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## Case Report

# The Contribution of Molecular Allergy Diagnostics to Identifying Cross-Reactive Allergies involving Profilin and nsLTP : A Case Report

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## KEYWORDS

CRD

Co-sensitization

Profilin

nsLTP

Molecular Allergy

Diagnostics

## Abstract

**Background:** The advent of molecular diagnostics, particularly Component Resolved Diagnosis (CRD), has been a significant advancement in the understanding of the underlying mechanisms of allergies. CRD is based on the identification of specific molecular allergens.

**Purpose:** This case aims to illustrate the value of molecular diagnostics in identifying co-sensitization and cross-reactivity profiles, as well as to discuss the practical implications for management, particularly in terms of prevention and patient education.

**Methods:** This report describes the case of a 25-year-old Algerian patient with pollen-food allergy syndrome, as well as co-sensitization to profilin and nsLTP.

**Results :** The patient presented with a history of allergic rhinitis and conjunctivitis during the spring. He reported experiencing dyspnea and urticaria immediately after ingesting peanuts and nuts, though sometimes he had no symptoms. He also experienced oral allergy syndrome, immediately after eating certain fruits, such as apples, peaches, and tomatoes. The results of specific IgE antibodies for allergen extracts indicated that the patient had a very broad sensitization profile. Subsequently, molecular allergen testing was performed using a customized allergen-chip based on the ISAC technology. Specific IgE against molecular components were positive, with most antibodies belonging to two molecular families: profilins and nsLTP. Profilin is often associated with moderate allergic reactions. In contrast, nsLTps proteins are linked to more severe clinical manifestations. Co-sensitization to Profilins and nsLTP can modulate clinical severity. Additionally, co-factors strongly influence the variable clinical expression of nsLTps.

**Conclusion:** This case clearly illustrates the importance of an allergen-specific diagnosis, based on allergenic molecules (CRD-component-resolved diagnosis), for identifying cross- and co-sensitization to multiple allergens. This approach allows for personalized, accurate diagnoses and optimized management of allergic diseases.

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## Introduction :

Pollen-food allergy syndrome (PFAS) is an IgE-mediated allergic reaction that occurs after ingesting fruits, vegetables, nuts, and seeds (1). It usually results from initial sensitization to a pollen allergen whose molecular structure is sufficiently similar to that of a food allergen to induce a cross-reaction (2). These proteins share common epitopes called pan allergens, which are mistakenly recognized as identical by the immune system, triggering an allergic response in sensitized individuals (3). The main respiratory sources involved are tree pollens such as birch, olive, cypress, and plane tree (2).

The advent of molecular diagnostics, particularly Component Resolved Diagnosis (CRD), has been a significant advancement in the understanding of the underlying mechanisms of allergies. CRD is based on the identification of specific molecular allergens. This approach allows for the distinction between co- sensitization and cross- sensitization, facilitates the assessment of clinical risk, and supports the development of targeted treatment strategies for each patient (4).

Of the 151 allergenic protein families that have been described, only a few contain cross-reactive allergens, some of which are well characterized from a molecular and clinical perspectives. Key pan allergen families involved in PFAS include pathogenesis-related protein class 10 (PR-10) proteins, profilins, nonspecific lipid transfer proteins (nsLTPs) and gibberellin-regulated proteins (GRPs) (5), each contributing to distinct patterns of cross-reactivity (1).

Profilin is involved in multiple pollen sensitivities and pollen-food-latex syndromes, and is often associated with moderate allergic reactions (4). In contrast, nsLTPs proteins are among the most common triggers of anaphylaxis and are linked to more severe clinical manifestations, particularly in monosensitized patients. However, when concomitant sensitization occurs with pattern allergen such as profilin or PR10, allergic symptoms tend to be less intense (6–8).

