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# Identification of a thaumatin-like protein as a new allergen in persimmon *(Diospyros kaki)* with cross-reactivity with banana *(Musa acuminata)*

*Identificação de uma proteína semelhante à taumatina como um novo alérgeno no caqui* (Diospyros kaki) *com reatividade cruzada com a banana* (Musa acuminata)

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**ABSTRACT**

Allergy to persimmon (*Diospyros kaki*) has been only rarely reported. The antigenic composition of the fruit is not entirely known. Thaumatin-like proteins (TLPs) have been described as allergens in pollens and various fruits, such as kiwi and banana, but not in persimmon. We report the case of a 22-year-old man, with persistent moderate-to-severe allergic rhinitis, sensitized to house dust mites. The patient describes an episode of oral mucosa and ear canal pruritus, followed by diffuse urticaria, which rapidly evolved to dysphonia, dyspnea, and dizziness, after eating raw persimmon. A few months later he developed similar cutaneous symptoms accompanied by nausea, vomiting, abdominal colic, and hypotension immediately after the intake of banana. The prick-prick test with raw persimmon and banana were positive, as well as the serum specific IgE to the extract of these fruits. The ImmunoCAP ISAC_112i test demonstrated a positive specific IgE against Act d 2 (kiwi thaumatin), which is homologous to banana TLP (Mus a 4). Serum IgE inhibition test with "sponge" of *Diospyros kaki* ImmunoCAP (f301) showed partial inhibition (40%) of IgE to Act d 2. This raises the suspicion that a TLP is at least partially responsible for the referred sensitization. This patient is sensitized to *Diospyros kaki* and *Musa acuminata*. An anaphylactic reaction to consumed persimmon, presumably as a result from cross-allergy with banana thaumatin was diagnosed in our patient. Thaumatin has not been previously described as an allergen of persimmon with cross-reactivity with banana, and in vitro with Act d 2 (kiwi TLP).

**Keywords:** Anaphylaxis, diospyros, food hypersensitivity, musa, cross reactions.

**RESUMO**

A alergia ao caqui (*Diospyros kaki*) tem sido raramente documentada, não sendo a composição antigênica da fruta totalmente conhecida. Proteínas semelhantes à taumatina (TLPs) foram descritas como alergênicos em pólens e várias frutas, como no kiwi e banana, mas não no caqui. Apresenta-se o caso de um doente de 22 anos, com rinite alérgica persistente moderada-grave, sensibilizado a ácaros do pó doméstico. O doente refere episódio de prurido na mucosa oral e canal auditivo, seguido de urticária generalizada, que rapidamente evoluiu para disfonia, dispneia e tontura, após ingestão de caqui. Poucos meses depois, desenvolveu sintomas cutâneos semelhantes, acompanhados de náuseas, vómitos, cólica abdominal e hipotensão imediatamente após ingestão de uma banana. O teste cutâneo por picada com caqui e banana em natureza foram positivos, bem como o doseamento de IgE específica. O teste ImmunoCAP ISAC_112i identificou a presença de IgE específica para Act d 2 (taumatina do kiwi), homóloga da TLP da banana (Mus a 4). O estudo de inibição ImmunoCAP ISAC com “esponja” de *Diospyros kaki* (f301) produziu uma inibição parcial (40%) da ligação de IgE a Act d 2, permitindo presumir que uma proteína semelhante à taumatina é, pelo menos, parcialmente responsável pela referida sensibilização. Este doente encontra-se sensibilizado a *Diospyros kaki* e *Musa acuminata*. Uma anafilaxia ao caqui ingerido, presumivelmente resultante de reatividade cruzada com a taumatina da banana foi diagnosticada. Não estão descritas na literatura TLPs como alergênicos do caqui com reatividade cruzada com a banana e com Act d 2 *in vitro* (TLP do kiwi).

