Armelline Almond Allergy: The First Reported Case

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A 7-year-old child developed severe anaphylaxis immediately after eating a dessert made of hazelnut, cocoa, milk, eggs, and amaretti cookies. He experienced vomiting, facial angioedema, diffuse urticaria, laryngeal edema, and wheezing. Oxygen saturation was 83%. The reaction resolved with adrenaline, aerosolized ß2 agonists, oral corticosteroids, and antihistamines.

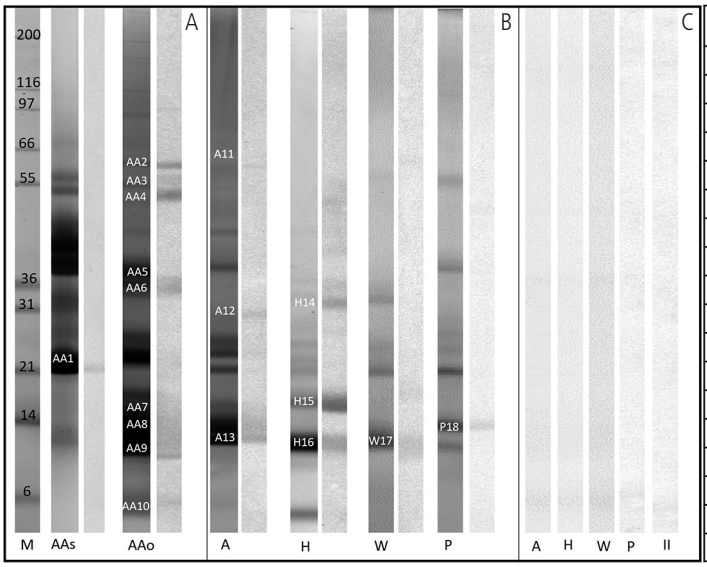
Since the patient usually consumed food containing milk, eggs, and cocoa, the diagnosis focused on almond, hazelnut, and armelline almonds (more commonly known as apricot kernel, the main ingredient of amaretti biscuits). The patient had regularly consumed hazelnut, almond, pistachio, pine nut, and cashew, as well as peach and apricot, before experiencing the severe reaction to amaretto but had never consumed peanut or walnut. Following the allergic reaction, the general practitioner recommended a diet free of nuts and peanut.

We performed a series of tests to characterize the allergic reaction, as follows: *i*) skin prick tests with hazelnut, almond, and respiratory allergens (grass pollen, birch pollen, olive pollen, composite mix, dust mite, molds, and epithelia); and *ii*) prick-by-prick tests with hazelnut, almond (raw, toasted, and processed in bakery products), the amaretto biscuit that caused the allergic reaction, and the inner and outer apricot kernel. All the skin test results were negative. Specific immunoglobulin E (sIgE) against hazelnut extract, Cor a 1, Cor a 8, Cor a 9, Cor a 14, and almond extract was determined using the automated ImmunoCap system (Thermo Fisher Scientific). All results were negative. In addition, to evaluate sensitization to other nuts and/or peanut, skin prick and prick-by-prick tests were performed with pistachio, cashew, pine nut, walnut, and peanut, as was sIgE with pistachio, pine nut, and walnut extracts and Jug r 1, Jug r 3, Ara h 1, Ara h 2, Ara h 3, Ara h 6, Ara h 8, and Ara h 9. The results of these tests were also negative. Afterwards, hazelnut and almond were reintroduced into the diet without reaction. The same was true for pistachio, cashew, and pine nut. Walnut and peanut were introduced for the first time and tolerated. The patient was warned to avoid consumption of foods containing armelline almond by carefully reading food labels.

Immunoblot analyses of water-soluble and oil-soluble armelline almond protein extracts were performed to identify any potential allergens not revealed by the skin tests. Oil-soluble hazelnut, almond, walnut, and peanut proteins were extracted and tested using immunoblotting to investigate any possible cross-reactivity between the nuts (see Supplemental Material for further detail). The study was reviewed and approved by the local ethics committee (approval no. 312 prot. no. 22050). All the families gave their written informed consent before the patients were enrolled in the study. The reactive bands were excised, digested with trypsin, and identified by means of liquid chromatography coupled with high-resolution tandem mass spectrometry, as published by Cirrincione et al [1], albeit with slight modifications (see Supplemental Material for further detail). Protein identifications are summarized in Table E1.

