Check for updates

Received: 25 August 2020 | Revised: 26 May 2021 | Accepted: 10 June 2021

DOI: 10.1111/pai.13579

ORIGINAL ARTICLE

WILEY

Oleosin Cor a 15 is a novel allergen for Italian hazelnut allergic children

Stefano Nebbia[1] | Cristina Lamberti[1] | Simona Cirrincione[1] | Alberto Acquadro[2] | Simona Abbà[3] | Marina Ciuffo[3] | Daniela Torello Marinoni[2] | Marcello Manfredi[4] | Emilio Marengo[4] | Roberta Calzedda[5] | Giovanna Monti[5] | Laura Cavallarin[1] | Maria Gabriella Giuffrida[1]

[1]Institute of Science of Food Production, National Research Council, Grugliasco, Italy

[2]Department of Agricultural, Forest and Food Sciences, University of Torino, Grugliasco, Italy

[3]Institute for Sustainable Plant Protection, National Research Council, Torino, Italy

[4]Center for Translational Research on Autoimmune and Allergic Disease - CAAD, University of Piemonte Orientale, Novara, Italy

[5]SC Pediatria, Ospedale Infantile Regina Margherita, Città della Scienza e della Salute, Torino, Italy

Correspondence
Laura Cavallarin, Institute of Science of Food Production, National Research Council, Largo Braccini 2, 10095 Grugliasco, Italy.
Email: laura.cavallarin@ispa.cnr.it.

Funding information
This study was supported by the CRT Foundation, Turin, Italy (grant no. 2014.2693).

Editor: Alexandra Santos

Abstract

Background: Hazelnut allergy, which is characterized by symptoms that range from mild to severe, is one of the most common allergies in children throughout Europe, and an accurate diagnosis of this allergy is therefore essential. However, lipophilic allergens, such as oleosins, are generally underrepresented in diagnostic tests. We therefore sought to characterize the IgE reactivity of raw and roasted hazelnut oleosins, using the sera of hazelnut-allergic pediatric patients.

Methods: Raw and roasted hazelnut oil body–associated proteins were analyzed by means of 1D and 2D electrophoresis and MS. Oleosin IgE reactivity was assessed by immunoblotting with the sera of 27 children who have confirmed hazelnut allergies and from 10 tolerant subjects. A molecular characterization of the oleosins was performed by interrogating the *C. avellana* cv. Jefferson and cv. TGL genomes, and through expression and purification of the recombinant new allergen.

Results: A proteomic and genomic investigation allowed two new oleosins to be identified, in addition to Cor a 12 and Cor a 13, in hazelnut oil bodies. One of the new oleosins was registered as a new allergen, according to the WHO/IUIS Allergen Nomenclature Subcommittee criteria, and termed Cor a 15. Cor a 15 was the most frequently immunorecognized oleosin in our cohort. Oleosins resulted to be the only immunorecognized allergens in a subgroup of allergic patients who showed low ImmunoCAP assay IgE values and positive OFC and PbP. Hazelnut roasting resulted in an increase in oleosin immunoreactivity.

Conclusion: A novel hazelnut oleosin, named Cor a 15, has been discovered. Cor a 15 could play a role in eliciting an allergic reaction in a subgroup of pediatric patients that exclusively immunorecognize oleosins. The high prevalence of hazelnut oleosin sensitization here reported further confirms the need to include oleosins in routine diagnostic procedures.

Nebbia and Lamberti contributed equally to this work. Laura Cavallarin and Maria Gabriella Giuffrida share co-seniorship.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.
© 2021 The Authors. *Pediatric Allergy and Immunology* published by European Academy of Allergy and Clinical Immunology and John Wiley & Sons Ltd.

KEYWORDS
Cor a 15, hazelnut allergy, oleosins, pediatric population, roasting

1 | INTRODUCTION

Hazelnut (*Corylus avellana L.*) allergy is the most common tree nut allergy in Europe[1], with a different prevalence being observed among European countries.[2] Associated oil body (OB) proteins have been receiving increasing interest, since oleosins have been described as major allergens in peanuts[3,4] and in sesame.[5,6] Moreover, they have been associated with severe symptoms as a result of the consumption of hazelnuts.[7,8] In 2015, the EuroPrevall project[2] established that hazelnut oleosin sensitization is quite common in Europe (which has from 10 to 25% of hazelnut-allergic patients), with a higher prevalence in children than in adults (34% vs 11.4%). Akkerdaas et al.[9] reported the two first allergenic oleosins in hazelnuts, Cor a 12 and Cor a 13.