**Descritores:** Anafilaxia, diospyros, hipersensibilidade alimentar, musa, reações cruzadas.

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## Introduction

Persimmon (Latin: *Diospyros kaki*) fruit is the edible fruit of the persimmon tree, which belongs to the *Ebenaceae* family. It is native to China and Japan, where it has been cultivated for centuries. In the mid-19th century, persimmon fruit was introduced into Europe.[1,2] Its color varies in different cultivars from yellow and orange to deep red. There are two types of varieties, astringent and non-astringent. The fruits of persimmon are rich in various nutrients and phytochemicals, such as carbohydrates, organic acids, vitamins, tannins, polyphenols, dietary fibers, triterpenoids, and carotenoids, which contribute significantly to taste, color, nutritive and medicinal value of these fruits.[3] Persimmons also have a high antioxidant potential that may have beneficial effects against oxidative stress in humans.[4]

Allergy to persimmon is rare and potentially serious. The first case of IgE-mediated persimmon allergy was published in 1991.[1] Since then, eight papers on allergic reactions to persimmon have been published, concerning a total of 12 patients.[2,5-12] Nine of these patients had anaphylaxis.

The antigenic composition of the fruit is not entirely known. So far, only three allergens have been identified: Dio k 1 (PR-10 protein, 19 kDa), Dio k 4 (profilin, 14 kDa), and Dio k IFR (isoflavone reductase, 38 kDa).[13] Cross-reactivity between birch pollen and persimmon fruit has been demonstrated.[6,7] Additionally, it was discovered that allergens from persimmon may cross-react with latex allergens, through the phenomenon known as latex-fruit syndrome.[11]

Thaumatin-like proteins (TLPs) have molecular masses of 20-30 kDa, with a particularly stable three-dimensional structure that is sustained by six disulphide bridges. They have been mentioned as plant defense proteins (PR-5 proteins) against pathogen attacks, particularly fungal ones, and reported as allergens in various fruits, such as apple, cherry, kiwi, olive, and banana, and in pollens, such as those of cypress and birch, among others. This family is thought to be a panallergen family responsible for cross-reactivity between pollen and fruit.[14-16] TLPs have not been reported as persimmon allergens.

## Ethical issues

Patient gave an informed consent to the use of his clinical data in an anonymous form.

## Case report

We present the case of a 22-year-old male patient, resident in Lisbon, with a persistent moderate-to-severe allergic rhinitis since childhood, which worsens during the autumn months.

In mid-2018, the patient was referred to our outpatient clinic in the Department of Immunoallergology after an allergic reaction to persimmon. He described, in the previous year, an episode of oral mucosa and ear canal pruritus, followed by diffuse urticaria, which rapidly evolved to dysphonia, dyspnea, and dizziness, after eating raw persimmon for the first time. For this reason, he was admitted to the emergency department, where, besides being medicated, he was kept on surveillance for 8 hours, with resolution of the condition. He denied involvement of any cofactors, such as physical exercise, alcohol consumption, or new drugs. Since then, he has been on a diet with an absolute avoidance of persimmon, with no other episodes.

A few months later, he developed similar cutaneous symptoms accompanied by nausea, vomiting, abdominal colic, and hypotension immediately after the intake of banana. He was also admitted to the emergency department, where he was medicated with antihistamine and intravenous corticosteroid, with resolution of symptoms. The patient denies complaints about eating other foods, namely tree nuts, other plant derivatives, or other fruits except persimmon. He also denied ever having eaten some fruits like kiwi.

As a part of the diagnostics performed in our immunoallergology consultation, skin prick tests were performed for common aeroallergens with commercial extracts (Bial-Aristegui™, Bilbao, Spain), namely house dust and storage mites (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Euroglyphus maynei*, *Lepidoglyphus destructor*, *Blomia tropicalis*, *Glycyphagus domesticus*, *Acarus siro*, *Tyrophagus putrescentiae*), dog and cat fur, *Alternaria alternata*, *Aspergillus fumigatus*, mixtures of grasses, *Phleum pratense*, platanus, *Olea europaea*, *Parietaria judaica*, *Artemisia vulgaris*, *Plantago lanceolata*, latex, and food allergens (peach lipid transfer protein [LTP], profilin, melon, avocado, and watermelon). Native skin tests with fresh foods (persimmon and banana) were also performed.