Co-sensitization to different allergenic families can modulate clinical severity, broaden the range of

affected foods, and complicate management. Here, we present an illustrative case of PFAS involving co-sensitization to the nsLTPs and profilin families. This case aims to illustrate the value of molecular diagnostics in identifying co-sensitization and cross-reactivity profiles, as well as to discuss the practical implications for management, particularly in terms of prevention and patient education.

## Results:

### Case Presentation:

A 25-year-old male living in Algiers, with no significant family history of allergic disease, was referred to the clinical allergology department at Beni Messous University Hospital for evaluation. The patient presented with a history of allergic rhinitis and conjunctivitis during the spring. He reported experiencing dyspnea and urticaria immediately after ingesting peanuts and nuts, though sometimes he had no symptoms. He also experienced oral allergy syndrome, which is characterized by a tingling sensation in the palate and lip swelling immediately after eating certain fruits, such as apples, peaches, and tomatoes.

Following the clinical evaluation, the patient was referred to the immunological department for biological assessment. The patient's serum was tested for specific IgE antibodies to the relevant airborne and food allergen extracts, using the IMMULITE® 2000 XPi system (Siemens).

The results indicated that the patient had a very broad sensitization profile (**Table 01**), with positive results for pollens from **timothy grass, olive, *Parietaria judaica***, as well as **peach, apple, tomato, peanut, sesame, walnut, hazelnut and almond**.

**Table 01:** Specific IgE results for airborne and food allergen extracts.

Due to the patient's extremely broad sensitization profile, he can be defined as a highly atopic, polysensitized subject.

The patient's serum was analyzed to exclude cross-reactivity due to Cross-reactive Carbohydrate Determinants (CCD) and was found to be negative for nAna c2 (Bromelain = 0.194 kU/L (class 0)).

Subsequently, molecular allergen testing was performed using a customized allergen-chip based on the ISAC technology, which includes a wide range of recombinant and native allergens. **Table 02** summarizes the positive specific IgE results, along with the sources and families to which each molecular allergen belongs.

| Code | Airborne allergens             | Specific IgE (kU/L) | Class |
|------|--------------------------------|---------------------|-------|
| D1   | Dermatophagoides pteronyssinus | < 0.1               | 0     |
| D2   | Dermatophagoides farinae       | 0.147               | 0     |
| E1   | Cat dander                     | 0.224               | 0     |
| E5   | Dog dander                     | < 0.1               | 0     |
| W19  | Parietaria Judaica pollen      | 3.43                | II    |
| G6   | Timothy grass pollen           | 41.69               | IV    |
| T9   | Olive pollen                   | 36.92               | IV    |
|      | Food allergen                  |                     |       |
| F49  | Apple                          | 64.2                | V     |
| F95  | Peach                          | 145                 | VI    |
| F25  | Tomato                         | 51.3                | IV    |
| F13  | Peanut                         | 23.9                | IV    |
| F17  | Hazelnut                       | 2.09                | II    |
| F20  | Almond                         | 1.37                | II    |
| F256 | Walnut                         | 2.11                | II    |
| F10  | Sesame                         | 8.62                | III   |

**Table 02:** Positive specific IgE results to recombinant molecular allergens

ISU-E: ISAC Standard Unit IgE

Specific IgE against molecular components were positive, with most antibodies belonging to two molecular families: **profilins** (rHev b 8 [latex], rPhl p 12 [timothy grass], rBet v 2 [birch], rSola I 1 [Tomato], rOle e 2 [olive], rAmb a 8 [ragweed]) and **nsLTPs** (rAra h 9 [peanut], rPru p 3 [peach], rArt v 3 [mugwort])

The patient's serum was also tested for IgE antibodies to other foods containing nsLTPs and profilins, and all tests returned positive results: Banana: 12,70 KU/l , Cashew nut: 5,29 KU/l , Pineapple: 4,26 KU/l , Avocado: 8,48 KU/l , Apricot: 3,38 KU/l , Kiwi: 15,91 KU/l , Orange: 9,39 KU/l.

