Specific IgE Values and Peach and Birch Single Recombinant Allergens with Spearman's Rank

IgE values	rPru p 1	rPru p 3	rPru p 4	rBet v 1	rBet v 2	rBet v 4
fennel	0.1897 $p = 0.1539$	0.4766 $p = 0.0002$	0.4738 $p = 0.0002$	0.1431 $p = 0.2839$	0.4725 $p = 0.0002$	0.3212 $p = 0.0140$

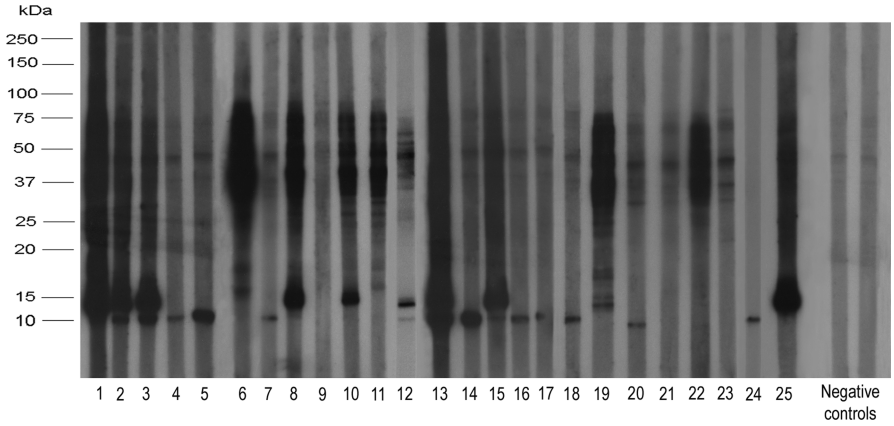

Figure 2. IgE immunoblotting of fennel extract using sera from 25 fennel-allergic patients and 2 negative controls.

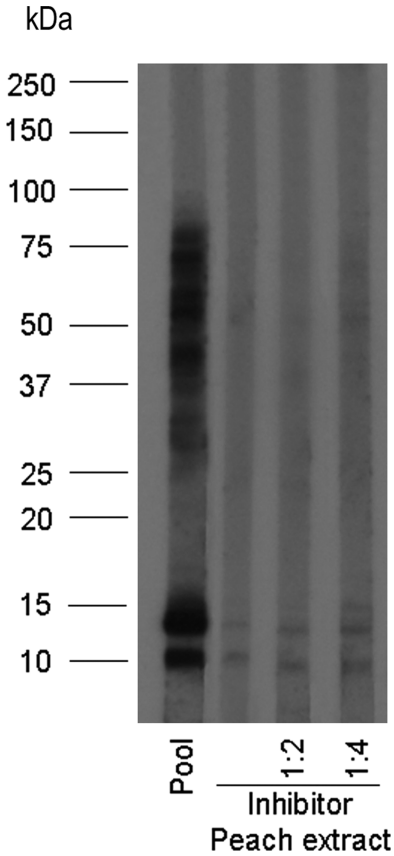

Figure 3. IgE Immunoblotting inhibition of fennel extract using pooled sera from patients 2, 5, 14, and 15 by peach extract at different concentrations.

Table 5. Main Allergens Identified in Fennel Extract and Their Homologous Proteins[a]

comparison between N-terminal amino acid sequences	sequence alignments	identity (%)	homology (%)
LTP fennel *Pru p 3 peach*	^{1}AITXGQVTSKLG^{12} *-ITCGQVSSALAP* ^{11}LGGCLGYLK^{19} *LAPCIPYVR* ^{20}GGGDPTPACCGGVK^{33} *GGGAVPPACCNGIR* ^{61}GINYGAASALPGK^{73} *GVNPNNAAALPGK* ^{74}CGISIPYPISPSTNCSR^{90} *CGVHIPYKISASTNCATVK*	58	74
PRP1 fennel *Pru p 1 peach*	GVQKSEVVITSA *GVFTYESEFTSE*	33	50
PRP1 fennel *Bet v 1 birch*	GVQKSEVVITSA *GVFNYETETTSV*	42	50
PRP1 fennel *Gly m 4 soy*	GVQKSEVVITSA *GVFTFEDEINSP*	42	42

[a]LTP, lipid-transfer protein; PRP1, pathogenesis-related protein 1.

sufficiently severe upon challenge to require epinephrine administration.

