

Allergy to persimmon (*Diospyros kaki*): A Chitinase and Thaumatin like protein, two new identified allergens

Betancor D¹, Gomez-Lopez A¹, Nuñez-Borque E¹, Fernandez-Bravo S^{1,2}, Barroso B¹, Esteban V^{1,2}, Pastor-Vargas C^{2,3*}, Cuesta-Herranz J^{1,2*}

¹Department of Allergy and Immunology, IIS-Fundación Jiménez Díaz, UAM, Madrid, Spain

²Red de asma, reacciones adversas y alérgicas (ARADyAL) RD16/0006/0013, Instituto de Salud Carlos III, Madrid, Spain

³Department of Biochemistry and Molecular Biology, Universidad Complutense de Madrid, Madrid, Spain

*Both authors have contributed equally in the manuscript.

Corresponding author:

Diana Betancor, MD

Allergy Department, Hospital Universitario Fundación Jiménez Díaz

Av Reyes Catolicos, 2, 28040, Madrid, Spain

E-mail: diana13_b@hotmail.com

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Persimmon or sharon fruit (*Diospyros kaki*) is a sweet tropical fruit from the Ebenaceae tree family. It is commonly cultivated in warm regions of the world, including China, Japan, Brazil, Turkey and Italy. Although its consumption is nowadays widespread in all industrialized countries due to its beneficial properties, few cases of allergy to persimmon have been reported. No persimmon allergen has been officially recognized (allergen.org). However, four allergens have been identified as being involved in isolated allergic reactions: nsLTP, Betv1-protein-like, profilin and IFR (isoflavone reductase) (the last three recognized in allergome.org) [1-3].

We present two case reports of systemic immediate reactions after ingestion of persimmon. The first one was a 34-year-old woman (P1) with a personal background of intermittent mild asthma due to mould (*Alternaria alternata*) and pollen (grass) allergy. She reported an episode of generalized urticaria, periocular angioedema and rhinitis few minutes after ingestion of a whole persimmon fresh fruit. The second patient (P2) was an 18-year-old woman who reported generalized urticaria, abdominal pain and shortness of breath while eating a persimmon fresh fruit. Both patients were treated in the emergency department with intramuscular antihistamine and corticosteroid treatment with rapid symptoms resolution.

Skin prick testing (SPT) to a battery of commercially available aeroallergen extracts (ALK, Spain) and prick-by-prick with natural persimmon was performed. Total serum IgE levels and persimmon specific IgE were measured with ImmunoCAP (Thermo Fisher Scientific). Immunoblot were carried out with natural persimmon extract. Oral food challenges were not performed due to systemic allergic symptoms along with a clear correlation with persimmon ingestion, and positive result to both persimmon specific IgE and skin prick-by-prick with natural persimmon. After the allergic reaction, both patients had tolerated normally other plant-derived food as fruit, nuts and vegetables.

SPT with aeroallergens was positive for P1 to *Lolium species* (5×7mm) and *Alternaria alternata*, (4×4 mm) and for P2: *Secalle cereale* (4×3mm) and *Cupressus arizonica* (7×4mm). Negative result was obtained to other pollens, dust mites and animal dander. Skin prick tests to purified allergens (Pho d 2[ALK, Spain] and Pru p 3[Roxall, Spain]) were negative in both patients.

Prick-by-prick testing with natural persimmon were positive to peel (P1 7x5 mm; P2 10x6 mm) and pulp (P1 6x4 mm; P2 8x7 mm) in both patients. Total IgE determination revealed a value of 39.4kU/L for P1 and 1200 kU/L for P2. Specific IgE to persimmon was 0.7kU/L for P1 and 0.95kU/L for P2. Specific IgE to rPru p 1, rPru p 3, rPru p 4, Bet v1, Bet v 2, Phl p 12 and Heb b 8 was negative in both patients.

Persimmon extract was prepared as previously reported [4]. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) of persimmon extract revealed multiple protein bands with molecular weights ranging from 10 to 100kDa (Figure in supplementary). Immunoblotting assays was performed under reduced conditions and the incubation with the patients' serum revealed an IgE-binding band of 16 kDa in P1 and another of 24 kDa in P2 (Figure 1). These bands were extracted from the gel and proteins were identified by mass spectrometry, as well as by searching a nonredundant protein sequence database (NCBI) using the Mascot program (<http://www.matrixscience.com>) in the Proteomic Service of Complutense University of Madrid, which is a member of the ProteoRed Network, as previously reported [4]. Results obtained revealed that 16 kDa IgE band corresponded to a thaumatin-like protein, while the 24 kDa protein correspond to chitinase.

Although persimmon allergy is not frequent, it can elicit allergic reactions in sensitized patients. In 2015, nLTP was for the first time identified as persimmon allergen [5]. In a series of 3 patients with persimmon allergy, IgE-inhibition assay showed a partly inhibition by the bromelain glycopeptide (carbohydrate determinants) in all patients and one of them also with Bet v 2 (profilin) suggesting its role as persimmon allergens [2]. Bolhaar et al. reported Bet v 1 as persimmon allergen in two patients with previous history of birch and grass-pollen rhinoconjunctivitis and plant food-related allergy (apple). They suggested the role of birch pollen as primary sensitizer in persimmon allergy [1].

More isolated persimmon allergy cases have been published. However, neither of them identified any allergen. A 30kDa and 60kDa IgE protein bands were demonstrated in a Japanese child with persimmon allergy [6]. An anaphylaxis case induced by this fruit demonstrated by in vivo and in vitro tests was published by Martinez et al [7].

Betv6 (*isoflavone reductases*) homologous protein bands were noted to be present in persimmon. It is responsible of cross-reactivity between birch pollen and some plant foods. Nevertheless, its role as allergen was not demonstrated in persimmon as it did in other plant foods as apple, peach, carrot... [1,3].

In the present study, two new allergens are identified in persimmon: thaumatin and chitinase. Lastly years, thaumatin is progressively having increased clinical relevance in pollens and plant-derived food, being considered as a panallergen. However, its cross-reactivity profile is still pending to be discovered. It is termstable, acid-resistant and intensely sweet-tasting protein of 23kDa present on pollens (cypress, birch, mugwort, olive, and plane), fruits (Mal d 2, Pru av 2, Act c 1 and Cap a 1) and other plant-derived food (Capa1) [8]. Chitinases are relatively small group of resistant proteins of about 30kDa. They are classified into different families and classes for which structural analyses and identification of epitopes have been only partially carried out. They have been identified as allergens in some fruits (banana, raspberry, pomegranate, avocado), vegetables (tomato), nuts (chestnuts), cereals (rice, wheat), insects (silkworm), aeroallergens (*D. farinae* and *D. pteronyssinus*) and other as latex. Precisely, these proteins are the main responsible of latex-fruits syndrome [9].

In conclusion, we report two allergens of 16kDa and 24kDa from persimmon, identified as thaumatin-like protein and chitinase, respectively. To the best of our knowledge, this is the first time that thaumatin and chitinase are reported as allergens in persimmon. More studies are needed to fully characterize persimmon's allergen and its cross-reactivity with other pollens.

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Conflict of interest

The authors have no conflicts of interest directly related to the present manuscript to declare.

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Figure 1. In vitro study with persimmon extract with both patients. Patients' immunoblotting were an IgE protein band of 16kDa in P1 (left) and 24kDa in P2 (right) were demonstrated.