Skin prick tests were positive for the mites allergen extracts (wheal average diameter in millimeters): *Dermatophagoides pteronyssinus* 12 mm, *Dermatophagoides farinae* 10 mm, *Euroglyphus*

*maynei* 8 mm, *Lepidoglyphus destructor* 14 mm, *Blomia tropicalis* 6 mm, *Glycyphagus domesticus* 7 mm, *Acarus siro* 14 mm, *Tyrophagus putrescentiae* 8 mm (histamine 8 mm, negative control 0 mm; a result was interpreted as positive in case of a wheal average diameter ≥ 3 mm). No skin reaction was observed for other tested allergen extracts, including pollens and latex. Skin prick tests with persimmon and banana in natura were positive, with mean papule diameters of 12 and 10 mm, respectively.

In vitro determination of the concentration of allergen specific IgE to house dust and storage mites was performed, as well as levels of specific IgE to selected allergens – banana, avocado, persimmon (*Diospyros kaki*), kiwi, and peach –, which were determined with the ImmunoCAP system (ThermoFisher Scientific, Uppsala, Sweden), using the Phadia100 equipment according to the manufacturer's instructions. Test was considered positive for values greater than 0.10 kUA/L.

The total IgE level was 93.9 kUA/L. Elevated levels of IgE (above 0.35 kUA/L) were found against all dust mites. The assay of specific IgE food levels using the ImmunoCAP method was positive for banana (0.21 kUA/L), avocado (0.13 kUA/L), *Diospyros kaki* (0.58 kUA/L), and kiwi (0.15 kUA/L), and negative for peach (0.01 kUA/L). These results are presented in Table 1.

**Table 1**
List of results for levels of specific IgE tested using the ImmunoCAP method

| Allergen | IgE level (kUA/L) |
|---|---|
| Banana | 0.21 |
| Avocado | 0.13 |
| *Diospyros kaki* | 0.58 |
| Kiwi | 0.15 |
| Peach | 0.01 |

The diagnostics was expanded by a determination of the level of specific IgE to allergen components, using the ImmunoCAP ISAC method, which identified the presence of specific IgE to Blot t 5 (1.9 ISAC standardized units for IgE [ISU-E]), Der f 1 (1.1 ISU-E), Der f 2 (7.7 ISU-E), Der p 1 (8.5 ISU-E), Der p 2 (11 ISU-E), Der p 23 (0.3 ISU-E), Lep d 2 (2.9 ISU-E), and Act d 2 (2.9 ISU-E) (Table 2).

A high level of specific IgE for Act d 2 (TLP) from kiwi[17] found in ImmunoCAP ISAC test was particularly noteworthy. It is worth to emphasize that Act d 2 presented cross-reactivity to Mus a 4 from banana[18], which is not available in ImmunoCAP ISAC_112i. There were no other elevated levels of IgE specific to components of allergens available in ImmunoCAP ISAC, including non-specific LTPs, PR10s, profilins and storage proteins.

ImmunoCAP ISAC has no persimmon or banana allergen components available, so it does not indicate if, in this particular case, the patient's allergy to persimmon was a result of a cross-reactivity with Mus a 4 or an allergy to another molecule, independent from banana or kiwi. To clarify this, an ImmunoCAP ISAC inhibition test was applied, using the "sponge" of *Diospyros kaki* (f301) allergen extract from ImmunoCAP as a source of allergen.

The baseline level of Act d 2 determined by ImmunoCAP ISAC_112i in patient serum was 2.9 ISU-E. After incubation of the patient's serum with "sponge" of ImmunoCAP *Diospyros kaki* (f301), the level of Act d 2 was 1.8 ISU-E (38% inhibition), which raises the suspicion that sensitization to LTPs is responsible (at least partially) to the referred sensitization. The results for non-inhibition of mites in the same array serve as an inhibition control. The results for these inhibition tests are presented in Table 3.

