# Kale allergy, a new member in LTP syndrome

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'Superfoods' have recently gained great popularity in terms of enhancing eating patterns. Among them, kale (*Brassica oleracea var acephala*) belongs to the Brassicaceae vegetable family, which also comprises broccoli, cauliflower, cabbage, kohlrabi, and Brussels sprouts as varying forms cultivated from wild mustard. [1] Despite its widespread consumption, only anecdotal cases of allergy to Brassicaceae's vegetables have been reported so far. [2-5] In particular, there are no reports of kale hypersensitivity. Several allergens responsible for Brassicaceae allergy have been described**.** Regarding mustard allergens, 2S albumins (Sin a 1 from yellow mustard and Bra j 1 from oriental mustard), 11S globulin (Sin a 2), lipid transfer protein (LPT; Sin a 3) and profilin (Sin a 4) have been identified. [6-8] Brassicaceae LTP, Bra o 3 (9 kd), has been described as a major allergen in broccoli, cauliflower, and cabbage. [2]

We reported a case of a 26 year-old woman with allergic rhinoconjunctivitis and bronchial asthma due to grass pollen as well as peach, melon, and watermelon allergy. She presented hives and palmoplantar pruritus within 10 minutes after eating a kale salad with walnuts, cranberries, and goat cheese. Symptoms subsided in one hour after dexchlorpheniramine administration. It was the first time she ate kale and she has never eaten it again. Besides from it, she has consumed all the ingredients again without symptoms. Regarding Brassicaceae

 
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family, she tolerated broccoli and cauliflower afterwards but has not consumed cabbage, mustard, or Brussels sprouts again.

Commercial extracts were used for skin prick testing to aeroallergens (pollen, animal epithelia, dust mites, fungi) and food (peach, melon, banana). *Alternaria alternata* (10 mm), *Cupressus Arizonica* (8 mm), *Cynodon Dactylon* (7 mm), cat, and dog epitheliums (5 mm and 8 mm), grasses mix (7 mm), *Phleum pratense* (8 mm), *Olea europea* (12 mm), *Platanus acerifolia* (10 mm), peach (3 mm), and melon (4 mm) were positive. Prick by prick with watermelon (4 mm), cabbage (8 mm), Brussels sprouts (10 mm), kale (12 mm), and mustard (7 mm) were also positive. Histamine (5 mm) and physiologic saline solution (0 mm) served as positive and negative controls, respectively. Blood test results as follows: total IgE 227 kU/L (measured with UniCap System; Phadia, Uppsala, Sweden); specific IgE for PhI p 12: 0.35 kU/L, Ole e7 LTP: 0.40 kU/L, Phleum p1+ p5b: 32 kU/L, Cor a 8: 0,83 kU/L, mustard: 0,16 kU/L, Ara h 9: 7.11 kU/L, rPru p3: 5.76 kU/L, Mal d 3: 7.07 kU/L and Pla a 3: 0,00 kU/L.

Kale, mustard, cabbage, and Brussels sprouts proteins were obtained by homogenization of 20 grams in 100 ml of phosphate-buffered saline (PBS). The homogenates were centrifuged for 30 minutes at 17700 rpm at 4ºC, and the supernatant was dialyzed against deionized water. Serum specific IgE analysis was performed using an IgE slot blot assay, which revealed IgE reactivity to peach peel, kale, cabbage, watermelon, and melon. IgE reactivity was not detected in Brussels sprouts, mustard, and *Artemisia vulgaris* extracts, or PBS (negative control). (Figure 1A). SDS-PAGE IgE immunoblotting assay was performed with the patient's serum (1:10 dilution) under reducing conditions revealing IgE reactivity with a molecular weight of 9 Kda in kale, cabbage, and Pru p 3 extracts. (Figure 1B). IgE bands from kale and cabbage extracts were cut from polyacrylamide gel (15% acrylamide, 2.6% acrylamide-bis-acrylamide cross-linking) for gel trypsin digestion and analyzed using MALDI-TOF mass spectrometry





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(MS) and LC-MS/MS. Protein identification based on MS or MS/MS spectra using the MASCOT software search algorithm resulted in high homology to peach LTP (pru p 3) in both extracts.