| Code      | Results (ISU-E) | Allergen source                            | Molecular family     |
|-----------|-----------------|--------------------------------------------|----------------------|
| rAmb a 8  | 0.83            | Ragweed ( <i>Ambrosia artemisiifolia</i> ) | <b>Profilins</b>     |
| rArt v 3  | 1.52            | Mugwort ( <i>Artemisia vulgaris</i> )      | <b>nsLTP</b>         |
| rBet v 2  | 9.2             | Birch ( <i>Betula pendula</i> )            | <b>Profilins</b>     |
| rHev b 8  | 12.93           | Latex ( <i>Hevea brasiliensis</i> )        | <b>Profilins</b>     |
| rOle e 1  | 13.55           | Olive ( <i>Olea europaea</i> )             | Common olive group 1 |
| rOle e 2  | 5.36            | Olive ( <i>Olea europaea</i> )             | <b>Profilins</b>     |
| rPhl p 1  | 1.33            | Timothy grass ( <i>Phleum pratense</i> )   | Grass group 1        |
| rPhl p 2  | 0.64            | Timothy grass ( <i>Phleum pratense</i> )   | Grass group 2        |
| rPhl p 12 | 9.87            | Timothy grass ( <i>Phleum pratense</i> )   | <b>Profilins</b>     |
| rAra h 9  | 8.63            | Peanut ( <i>Arachis hypogaea</i> )         | <b>nsLTP</b>         |
| rPru p 3  | 1.94            | Peach ( <i>Prunus persica</i> )            | <b>nsLTP</b>         |
| rSola I 1 | 6.78            | Tomato ( <i>Solanum lycopersicum</i> )     | <b>Profilin</b>      |

## Discussion:

This report describes the case of a 25-year-old Algerian patient with pollen-food allergy syndrome, as well as co-sensitization to profilin and nsLTP.

Profilin is a protein of 12- 15 kDa in size present in all eukaryotic cells and involved in the organization of cytoskeleton as well as in signal transduction (4). Profilins from higher plants constitute a family of highly conserved proteins showing sequence identities of at least 75% even between members from distantly related organisms. In view of the high sequence homology, cross- reactivity between profilins is extremely common and involves virtually every plant source (**Figure 01**). Thus, profilin can be considered the archetypal pan-allergen (4). They are widely distributed across the plant kingdom, found in both pollen and plant foods sources.