## Discussion

This patient is sensitized to *Diospyros kaki* and *Musa acuminata*, having had two reported episodes of anaphylaxis after the consumption of a persimmon fruit and a banana, respectively. It was confirmed that the *Diospyros kaki* extract inhibited Act d 2 by ~ 40%, which makes us presume the participation of cross-reaction with other TLP – banana TLP (Mus a 4) – in the development of symptoms.

Considering that the reaction with persimmon occurred at the patient's first exposure to it, and that the patient was not sensitized to any pollens, we assume that this reaction resulted from a primary sensitization to banana. We informed the patient to avoid persimmon, banana, and kiwi, and provided an epinephrine autoinjector for prehospital treatment of anaphylaxis in case of an emergency.

**Table 2**
Results for the ImmunoCAP ISAC test. No increased levels of allergen-specific IgE were found for other allergen components included in the ImmunoCAP test

| Allergen source | Allergen component | Allergen type | IgE level (ISU-E) |
|---|---|---|---|
| *B. tropicalis* (house dust mite) | rBlo t 5 | Mites gyroup 5 | 1.9 |
| *D. farinae* (house dust mite) | rDer f 1 | Cysteine protease | 1.1 |
| | rDer f 2 | NPC2 family | 7.7 |
| *D. pteronyssinus* (house dust mite) | rDer p 1 | Cysteine protease | 8.5 |
| | rDer p 2 | NPC2 family | 11 |
| | rDer p 23 | Peritrophin-like proteins | 0.3 |
| *L. destructor* (storage mite) | rLep d 2 | NPC2 family | 2.9 |
| Kiwi | nAct d 2 | Thaumatin-like protein | 2.9 |

ISU-E: ISAC standardized units for IgE.

**Table 3**
Results for the ImmunoCAP ISAC test performed with serum inhibited with “sponge” of ImmunoCAP *Diospyros kaki* (f301)

| Allergen source | Allergen component | Allergen type | IgE level (ISU-E) / (% inhibition) |
|---|---|---|---|
| *B. tropicalis* (house dust mite) | rBlo t 5 | Mites group 5 | 1.8 / (5%) |
| *D. farinae* (house dust mite) | rDer f 1 | Cysteine protease | 1.1 / (0%) |
| | rDer f 2 | NPC2 family | 7.6 / (1%) |
| *D. pteronyssinus* (house dust mite) | rDer p 1 | Cysteine protease | 8.3 / (2%) |
| | rDer p 2 | NPC2 family | 11 / (0%) |
| | rDer p 23 | Peritrophin-like proteins | 0.3 / (0%) |
| *L. destructor* (storage mite) | rLep d 2 | NPC2 family | 2.5 / (14%) |
| Kiwi | nAct d 2 | Thaumatin-like protein | 2.9 / (38%) |

ISU-E: ISAC standardized units for IgE.

According to previously published reports in the literature, most cases of allergy to persimmon occurred in patients with allergic rhinitis/rhinoconjunctivitis, sensitization to birch and grass pollen, and allergy to other plant-derived foods[2,5-12], or with a cross-reaction with latex allergens[11], which did not happen in this patient, who was not sensitized to any pollens or to latex (in skin and/or serological tests). There has also been no documented sensitization to pollen allergens, latex, or proteins in the LTP group (such as Pru p 3) in ImmunoCAP ISAC_112i, neither to other fruit allergens available in ISAC, except for kiwi TLP (Act d 2).

The presented case is interesting because TLPs, as far as we know, have not been previously described as persimmon allergens with cross-reactivity to banana, although, in our patient, the specific allergen has not been isolated and characterized.

Identification of food allergens is a priority in the management of food allergy, considering that well-characterized relevant allergens might replace allergen extracts in a component-based diagnosis of allergy.[19] The rapid progress made in the field of molecular allergen characterization appears to considerably improve the use of recombinant allergens in diagnosis and specific immunotherapy.[20] Thus, further research is needed to fully isolate and characterize the allergen and understand the cross-reactivity of persimmon TLP with banana and/or other foods.

The level of knowledge about persimmon allergens is surely unsatisfactory, and further studies are required. Persimmon fruit certainly contains cross-reacting allergens, but the presence of species-specific allergens cannot be excluded.

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