The immunoblot analysis revealed 18 bands that were recognized by the patient's serum in water-soluble armelline extract (1 band) and oil-soluble protein extract (9 bands), as well as in oil-soluble protein extracts of almond, hazelnut, walnut, and peanut (respectively 3, 3, 1, and 1 bands) (Figure). The only water-soluble armelline allergen recognized by the patient's IgE was a cupin type-1 domain–containing protein (band AA1). Cupins belong to the 11S seed storage protein family and form hexamers made by subunits derived from a single precursor linked by a disulfide bond. They are well-known allergens. For example, almond Pru du 6 [2], known as amandin, has more than 90% of similarity with cupin from armelline seeds. However, as the patient in the present case tolerated almonds, we hypothesized that this allergen was not involved in eliciting the allergic reaction. The main protein contained in the immunoreactive high-molecular-weight bands was 11-ß-hydroxysteroid dehydrogenase (11β-OHSD2, UniProt entry A0A6J5XIH0), while 2 oleosins were identified at a low molecular weight (UniProt entries A0A6J5UZ74 and A0A6J5UGK6). The proteins recognized in the other oil-soluble tree nut extracts, probably because of cross-reactivity, were oleosins. The role of oleosins as allergens has already been described, especially in peanuts and hazelnuts [3-5], and although they have already been found in walnut and almond at protein level, no evidence of their role as allergens has been reported.

To investigate the role of 11β-OHSD2 as an allergen, the degree of sequence similarity with known allergens was assessed by means of COMPASS (comparative sequence



BAND	FOOD	UNIPROT ENTRY	PROTEIN NAME
AA1	Armelline almond	A0A6J5Y7I7	Cupin type-1 domain-containing protein
AA2	Armelline almond	A0A6J5XIH0	11-β-hydroxysteroid dehydrogenase
AA3	Armelline almond	A0A6J5WQN7	Fungal lipase-like domain-containing protein
AA4	Armelline almond	A0A6J5XIH0	11-β-hydroxysteroid dehydrogenase
AA5	Armelline almond	A0A6J5TVS9	11-β-hydroxysteroid dehydrogenase
AA6	Armelline almond	A0A6J5XIH0	11-β-hydroxysteroid dehydrogenase
AA7	Armelline almond	A0A6J5UZ74	Oleosin
AA8	Armelline almond	A6J50AUGK6	Oleosin
AA9	Armelline almond	A0A6J5UGK6	Oleosin
AA10	Armelline almond	A0A6J5UGK6	Oleosin
A11	Almond	Q43804	Oleosin
A12	Almond	A0A5E4ET55	Oleosin
A13	Almond	Q43608	Oleosin
H14	Hazelnut	C0HM28	Oleosin Cor a 15
H15	Hazelnut	C0HM28	Oleosin Cor a 15
H16	Hazelnut	Q84T91	Oleosin Cor a 13
W17	Walnut	G8H6H9	Oleosin
P18	Peanut	Q647G5	Oleosin Ara h 10

Figure. A, LDS-PAGE of armelline almond soluble proteins (AAs) and armelline almond oleosome (AAo), with the results of the corresponding immunoblotting against the serum of the allergic patient. B, LDS-PAGE of almond oleosome (A), hazelnut oleosome (H), walnut oleosome (W), and peanut oleosome (P). The corresponding immunoblot for the sera of the allergic patient is shown beside each LDS-PAGE lane. C, Pool of 5 nonallergic patients used as a negative control. The numbers 1 to 18 refer to the immunoreactive bands. M indicates molecular weight markers; LDS-PAGE, lithium dodecyl sulfate polyacrylamide gel electrophoresis.

search software, https://comparefasta.comparedatabase.org/). The results matched with a seed maturation-like protein precursor also known as glucose and ribitol 11 dehydrogenase (UniProt entry A0A0A6YYW2) from *Sesamum indicum*, with 27.5% sequence identity, 63.8% similarity, and an E-score of $2.9e^{-18}$. This protein has already been reported to be a potential minor allergen in sesame allergy by Beyer et al [6], and its sequence was submitted to the NCBI database by Grishina et al in 2008 (https://www.uniprot.org/citations/CI-F5R3IGCA0QMCU). Hamada et al [7] reported 11β-OHSD2 as a novel wheat allergen in patients with baker's respiratory allergy [8]. Since it is part of the steroleosin family [9], there is evidence that steroleosins could also be food allergens.

Armelline almond is present in numerous baked products and is labeled as either *armelline almond* or *apricot almond*. This can cause confusion, leading to unnecessary eliminations from the diet or, conversely, failure to identify potentially dangerous food.

We report the first case of allergy to armelline almond in a patient with no other food allergies.

Since the patient tolerates nuts and *Prunus* fruit, we consider that immunoreactivity to armelline oleosins is not a cross-reaction but could be due to primary sensitization to armelline seeds. Moreover, the discovery of a new immunoreactive protein, 11β-OHSD2, which was undetectable in the other nuts analyzed, leads us to speculate about its specific role in armelline almond allergy. Further research should be performed to clarify the role these proteins play, and their relevance as allergens should be demonstrated in a cohort of patients.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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