ORIGINAL ARTICLE

(R)-Mandelonitrile Lyase, a Homolog of Pru du 10, Is a Major Peach Allergen

López-Matas MA[1]*, Vílchez-Sánchez F[2]*, Álvarez F[1], Rodriguez-Perez R[2], Dominguez-Ortega J[2], Carnés J[1], Pedrosa M[2,3]

[1]R&D Unit, LETI Pharma S.L.U., Tres Cantos, Madrid, Spain
[2]Department of Allergy, Hospital Universitario La Paz, Institute for Health Research (IdiPAZ), Madrid, Spain
[3]Center for Biomedical Research Network on Rare Diseases (C IBERER U754), Madrid, Spain
*Both authors contributed equally

J Investig Allergol Clin Immunol 2025; Vol. 35(2): 122-129
doi: 10.18176/jiaci.0972

Abstract

Background: Peach allergy is a prevalent cause of food allergy. Despite the repertoire of allergens available for molecular diagnosis, IgE to individual peach allergens may still be undetectable in some patients who present symptoms after ingestion. The objective of this study was to investigate the allergen profile of patients with symptoms induced by peach.
Materials and Methods: We performed an exploratory retrospective study of 42 patients presenting symptoms after ingestion of peach. The allergen profile of individual patients was investigated using immunoblot. A serum pool was prepared with sera that recognized a 70-kDa band. The pool was used to detect this protein in peach peel and pulp and to identify the 70-kDa protein in 2D immunoblot. Spots recognized in the 2D immunoblot were sequenced using LC-MS/MS. Inhibition studies were performed with peach peel and almond.
Results: The immunoblot revealed that 22 patients (52.4%) recognized the 70-kDa protein in peach peel and pulp. Two spots were observed in 2D-PAGE, and both were identified as (R)-mandelonitrile lyases (RMLs) showing high amino acid similarity with Pru du 10. The RMLs were partially inhibited with an almond extract. No association was found between symptoms and sensitization to RML. RML-sensitized patients were older and reported pollen-associated respiratory symptoms more frequently than patients whose results were negative for this protein.
Conclusions: We identified a new peach allergen, an RML, which was a homolog of Pru du 10 and was recognized by 52% of the population.

Key words: Peach allergy. Peach allergen. Mandelonitrile lyase. Food allergy. Homolog of Pru du 10.

Resumen

Antecedentes: La alergia a melocotón es una causa frecuente de alergia alimentaria. A pesar de que hay varios alérgenos disponibles para el diagnóstico molecular, existen pacientes con niveles indetectables de IgE a estos alérgenos, pero que presentan síntomas tras la ingesta de melocotón. El objetivo de este estudio fue investigar el perfil alergénico en una población de pacientes con síntomas producidos por el melocotón.
Materiales y Métodos: Se realizó un estudio exploratorio retrospectivo con pacientes que presentaron síntomas tras la ingesta de melocotón. Se incluyeron en el estudio cuarenta y dos pacientes. El perfil alergénico de cada paciente se investigó mediante inmunoblot. Se preparó un *pool* con los sueros que reconocían una banda de 70 kDa. Este *pool* se utilizó para detectar esta proteína en piel y pulpa de melocotón, y para identificar la proteína de 70 kDa en un inmunoblot 2D. Los puntos reconocidos en el blot 2D se secuenciaron mediante LC-MS/MS. Se realizaron estudios de inhibición entre piel de melocotón y almendra.
Resultados: Veintidós pacientes (52,4%) reconocieron la proteína de 70 kDa en el inmunoblot. Esta proteína fue reconocida tanto en piel como en pulpa. Se observaron dos puntos en 2D-PAGE, ambos se identificaron como (R)-mandelonitrilo liasas (RML) con alta similitud de aminoácidos con Pru du 10. Las RML de melocotón se inhibieron parcialmente con un extracto de almendra. No se encontró asociación entre ningún síntoma y la sensibilización a RML. Los pacientes sensibilizados a RML tuvieron mayor edad y reportaron síntomas respiratorios asociados al polen con mayor frecuencia que los pacientes negativos.
Conclusiones: Se ha identificado un nuevo alérgeno del melocotón, una RML, homóloga de Pru du 10, reconocida por el 52% de la población.

Palabras clave: Alergia a melocotón. Alérgeno de melocotón. Mandelonitrilo liasa. Alergia a alimentos. Homólogo de Pru du 10.

Summary box

- **What do we know about this topic?**
  Five allergens have been associated with the symptoms induced by peach ingestion. However, the symptoms are not associated with these allergens in some patients. Therefore, more allergens are involved in peach allergy.
- **How does this study impact our current understanding and/or clinical management of this topic?**
  The identification of a new peach allergen adds to our knowledge of peach allergy, thus improving diagnosis. Also important is the finding that the new allergen cross-reacts with almond, enabling us to make recommendations to patients sensitized to this fruit.

Introduction

The prevalence of food allergy has increased significantly over recent decades, and peach is one of the most important causes in European countries, particularly in the Mediterranean area [1].