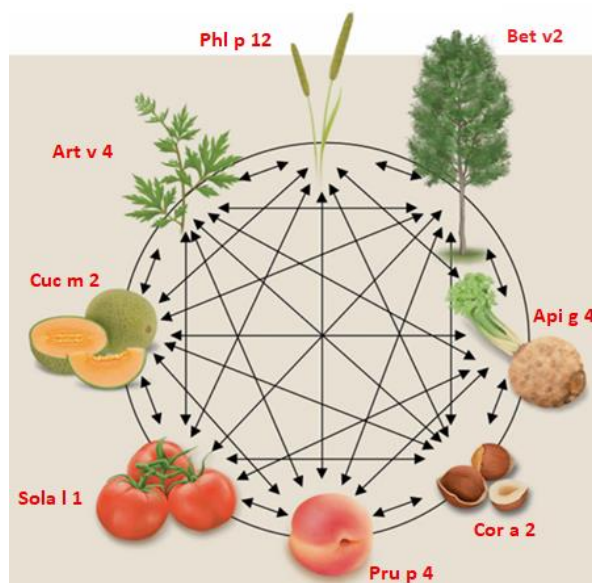


**Fig 01 :** Cross- reactivity between profilins from different pollen sources and plant foods (4)

Among pollen sources, profilins are present in various botanical families, including *Fagales* (birch, hazel tree, alder, hornbeam, oak, beech), *Graminae* (timothy grass and other grass species), *Asteraceae* (mugwort, ragweed), *Urticaceae* (pellitory), *Oleaceae* (olive, ash), *Cupressaceae* (cypress), *Euphorbiaceae* (annual mercury, date palm). Each species is associated with specific profilin allergens, such as *Bet v 2* for birch or *Phl p 12* for grasses (4). Up to 50% of pollen allergic patients are sensitized to profilin (4). As a minor pollen allergen, sensitisation to profilins occurs after sensitisation to a primary and major pollen source. Grass pollen is usually responsible for this hypersensitivity, but depending on geographical location, birch, ragweed or mugwort pollen may also act as primary sensitizers (9). The clinical significance of profilins as an airborne allergen remains unclear. Provocation tests (conjunctival, nasal and bronchial) have shown that profilins could act as an aeroallergen (4,10,11), but their real impact seems to be limited to the season of the main sensitising pollen (12). However, in some regions, it may indicate more severe respiratory allergies (13).

In our case, the patient's primary sensitization appears to be to olive and Timothy grass pollens, as their major allergens (**rOle e 1** and **rPhl p 1**, respectively) tested positive. These sensitizations are consistent with the patient's symptoms, which occurred exclusively during the spring season, corresponding to the pollination periods of olive

trees (April-June) and Timothy grass (May-August). Additionally, the patient's serum showed strong reactivity to molecular allergens of the profilin family, particularly **Phl p 12** (Timothy grass) and **Ole e 2** (olive tree). The additional positivity to birch profilin (**Bet v 2**), despite the absence of birch pollen in Algeria, indicates a cross-reactivity mechanism mediated by profilins, which are highly conserved panallergens shared among different pollen sources. This pattern reflects sensitization to a common profilin epitope rather than to species-specific pollen allergens.

In addition to pollen profilin sensitization, biological sensitization was also observed to profilins present in Tomato (**rSola I 1**), thus defining the framework of pollen-food allergy syndrome (PFAS) (14). Profilins are widely distributed across numerous botanical families, including *Rosaceae* (apple, peach, pear), *Cucurbitaceae* (melon), *Actinidiaceae* (kiwi), *Apiaceae* (celery, carrot), *Rutaceae* (orange), *Leguminosae* (peanut, soybean), *Solanaceae* (tomato), *Bromeliaceae* (pineapple), *Corylaceae* (hazelnut), *Brassicaceae* (yellow mustard), *Asteraceae* (sunflower), and *Moraceae* (fig). Although present in virtually all plant-derived foods, profilins have long been underestimated as allergens. It has been reported that up to 50% of profilin-sensitized patients may have food allergies, especially those allergic to foods such as melon, watermelon, citrus fruits, bananas, pineapple, persimmons, zucchini, and tomatoes (14). Clinically, profilin sensitization most often manifests as OAS, characterized by itching and swelling of the lips, mouth, and throat immediately after ingestion of raw plant-derived foods (14). This was also the case for our patient, whose symptoms were limited to OAS and disappeared when the same foods were consumed after cooking. This pattern reflects the intrinsic lability of profilins, which are highly sensitive to heat, pepsin, and enzymatic digestion, leading to denaturation during food processing and explaining the general tolerance of cooked or processed foods (15). In most individuals, profilin-related reactions remain mild and localized; however, in rare cases, severe systemic responses have been described following ingestion of small quantities of purified profilin, particularly in regions with high grass pollen sensitization (16). Such responses may also occur under specific

conditions, notably in the presence of particular cofactors (17).

The patient also tested positive for **Hev b 8** profilins in latex. This could be regarded as a potential risk for intraoperative anaphylactic reactions. However, it has been reported that patients with specific IgE reactivity only to profilin in natural latex can undergo surgery or other medical procedures without any risk (15).

The second molecular family to which our patient was sensitized corresponds to nonspecific lipid transfer proteins. Among the five nsLTP components present on the allergen chip (Ole e 7, Art v 3, Par j 2, Ara h 9, and Pru p 3), the patient exhibited specific IgE reactivity to three of them: **Art v 3** (mugwort), **Ara h 9** (peanut), and **Pru p 3** (peach).