In order to demonstrate the presence of cross-reactivity between pru p 3 and kale or cabbage extracts, SDS-PAGE immunoblotting-inhibition assays were performed. IgE binding was not completely inhibited by kale or cabbage extracts, but peach peel did (Figure 1B).

Mustard tolerance was confirmed by an open oral challenge. Increasing doses of mustard sauce were administered reaching a total dose of 10 g without symptoms.

Regarding food allergy, LTP is the most frequent cause in adults in the Mediterranean. LTP-sensitized patients may experience symptoms with the consumption of a large variety of plant foods due to their wide distribution and the high homology between LTPs from a large list of unrelated fruits and vegetables. Nevertheless, the clinical expression of this sensitization could be variable, comprising patients who tolerate most foods despite being strongly sensitized or who only experience reactions in the presence of co-factors, and others who, despite showing minimal sensitization, present significant allergic reactions. [9]

Palacin et al. identified Bra o 3, cabbage LTP, which shows cross-reactivity with other plant foods, such as mustard or peach, sharing 50% of identity with Pru p 3. [2] Sin a 3 shares 65% and around 55% of identity with Bra o 3 and Pru p 3 respectively. [9] These findings confirmed cross-reactivity between Rosacea and Brassicaceae LTPs.

According to recent publications, in peach allergic patients with or without peanut allergy, determining the IgE and IgG4 recognition patterns against linear al B-cell epitopes in different LTPs could differentiate between patients with different phenotypes of LTPs tolerance. [10]

All these facts reveal the importance of identifying and characterizing the involved allergens in an allergic reaction in order to narrow down avoidance measures and indications.





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As far as we know, this is the first case of kale allergy identifying a LTP as the culprit allergen.

## Conflicts of interest

The authors declare no conflicts of interest.





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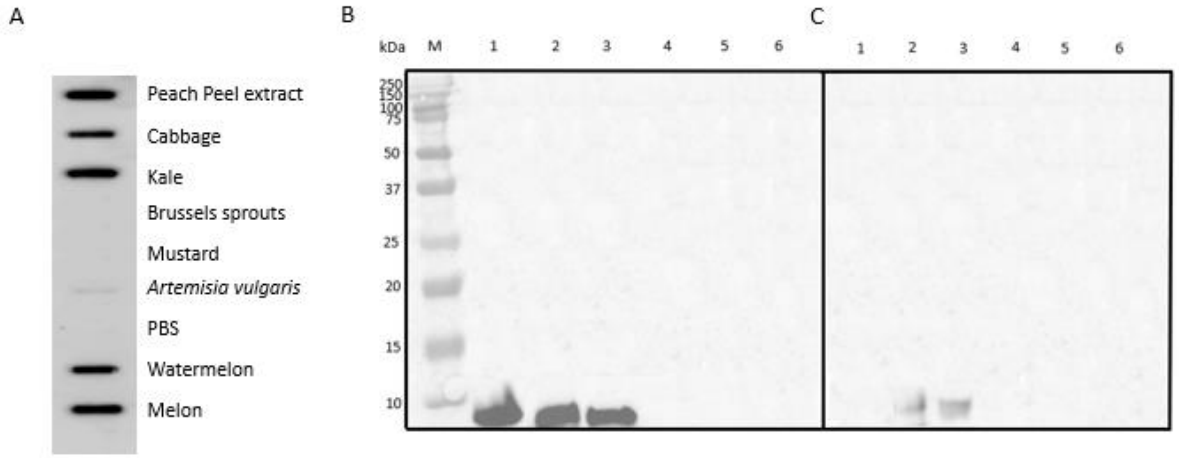
**Figure 1.** Detection of IgE reactivity protein from the Kale-allergic patient´s serum. **A.** Slot Blot; IgE binding to peach peel extract, cabbage, kale, mustard, brussels sprouts, Artemisia vulgaris, watermelon and melon extracts and PBS as negative control. **B.** Western blot of peach peel (line 1), cabbage (line 2), kale (line 3), mustard (line 4), brussels sprouts (line 5), Artemisia vulgaris (line 6). **C.** SDS-PAGE immunoblotting-inhibition with purified peach peel LTP in the inhibitory phase and the same extracts that in western blot in the solid pase.





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