Five distinct food allergens have been described in peach, as follows: Pru p 1 (pathogenesis-related protein, PR-10); Pru p 2 (thaumatin-like protein); Pru p 3 (nonspecific lipid transfer protein 1 [nsLTP1]); Pru p 4 (profilin); and Pru p 7 (gibberellin-regulated protein). Two other components have been described as respiratory allergens, namely, Pru p 9 (pathogenesis-related protein PR-1) and Pru p 10 (polygalacturonidase) [2]. The allergen Pru p 3 is the main sensitizer in Mediterranean countries [3,4] and is involved in primary food allergy, producing symptoms ranging from oral allergy syndrome (OAS) to anaphylaxis [5]. Pru p 1, on the other hand, is the main sensitizer in Central Europe and is associated with cross-reactivity to birch pollen [4]. Pru p 2, Pru p 4, and Pru p 7 are further involved in secondary sensitization to peach by cross-reactivity with pollen allergens [6].

A substantial repertoire of allergens is available for molecular diagnosis. However, some patients present a wide range of symptoms after ingestion of peach despite having undetectable serum IgE levels against any previously described allergens [7]. In these patients, specific diagnosis remains elusive, although it is crucial to offer an appropriate recommendation for treatment and to prevent future allergic reactions. It is also relevant to rule out cross-reactivity with other fruits and/or vegetables. The objective of the present study was to investigate the allergen profile of a population with peach allergy. We discovered a new peach allergen recognized by the patients' sera and identified it using mass spectrometry.

Materials and Methods

Patient Population

We performed an exploratory retrospective study from February 2020 to September 2021 at Hospital La Paz (Madrid, Spain). The study population comprised patients of any age who had been diagnosed with peach allergy. Diagnosis was made based on a suggestive clinical history (oral allergy syndrome, urticaria/angioedema, rhinoconjunctivitis/asthma, gastrointestinal symptoms, or anaphylaxis) occurring within 2 hours after peach intake or contact, in addition to positive skin prick test (SPT) and/or sIgE results for peach extract. The study was approved by local ethics committee (PI-4513). Demographic and clinical data were extracted from medical records.

SPT was performed with commercial peach peel and pulp, profilin (Pho d 2, ALK Abello), birch pollen extract (LETI Pharma S.L.U.), and purified Pru p 3 (10,000 DPU/mL, Roxall). A wheal of 3 mm or greater was considered positive. Total IgE and specific IgE to peach extract, Pru p 1, Pru p 3, Pru p 4, and Pru p 7 were determined using ImmunoCAP (Thermo Fisher Scientific).

Extract Manufacturing

Peaches were purchased at a local market and carefully peeled to prepare peel and pulp extracts according to in-house manufacturing procedures (LETI Pharma). In short, peach peel and pulp were homogenized separately and extracted for 4 hours in phosphate-buffered saline (PBS)/polyvinylpolypyrrolidone buffer under continuous magnetic stirring at 4°C. Extracts were then centrifuged for 30 minutes at 15 000*g*, and supernatants were collected, dialyzed, filtered, frozen, and freeze-dried. Protein content and protein profile were analyzed using the Bradford and SDS-PAGE assays, respectively.

Allergen Profile

The allergen profile of individual patients was investigated using immunoblot. Briefly, the proteins contained in 100 µg of lyophilized peach peel extract were separated according to their molecular weight in Invitrogen NuPAGE PreCast 4-12% BisTris gels (Thermo Fisher Scientific) under reducing conditions, electrotransferred onto a PVDF membrane using a Trans-Blot Turbo Transfer Pack (Bio-Rad), and dried at room temperature. Membranes were then incubated overnight with the individual sera diluted 1/2 in PBS-0.1% Tween. After 2 hours of incubation with monoclonal antihuman-IgE-PO (Southern Biotech), the reaction was developed with ECL Prime Western Blotting Detection (Amersham) and visualized using chemiluminescence. A pool of peach-positive plasma sera (Plasmalab International) was used as a positive control. A serum pool was prepared from 22 individual sera that showed a 70-kDa band during immunoblot testing. This pool was used

to determine whether the same protein was present in pulp extract. ImmunoCAP was performed with peach extract and cross-reactive carbohydrate determinants (CCDs) (MUXF3, Thermo Fisher Scientific) to characterize the pool of sera.

2D Electrophoresis and Immunoblot

Peach peel extract was purified and concentrated with ammonium sulfate in 2 different steps until a saturation percentage of 40% and 80% was attained, then maintained at 4°C overnight. The sample was centrifuged at 10 000*g* for 5 minutes at 4°C, and the pellet was collected and reconstituted in ultrapure water. Concentrated extract was cleaned with the ReadyPrep 2-D Cleanup Kit (Bio-Rad), and proteins were separated according to their isoelectric point (pI) on ReadyStrip IPG Strips (Bio-Rad) in a pH range of 3-10 using Protean IEF Cell (Bio-Rad). Two strips were processed simultaneously; after the first-dimension run, they were equilibrated with ReadyPrep 2-D Kit buffers (Bio-Rad). The proteins were then separated in the second dimension according to their molecular weight. After the second dimension, one of the gels was stained with Oriole fluorescent gel stain (Bio-Rad) to study the spot protein profile, and the second gel was used to determine the allergen profile by 2D immunoblot, as described above.