The International Union of Immunological Societies (IUIS) lists 46 allergenic nsLTPs from fruits, pollens, vegetables, nuts, seeds, and latex (4). They are the main allergens in the *Prunoideae* subfamily (peach, apricot, plum, cherry...) and exhibit strong IgE cross-reactivity within the *Rosaceae* family, as well as with citrus fruits, grape, tomato, vegetables (such as asparagus and lettuce), nuts (hazelnut, walnut, peanut), maize, onion, carrot, rice, and spelt (with partial cross-reactivity) (16). Their concentration varies depending on the fruit's ripeness, storage, and cultivar (17).

Some pollens (*Parietaria*, *Artemisia*, *Olea*) also contain nsLTPs (**Par j 1**, **Art v 3**, **Ole e 7**) but their cross-reactivity with Pru p 3 is variable, depending on sequence homology and structural similarity (**Figure 02**).

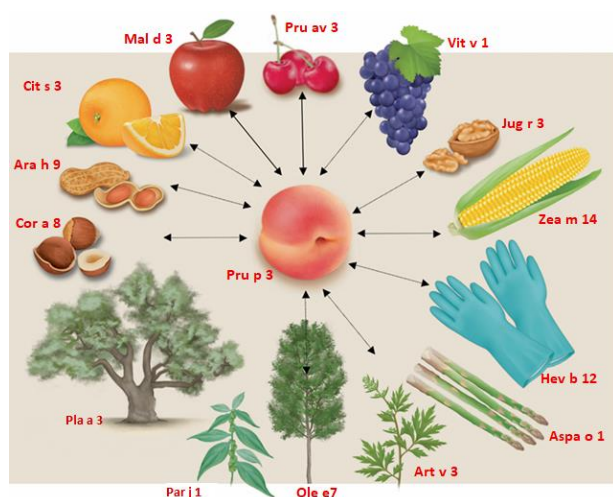


**Fig 02:** Cross- reactivity between nsLTP from different allergenic sources. Continuous lines indicate a high degree of cross- reactivity among the *Rosaceae* family. Dashed lines indicate partial cross- reactivity (4).

Our patient tested positive for Art v 3 but negative for Par j 1 and Ole 7. These results are consistent with known cross-reactivity patterns: Par j 1 shows no cross-reactivity with nsLTPs from other sources, Ole e 7 shares less than 20% amino acid sequence identity with Pru p 3, while Art v 3 exhibits partial cross-reactivity with Pru p 3.

In Mediterranean regions, sensitization to nsLTPs is often initiated by peach Pru p 3, and patients subsequently tend to develop cross-sensitization to nsLTPs from other plant foods. Pru p 3, the major allergen in peach, was the first nsLTP identified as a clinically relevant food allergen. It is mainly found in the pericarp of fruits, which contains about 220 times more Pru p 3 than the pulp. It is a small basic protein (91 amino acids, 9.2 kDa) belonging to the prolamin superfamily, and it contains a highly conserved domain composed of alpha-helices that harbor eight cysteine residues forming four intramolecular disulfide bridges. This compact structure provides great stability and resistance to heat (in vitro, its ability to bind to IgE is maintained after 30 minutes at 121°C and 160 minutes at 100°C), pH variations, and proteolytic digestion (approximately 35% of the molecule remains intact after treatment with trypsin) (18). This stability explains its ability to maintain binding capacity to IgE and its high allergenic potential (19). As part of adapting avoidance measures, peeling these fruits significantly reduces the intake of nsLTP in sensitized individuals.

Following primary sensitization to Pru p 3, cross-reactivity can extend to homologous nsLTPs from other plant sources, including peanut Ara h 9, as observed in our patient. Among the peanut molecular allergens tested (Ara h 1, Ara h 2, Ara h 3, Ara h 6, Ara h 8, and Ara h 9), only Ara h 9 was positive, highlighting the relevance of specific IgE to nsLTP. Determination of Ara h 9 is therefore particularly important in patients from Mediterranean regions, where nsLTP sensitization represents a predominant allergic phenotype, and should be systematically included in the diagnostic workup of peanut allergy.