Our major result was the highly significant statistical correlation between the severity of symptoms to fennel and peach ($p = 0.0009$). This is particularly relevant in light of the role played by peach allergy in Mediterranean populations, in which severe peach allergy is common. Thus, in addition to the intrinsic risks of severe symptoms in response to peach ingestion, peach allergy should also be considered a risk factor

Table 4. Allergens Identified by N-Terminal Amino Acid Sequence and LC−MS/MS Techniques and Number and Percentage of Reacting Patients[a]

SDS−PAGE band no.	MW (kDa)	N-terminal amino acid sequences	LC−MS/MS	sequence coverage (%)	reacting patient no.	protein identification
1	15−17	^{1}GVQKSEVVITSA^{12}		13	11 (44%)	100% homology with parsley (*P. crispum*) PRP1 (Q40795)
2	9	^{1}AITXGQVTSKLG^{12}	^{11}LGGCLGYLK^{19} ^{20}GGG**D**PTPACCGGVK^{33} ^{61}GINYGAASALPGK^{73} ^{74}CGI**S**IPYPISPST**N**CSR^{90}	68	15 (60%)	89% homology with celery (*Apium graveolens*) nsLTP1 precursor (E6Y8S8)

[a]PRP1, pathogenesis-related protein 1; LTP, lipid-transfer protein. In bold font, amino acids differing from celery LTP.

for severe allergic reactions to other plant foods, such as fennel. The high association between fennel and peach allergy is also demonstrated by the high correlation between fennel- and peach-specific IgE levels ($p < 0.000\,05$). Furthermore, the higher levels of peach than fennel IgE levels likely reflect these patients primarily presented with OAS to peach. These *in vivo* and *in vitro* results suggest that fennel allergy should always be investigated in peach-allergic patients.

We also investigated whether the severity of fennel-induced symptoms could be related to the types of allergens involved. As shown in Figure 1, the more relevant fennel allergens that we identified included a LTP of 9 kDa and a PRP1 of 15 kDa belonging to the Bet v 1 family with a high homology to the parsley antigen. Because fennel LTP and Bet v 1 homologues were not available for quantitative IgE antibody detection, we measured IgE levels to rPru p 3 and rPru p 1 as substitutes, to evaluate the number of fennel-allergic patients who had positive responses to these allergens. Among the 58 investigated patients, we found positive IgE responses to rPru p 3 and rPru p 1 in 34 and 38 patients, respectively. Furthermore, 22 of the 58 patients exhibited positive rPru p 4 (profilin) IgE levels. The high rate of positivity to the Bet v 1 homologues and profilin were not surprising because these allergens have already been described as relevant Apiaceae allergens[20] and demonstrated in fennel.[6] However, the observation that LTP is a major fennel allergen is a new and unique finding in our study. Specifically, we found highly statistically significant correlations between fennel IgE levels and rPru p 3 ($p = 0.0002$), rPru p 4 ($p = 0.0002$), and rBet v 2 IgE ($p = 0.0002$) values. These correlations indicate that, in our study population, LTP and profilin are the most relevant proteins that determine the sensitization to fennel. We were surprised not to find a significant correlation between fennel IgE levels and rPru p 1 ($p = 0.1539$) or rBet v 1 ($p = 0.2839$) IgE values, because Bet v 1 homologous allergens would be expected to play a crucial role in this Apiaceae family member. The amino acid homology analysis of the peach and fennel proteins may provide an explanation. As shown in Table 5, Bet v 1 and fennel PRP1 are only 50% homologous and 42% identical. The low similarity may explain why fennel sensitization does not seem to be related to Bet v 1 nor Pru p 1 sensitization. On the contrary, fennel and peach LTP are 74% homologous and 58% identical, which is consistent with the correlation in IgE levels and especially in symptom severity between fennel and peach. In fact, the percentage of identity between LTP of fennel and peach, which belong to different botanical sources, is quite similar to that between peach and apple LTPs (63%), which are known to be highly cross-reactive.

Despite the statistical association between symptom severity to peach and fennel, we did not find a significant association between rPru p 3 IgE values and fennel symptom severity. Because we previously observed a correlation between symptom severity to peach and the positivity to rPru p 3 in the same cohort,[8] we expected to observe a correlation between rPru p 3 IgE levels and fennel symptom severity. We ascribed this apparent discrepancy to either an insufficient number of fennel allergic patients or an incomplete overlap of the rPru p 3 sequence used as a fennel LTP marker. However, other factors could play a role in modifying the symptoms. For example, we found that 10 of the 23 patients with fennel severe symptoms did not demonstrate specific IgE antibodies to fennel LTP but only to the 14−17 kDa Bet v 1 homologue. We can thus infer that sensitization to the fennel 14−17 kDa Bet v 1 homologous allergen[21] could determine severe symptoms, as already observed in birch- and soy-allergic patients sensitized to soy Bet v 1 homologous proteins.

In conclusion, severe allergic reactions to fennel are significantly related to the severity of peach-induced symptoms. Furthermore, we found that a newly identified IgE binding LTP, which is highly cross-reactive with Pru p 3, is a clinically relevant fennel allergen that in the future has to be studied with respect to digestion stability. For these reasons, fennel should be included in the list of foods that cause "LTP syndrome".

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Notes

The authors declare no competing financial interest.

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