Allergen Identification

Spots recognized in the 2D immunoblot were excised from the gel, digested with trypsin, analyzed using liquid chromatography mass spectrometry/mass spectrometry (LC-MS/MS) in a Q Exactive HF spectrometer (Thermo Fisher Scientific) and identified with Proteome Discoverer software (Thermo Fisher Scientific) in the Proteomic Unit of the Complutense University (Madrid, Spain).

Immunoblot Inhibition

Inhibition studies were performed with peach peel extract and almond extract using the pool of sera. Briefly, 100 µg of lyophilized peach peel extract corresponding to approximately 6 µg of protein was electrophoresed and electrotransferred, as explained above in *Allergen Profile*. Almond extract (30 µg of protein, LETI Pharma) or peach peel extract (30 µg of protein, positive control) was preincubated with the pool of sera (dilution 1/2) for 2 hours. Afterwards, the inhibition mixtures were incubated with the membrane for 2 hours, washed, and developed by chemiluminescence. The percentage of recognition of the 70-kDa band was calculated using densitometry with the software ImageQuant TL 8.1 (Cytiva).

Table 1. Description of the Study Population.[a]

	Total (N=42)	RML-sensitized (n=22)	RML-negative (n=20)	*P* Value
Female sex	22 (52.4)	13 (59.1)	9 (45)	.361[b]
Median (range) age, y	9.59 (0.86-46.91)	11.5 (5.22-46.91)	6.98 (0.86-38.6)	.014[c]
Anaphylaxis	12 (31.6)	9 (42.9)	3 (17.6)	.096[b]
OAS only	27 (71.1)	13 (61.9)	14 (82.4)	.153[b]
Symptoms with other fruits				
Kiwi	9 (21.4)	6 (27.3)	3 (15)	.460[b]
Melon/watermelon	8 (19.0)	8 (36.4)	0 (0)	.004[b]
Banana	3 (7.1)	3 (13.6)	0 (0)	.233[b]
Rosaceae (other)	8 (19.0)	6 (27.3)	2 (10)	.243[b]
Nuts/peanut	13 (31.0)	7 (35)	6 (27.3)	.741[b]
Pollen-related respiratory symptoms	23 (52.3)	17 (77.3)	6 (30)	.003[b]
SPT positive				
Peach peel	34 (81.0)	16 (76.2)	18 (100)[d]	.027[b]
Peach pulp	8 (19.0)	2 (10)	6 (33.3)	.078[b]
LTP	34 (81.0)	16 (76.2)	18 (100)	.027[b]
Profilin	6 (14.3)	4 (19)	2 (11.8)	.540[b]
Birch	11 (26.2)	7 (38.9)	4 (22.2)	.278[b]
Median (range) total IgE, kU/L	97.8 (1.40-4112)	229 (14.8-4112)	47.1 (1.40-403)	.002[c]

Abbreviations: LTP, lipid transfer protein; OAS, oral allergy syndrome; RML, (R)-mandelonitrile lyase; SPT, skin prick test.
[a]Data presented as No. (%) of patients in each group unless stated otherwise.
[b]χ^2 test.
[c]Mann-Whitney test.
[d]This assay was performed in only 18 patients.

Statistical Analysis

Descriptive statistical analyses (median and range) were performed for numerical variables (age and kU/L of total or specific IgE). The χ^2 or Mann-Whitney test was used to compare groups of individuals (reactors and nonreactors to the new allergen).

Results

Patient Population

The study population comprised 42 patients (22 females [52.4%]) with symptoms ranging from OAS to anaphylaxis after ingestion of peach. The median (IQR) age was 9.59 years (5.90-14.50 [min-max, 1-46] years). Table 1 shows the population characteristics. Of note, none of the patients reported symptoms after ingestion of almond.

Allergen Profile of Patient Sera

Recognition profiles varied widely (Figure 1), although 22 of 42 individual serum samples (52.4%) recognized a protein of approximately 70 kDa in the immunoblots (Figure 1A). Only 1 patient (patient 42) was monosensitized to this protein (Figure 1).

The pool of patient sera recognized the band of approximately 70 kDa in peach peel and pulp (Figure 1B). However, the protein recognized in the pulp was slightly smaller than that recognized in the peel.

The pooled sera had an sIgE of 5.4 kU_A/L to peach extract and 0.1 kU_A/L (negative) to the molecular component MUXF3 (marker of sensitization to CCDs).