Since Ara h 9, like Pru p 3, possesses thermal and digestive stability, affected patients can develop systemic symptoms. The clinical manifestations are highly heterogeneous, ranging from local oropharyngeal symptoms to anaphylaxis (8). Our patient reported dyspnea and urticaria immediately after ingesting peanuts and nuts, and only local oropharyngeal symptoms after ingesting peaches. According to I. Mota et al. (20), the clinical expression of allergy in individuals sensitized to

nsLTPs may vary depending on the plant food consumed.

In addition, reactions are generally more severe in patients who are monosensitized to nsLTPs and tend to be milder in cases of concomitant sensitization to profilin or PR-10 (6,7). One plausible explanation for this observation is that, in nsLTP mono-sensitized patients, the high density of nsLTP-specific IgE on effector cells allows Pru p 3 to efficiently trigger mediator release, which is associated with a higher risk of severe reactions(21). Also, the ratio of nsLTP-specific IgE to total IgE may modulate cell activation and the severity of clinical reactivity (22). However, in cases of co-sensitization to nsLTPs along with profilins or the PR-10 protein family, IgE directed against nsLTPs is often accompanied by IgE targeting other allergens. Co-sensitization to unrelated allergens, from the same or different sources, therefore appears to exert a “protective” effect against the clinical expression of nsLTP allergy (21). (Fig03)

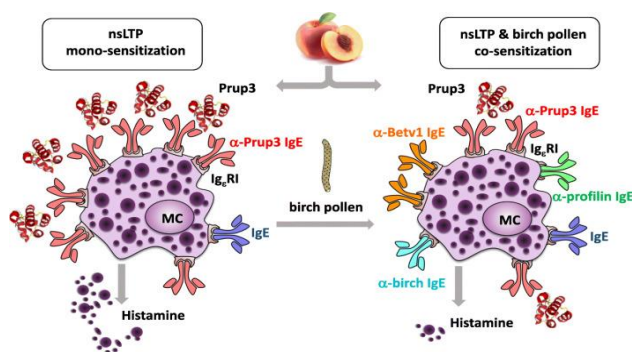


**Fig03:** Model of nsLTP mono-sensitization (left) vs. co-sensitization to unrelated allergens (right). (22)

Furthermore, the clinical expression is also often modulated by cofactors such as physical exercise, nonsteroidal anti-inflammatory drugs or alcohol intake (25). The presence or absence of these cofactors may explain why our patient tolerated nsLTP-containing foods on some occasions but experienced severe reactions on others.

The patient's serum was also tested for IgE antibodies to other foods containing nsLTPs, and all tests returned positive results for Banana, Cashew nut, Pineapple, Avocado, Apricot, Kiwi and Orange. Although the patient did not show any symptoms after ingesting these foods. This positivity is likely due to cross-reactivity between nsLTPs from different allergenic sources. It should be noted that sensitization is not synonymous with allergy, and the presence of nsLTPs in a plant-based food does not justify its elimination if it has been tolerated, since contact with the intestinal immune system may contribute to maintaining tolerance (4). In such cases, the oral food challenge remains the gold standard for confirming clinical

relevance and, in some instances, should be performed in the presence of cofactors.

## Conclusion:

This case clearly illustrates the importance of an allergen-specific diagnosis, based on allergenic molecules (CRD — component-resolved diagnosis), for identifying cross- and co-sensitization to multiple allergens. This approach allows for personalized, accurate diagnoses and optimized management of allergic diseases. It also improves our understanding of the clinical manifestations associated with nsLTP/profilin co-sensitization, which appears to reduce the risk of severe allergic reactions to nsLTP. Finally, cofactors strongly influence the variable clinical expression of nsLTPs.

## Declaration of Interests

The authors declare that they have no conflict of interest.

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