2D Electrophoresis and Immunoblot

The pool of sera recognized an area corresponding to a pI of 5-6 at ~70 kDa (Figure 2B). This area corresponded

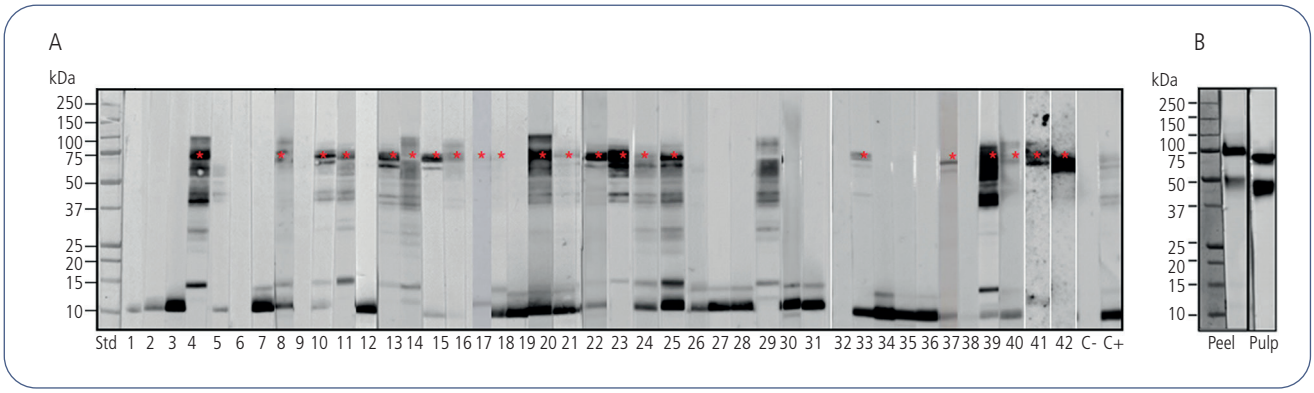

Figure 1. Allergen profile. A, Peach peel with individual sera (1 to 42), a no-serum negative control (C–), and a positive control with a pool of commercial plasma from patients reactive to peach (C+). All sera were diluted 1/2. The bands at 70 kDa are marked with a red asterisk (*). B, Immunoblot of peel and pulp of peach using a pool of sera from the patients with a 70-kDa reactive band in A.

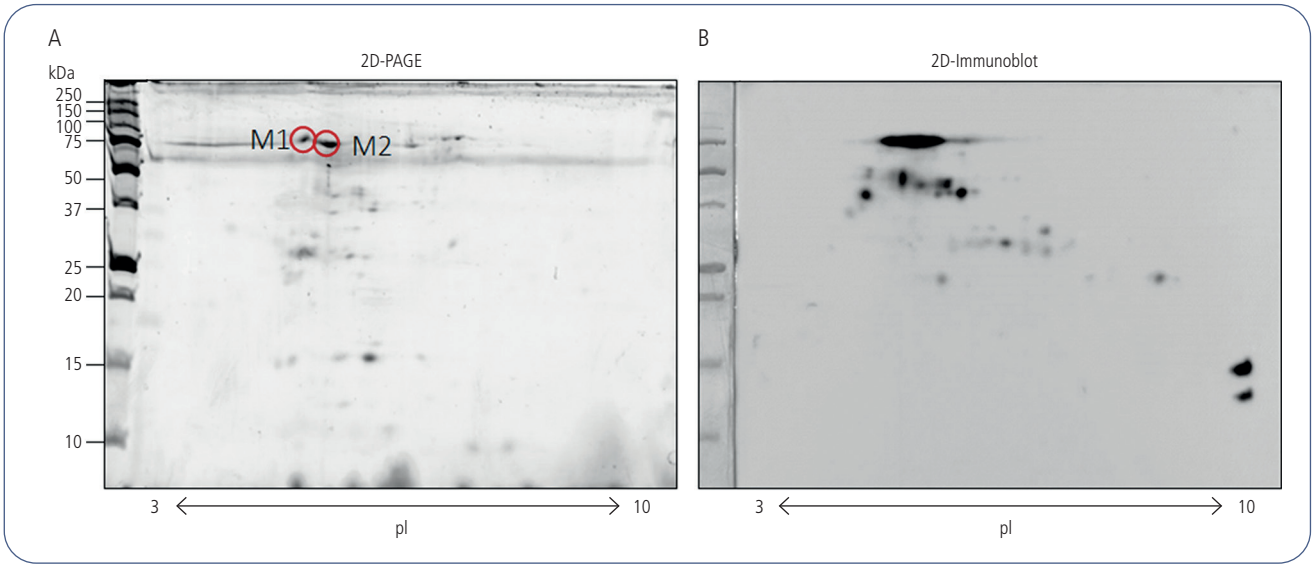

Figure 2. 2D protein and allergen profile of the peach peel extract. A, 2D electrophoresis of peach peel extract; spots identified by LC/MS-MS are marked with a red circle and named M1 and M2. B, 2D immunoblot of peach peel using a pool of sera made up of patients with a 70-kDa band in Figure 1A, diluted 1/2.

to 2 spots (M1 and M2) in the 2D-PAGE (Figure 2A), which were identified as (R)-mandelonitrile lyases (RMLs) by LC-MS/MS analysis. Fifteen peptides from M1 corresponded to the protein A0A251QUN8, with a sequence coverage of 25.9%, and 16 peptides from M2 corresponded to the protein A0A251QUN1, with a sequence coverage of 31.5% (Figure 3 and Table 2). Both are isoforms of the peach RML with amino acid identity to the almond allergen Pru du 10 of 70% and 70.5%, respectively.

Immunoblot Inhibition

Given the high identity of RML with Pru du 10, we performed an immunoblot inhibition assay to study cross-reactivity. The band corresponding to RML detected in the peach peel extract was inhibited by approximately 58% with an almond extract (Figure 4).

(R)-Mandelonitrile Lyase–Sensitized Patients

Of the 22 patients whose serum reacted with the newly identified peach allergen RML, 13 were women (59%), with a median age of 11.5 years (range, 5.2-46.9). Ten were children aged ≤10 years, 7 were adolescents aged between 11 and 18 years, and 5 were adults >18 years.

More than half of the patients reported OAS as the only symptom (13 [61.9%]). Interestingly, 9 patients reported systemic reactions (42.9%), although no association was found

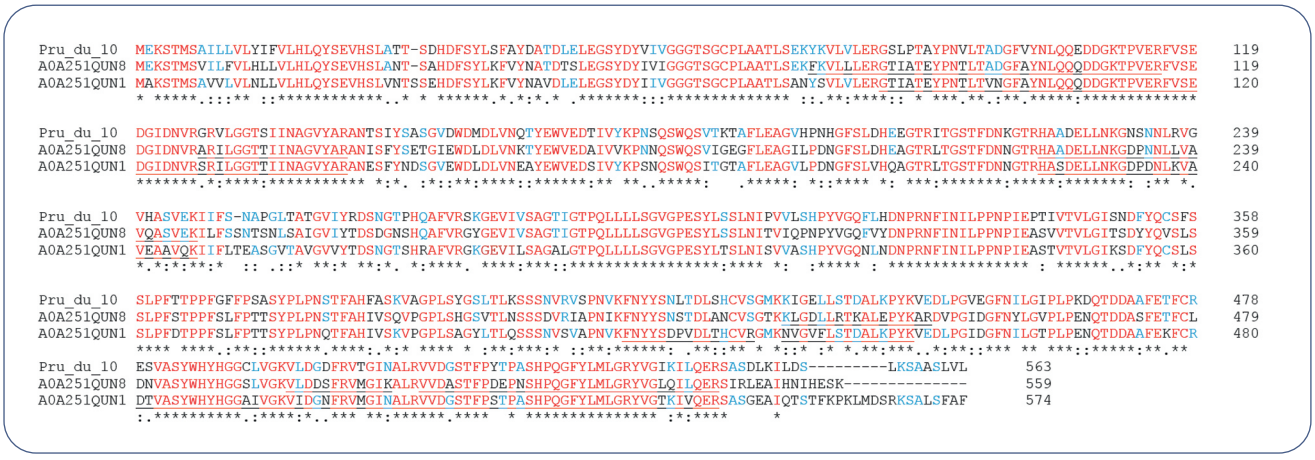

Figure 3. Protein identification by LC/MS-MS. The spot M1 corresponded to the protein A0A251QUN8, and the spot M2 was identified as A0A251QUN1. Both proteins are (R)-mandelonitrile lyases, homologs of Pru du 10. Peptides identified by LC/MS-MS are underlined. Identical amino acids in the 3 proteins are marked in red; amino acids of Pru du 10 that matched one of the identified peach proteins are marked in blue.

Table 2. Peptides Identified by LC/MS-MS in Spots M1 and M2.

Spot M 1: A0A251QUN8	Spot M2: A0A251QUN1
ARILGGTTIINAGVYAR	DTVASYWHYHGGAIVGK
FKVLILER	FNYYSDPVDLTHCVR
GDPNNLLVAVQASVEK	FVSEDGIDNVR
GTIATEYPNTLTADGFAYNLQQQDDGK	GDPDNLKVAVEAAVQK
GTIATEYPNTLTADGFAYNLQQQDDGKTPVER	GMKNVGVFLSTDALKPYK
HAADELLNK	HASDELLNK
HAADELLNKGDPNNLLVAVQASVEK	HASDELLNKGDPDNLK
KLGDLIR	HASDELLNKGDPDNLKVAVEAAVQK
ILGGTTIINAGVYAR	ILGGTTIINAGVYAR
TKALEPYK	NVGVFLSTDALKPYK
TKALEPYKAR	SRILGGTTIINAGVYAR
VLDDSFR	VAVEAAVQK
VLDDSFRVMGIK	VIDGNFRVMGINALR
VVDASTFPDEPNSHPQGFYLMLGR	VMGINALR
YVGLQILQER	VVDGSTFPSTPASHPQGFYLMLGR
	YVGTKIVQER

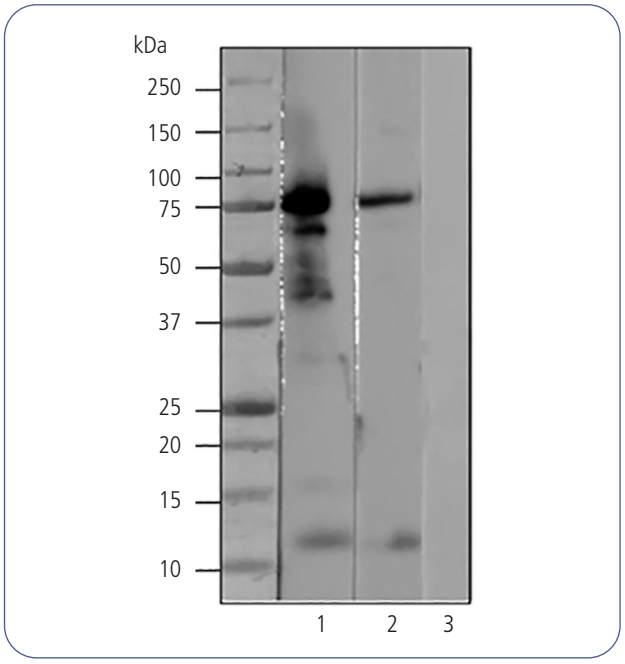

Figure 4. Immunoblot inhibition with almond. Lane 1, Immunoblot with peach peel in solid phase and the pool of RML-sensitized sera diluted 1/2. Lane 2, Immunoblot with peach peel in the solid phase and the pool of RML-sensitized sera (diluted 1/2) inhibited with almond extract; Lane 3, Immunoblot with peel peach in the solid phase and the pool of sera (diluted 1/2) inhibited with peach peel (positive control).

between any specific reported symptom and sensitization to RML (data not shown).

Thirteen patients were sensitized to Pru p 3 (59.1%), 2 to Pru p 1 (9.1%), 4 to Pru p 4 (18.2%), and 3 to Pru p 7 (13.6%) (Table 3). Only 1 patient was monosensitized to RML. Seventeen patients (77.3%) reported respiratory symptoms upon exposure to pollen.

Comparative results between RML-sensitized and -negative patients are summarized in Table 1. Of note, RML-sensitized patients were older (median, 11.5 vs 7.0 years; *P*=.014) and reported pollen-associated respiratory symptoms more frequently (77.3% vs 30%, *P*=.005). RML-sensitized patients reported anaphylaxis more frequently than RML-negative patients. SPT with peach peel and Pru p 3 yielded positive results in all the patients who tested negative for RML. Conversely, SPT with peach pulp, profilin, and birch, although not statistically significant, yielded positive results in a higher percentage of patients in the RML-sensitized group. Accordingly, levels of total and specific IgE for peach and its components were higher in RML-sensitized patients, except for Pru p 3 (Table 3).

Patients whose serum reacted with RML also reported symptoms upon ingestion of Cucurbitaceae fruits more frequently than those who tested negative (36.4% vs 0%, *P*=.04).

Discussion

A deeper knowledge of peach allergens is essential if we are to improve the products used to diagnose allergy and better understand the source of symptoms. Although molecular in vitro diagnostic assays have been developed for 4 peach allergens (Pru p 1, Pru p 3, Pru p 4, and Pru p 7), we found some patients who had symptoms after consuming peaches despite testing negative to all 4 peach allergens. We identified and characterized a new peach allergen, RML, which reacted with serum from 22 of 42 tested patients (52.4%) who developed symptoms after peach ingestion. To the best of our knowledge, this is the first time that this protein has been described in peach. Moreover, the high prevalence of sensitization to RML in the study population indicates that this protein could act as a major allergen with a high incidence in our area. The prevalence of RML was even higher than that of Pru p 1 (4.8%), Pru p 4 (9.5%), and Pru p 7 (9.5%) and was only surpassed by that of Pru p 3 (76.2%).

Mandelonitrile lyases belong to the family of hydroxynitrile lyases involved in cyanogenesis, which is the process plants use to degrade α-cyanohydrins into hydrocyanic acid and the respective aldehyde or ketone. This reaction is catalyzed by hydroxynitrile lyases, and the release of hydrocyanic acid functions as a defense mechanism against herbivores and microbial attack [8,9]. In fact, ingestion of cyanogenic plants can produce acute cyanide poisoning in animals and in

Table 3. Prevalence of Sensitization to Peach Allergens by In Vitro Tests.[a]

	Total (N=42)		RML-sensitized (n=22)		RML negative (n=20)		*P* Value	
	Patients[b]	Median (range) kU_A/L	Patients[b]	Median (range) kU_A/L	Patients[b]	Median (range) kU_A/L	Number (χ^2test)	sIgE (Mann-Whitney test)
Peach	35 (83.3)	2.10 (0-29.3)	19 (86.4)	2.16 (0.09-23.3)	16 (80)	1.67 (0.0-29.3)	.691	.811
Pru p 1	2 (4.8)	0 (0-9.14)	2 (9.1)	0 (0-9.14)	0 (0)	0 (0-0)	.489	.208
Pru p 3	32 (76.2)	1.73 (0-35.3)	15 (85)	1.16 (0.01-23.9)	17 (68.2)	1.96 (0-35.3)	.284	.003
Pru p 4	4 (9.5)	0.01 (0-17.7)	4 (18.2)	0.01 (0-17.7)	0 (0)	0 (0-0.01)	.109	.002
Pru p 7	4 (9.5)	0.02 (0-15)	3 (13.6)	0.05 (0-15)	1 (5.0)	0.01 (0-6.72)	.610	.033

Abbreviation: RML, R-mandelonitrile lyase.
[a]Data presented as No. (%) of patients in each group.
[b]The patient is considered to be sensitized if sIgE >0.34 kU_A/L.

humans [10]. RML is a major seed protein, although we found this protein in both the peel and the pulp of peaches.

An allergen from the RML family has been described in almond (Pru du 10) [11]. The new peach allergen has a similar molecular weight (~70 kDa) and was inhibited with an almond extract, indicating that it is homologous to Pru du 10. Although Pru du 10 is a glycoprotein, the authors demonstrated that IgE bound to protein and not to glycosides [11]. In the case of the new peach allergen, we measured sIgE against MUXF3 (a marker of sensitization to CCDs) in the pool of sera. The result was negative, indicating that the patients recognized the protein and not the glycoside residues.

Patients who reacted to RML were older than those who did not, indicating late sensitization to RML. This finding is corroborated by the presence of only 1 patient who was monosensitized to RML. Our hypothesis is that most of the patients who were previously sensitized to different allergens were subsequently sensitized to RML, although the relatively small number of adult patients included in the study population made this finding difficult to confirm. Further studies with a greater number of adults are needed to replicate these results. The greater incidence of pollen-associated respiratory symptoms in the RML-sensitized patients could indicate secondary sensitization to peach, as described for Pru p 1 in relation to Bet v 1 [4,12,13] and in Pru p 7 with respect to Cupressaceae pollinosis [14-16], although this relationship has not been found in the Mediterranean area [17-18] or for the panallergens Pru p 2 [19] and Pru p 4 [20]. As this study was not intended to cover specific pollen sensitization, not all patients were systematically tested against the same pollen extracts; therefore, no hypothesis can be drawn from the possible relationship between any given pollen and sensitization to RML. Nevertheless, the fact that RML-sensitized patients reported pollen allergy symptoms more frequently and were older than the RML-negative patients suggests the possibility that sensitization to RML develops over time and accompanies pollen sensitization, although no homolog to this allergen in pollens has been identified to date.

No association could be established between any given symptom reported by patients and sensitization to RML. However, RML-sensitized patients reported anaphylaxis more frequently than the RML-negative patients (the difference was nonsignificant). Of note, the RML-monosensitized patient reported anaphylaxis upon peach ingestion. One of the drawbacks of the study is the lack of systematic oral food challenge for all patients. Consequently, we cannot ensure the clinical relevance of this allergen. Nevertheless, our data show it to be a potentially highly relevant allergen. Further studies are needed to confirm its clinical relevance.

Interestingly, patients sensitized to RML reported a significantly greater frequency of symptoms upon ingestion of fruits belonging to the Cucurbitaceae family. To the best of our knowledge, no allergen belonging to the mandelonitrile lyase family has been described in these fruits. Whether this association is truly related to sensitization to RML or not is unknown. Cucurbitaceae allergy is related to sensitization to profilin in pollen-allergic patients [21], and the higher incidence of allergy to these fruits can be attributed to the fact that these patients are pollen-allergic. However, the prevalence of sensitization to profilin in RML-sensitized patients was not higher than that of the RML-negative ones. The prevalence of sensitization to profilin in peach-allergic patients has previously been reported to be around 34% [22]. In our study, however, the frequency of sensitization to profilin was lower (9.5%), in line with previously reported data in peach-allergic children in Spain [23]. These differences may be related to patient age, given that the first study [22] was performed in adults. The frequency of Cucurbitaceae allergy in our population was much higher (36.4%) than that reported to profilin and, therefore, higher than usually reported in pollen-allergic patients. Larger studies should be performed to clarify this possible association.

In conclusion, we identified a previously unknown peach protein that could act as an allergen in peach-allergic patients. The protein was found to be RML, which is homologous to the almond allergen Pru du 10. Given that the new allergen was recognized by 52.4% of the study population, RML could be considered a major peach allergen. More studies are necessary to fully understand the role of this allergen in symptoms induced after peach ingestion.

Funding

The authors declare that no funding was received for the present study.

Conflicts of Interest

M.A. López-Matas, F. Álvarez, and J. Carnés are employees of LETI Pharma.

The remaining authors declare that they have no conflicts of interest.

Previous Presentation

The clinical results of this study were presented as an oral communication at the EAACI Congress, Hamburg 2023. Data on the identification and characterization of the new allergen were presented in poster format at the EAACI Congress, Hamburg 2023.

References

1. Lyons SA, Clausen M, Knulst AC, Ballmer-Weber BK, Fernandez-Rivas M, Barreales L, et al. Prevalence of Food Sensitization and Food Allergy in Children Across Europe. J Allergy Clin Immunol Pract. 2020;8:2736-46 e9.
2. WHO/IUIS Allergen Nomenclature Sub-Committee (http://www.allergen.org).
3. Lyons SA, Burney PGJ, Ballmer-Weber BK, Fernandez-Rivas M, Barreales L, Clausen M, et al. Food Allergy in Adults: Substantial Variation in Prevalence and Causative Foods Across Europe. J Allergy Clin Immunol Pract. 2019;7:1920-8 e11.
4. Barni S, Caimmi D, Chiera F, Comberiati P, Mastrorilli C, Pelosi U, et al. Phenotypes and Endotypes of Peach Allergy: What Is New? Nutrients. 2022;14(5):998.
5. Egger M, Hauser M, Mari A, Ferreira F, Gadermaier G. The role of lipid transfer proteins in allergic diseases. Curr Allergy Asthma Rep. 2010;10:326-35.

6. Poncet P, Senechal H, Charpin D. Update on pollen-food allergy syndrome. Expert Rev Clin Immunol. 2020;16:561-78.
7. Balsells-Vives S, San Bartolome C, Casas-Saucedo R, Ruano-Zaragoza M, Rius J, Torradeflot M, et al. Low Levels Matter: Clinical Relevance of Low Pru p 3 sIgE in Patients With Peach Allergy. Front Allergy. 2022;3:868267.
8. Nahrstedt A. Cyanogenic compounds as protecting agents for organisms. Plant System Evol. 1985;150:35-47.
9. Jones DA. Cyanogenesis in animal-plant interactions. Ciba Found Symp. 1988;140:151-70.
10. Swain E, Poulton JE. Immunocytochemical Localization of Prunasin Hydrolase and Mandelonitrile Lyase in Stems and Leaves of Prunus serotina. Plant Physiol. 1994;106:1285-91.
11. Kabasser S, Hafner C, Chinthrajah S, Sindher SB, Kumar D, Kost LE, et al. Identification of Pru du 6 as a potential marker allergen for almond allergy. Allergy. 2021;76:1463-72.
12. Fuhrer S, Trimmel S, Breuker K, Tollinger M. NMR resonance assignments of the pathogenesis-related peach allergen Pru p 1.0101. Biomol NMR Assign. 2019;13:127-30.
13. Pasini G, Curioni A, Vegro M, Pagani M, Masi A, Schievano E, et al. Extraction and mass spectrometry identification of a major peach allergen Pru p 1. J Sci Food Agric. 2012;92:570-6.
14. Klingebiel C, Chantran Y, Arif-Lusson R, Ehrenberg AE, Ostling J, Poisson A, et al. Pru p 7 sensitization is a predominant cause of severe, cypress pollen-associated peach allergy. Clin Exp Allergy. 2019;49:526-36.
15. Poncet P, Aizawa T, Senechal H. The subtype of Cupressaceae pollinosis associated with Pru p 7 sensitization is characterized by a sensitization to a cross-reactive gibberellin-regulated protein in cypress pollen: BP14. Clin Exp Allergy. 2019;49:1163-6.
16. Iizuka T, Barre A, Rouge P, Charpin D, Scala E, Baudin B, et al. Gibberellin-regulated proteins: Emergent allergens. Front Allergy. 2022;3:877553.
17. Asero R, Abbadessa S, Aruanno A, Barilaro G, Barzaghi C, Bignardi D, et al. Sensitization to Gibberellin-Regulated Protein (Peamaclein) Among Italian Cypress Pollen-Sensitized Patients. J Investig Allergol Clin Immunol. 2021;32:40-7.
18. Betancor D, Fernández-Bravo S, Nuñez-Borque E, González-Cano B, Esteban V, Pastor-Vargas C, et al. Is Pru p 7 (peamaclein) sensitization a predominant cause of cypress pollen-associated peach allergy in Spain? J Investig Allergol Clin Immunol. 2024;34(1):53-5.
19. Palacin A, Rivas LA, Gomez-Casado C, Aguirre J, Tordesillas L, Bartra J, et al. The involvement of thaumatin-like proteins in plant food cross-reactivity: a multicenter study using a specific protein microarray. PLoS One. 2012;7:e44088.
20. Rodriguez-Perez R, Fernandez-Rivas M, Gonzalez-Mancebo E, Sanchez-Monge R, Diaz-Perales A, Salcedo G. Peach profilin: cloning, heterologous expression and cross-reactivity with Bet v 2. Allergy. 2003;58:635-40.
21. Rodriguez Del Rio P, Diaz-Perales A, Sanchez-Garcia S, Escudero C, Ibanez MD, Mendez-Brea P, et al. Profilin, a Change in the Paradigm. J Investig Allergol Clin Immunol. 2018;28:1-12.
22. 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;112:789-95.
23. Boyano-Martínez T, Pedrosa M, Belver T, Quirce S, García-Ara C. Peach allergy in Spanish children: tolerance to the pulp and molecular sensitization profile. Pediatr Allergy Immunol. 2013;24:168-72.

Manuscript received July 19, 2023; accepted for publication November 15, 2023.

Jerónimo Carnés

Research & Development Allergy and Immunology Unit
Laboratorios LETI, S.L.U.
Calle del Sol 5
28760 Tres Cantos, Spain
E-mail: jcarnes@leti.com