Oleosins are usually underrepresented in skin prick test (SPT) extracts and in vitro reagents, thereby giving rise to false negatives in hazelnut allergy diagnosis.[7,10] Their absence in test reagents is due to their hydrophobic structure, which results in their eluding the standard protein purification procedures. Zuidmeer-Jongejan et al.[7] pointed out the absence of Cor a 12 and Cor a 13 in nine of the most commonly used SPT extracts.

The IgE binding potential of peanut oleosins has recently been found to be increased by in-shell roasting.[4] However, the effect of roasting on hazelnut oleosin immunorecognition remains unknown.

Our aim was to investigate the contribution of oleosins to hazelnut pediatric allergies and to assess the effect of roasting on hazelnut oleosin immunogenicity.

Key Message

The manuscript deals with the discovery of a new hazelnut allergen, Cor a 15. It also provides novel evidence concerning the role of oleosins in hazelnut allergy, the most common tree nut allergy in Europe. The research findings are relevant for clinical practice, since they confirm the need to include lipophilic allergens, such as oleosins, in the protein extracts used for routine diagnostic tests, as already found for peanuts.

2 | METHODS

Details about the methods used for most of the conducted experiments are available in the Online Repository.

2.1 | Study population and clinical methods

Pediatric subjects with a convincing history of hazelnut allergy (HA patients, no. = 27) and pediatric subjects sensitized but tolerant to hazelnuts (HS patients, no. = 10) were retrospectively recruited from the Paediatric Allergy Unit, Regina Margherita Children's Hospital (Città della Salute e della Scienza, Turin, Italy). Hazelnut allergy was defined as a convincing history of allergic symptoms (oral allergy syndrome (OAS), urticaria, angioedema, respiratory, cardiovascular, and/or gastrointestinal symptoms) appearing within 1 h after hazelnut ingestion. The allergy diagnosis was confirmed by means of an oral food challenge (OFC) (except for patients with severe anaphylaxis), by prick by prick (PbP), and by hazelnut sIgE dosed with ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden) (Table 1).

All the HA patients reported having had a reaction to foods containing roasted hazelnuts (cocoa, hazelnut spreads, chocolate, hazelnut ice cream, chocolate pralines with whole roasted hazelnuts, or hazelnut nougat). For this reason, the OFC was performed with finely ground roasted hazelnuts.

The OFC was initiated using one drop of physiological solution mixed with the hazelnut powder and placing the solution on the inner side of the lower lip. The challenge was continued with increasing doses of hazelnut powder (0.2 g, 0.4 g, 1 g, 2 g, 3 g, 6 g, 8 g, and 10 g) administered in 20-min intervals, unless symptoms occurred or until a total cumulative dose of 30 g of hazelnut powder (equivalent to 15 hazelnuts). The OFC procedure was stopped after observation of the first objective symptom. In the case of mild objective or subjective symptoms, it was decided to wait longer for the subsequent dose and/or to repeat the same dose, and the OFC procedure was stopped in the case of persistent symptoms (lasting longer than 30 minutes) and/or worsening symptoms and in the case of repeated elicitation (over 3 doses). The scoring of the severity of the reaction to the OFC was evaluated independently by two allergy specialists (G.M. and R.C.), and the results were coded as mild, moderate, or severe, according to the Integrated Grading System for Scoring Reaction Severity of Oral Food Challenge Reactions of Zhu et al.[11]

All the patients underwent PbP, using both raw and roasted hazelnuts. Histamine (10 mg/ml) was used as a positive control, and a saline solution was used as the negative control. PbP was considered positive if the wheal was higher than 3 mm, and there was no reaction to the negative control.

The IgEs sera were assayed against a hazelnut extract, rCor a 1, rCor a 8, rCor a 9, rCor a 14, and rBet v 1 using ImmunoCAP. The cut-off for positivity was set at 0.10 KU/l. rCor a 1, rCor a 8, rCor a 9, and rCor a 14 sIgE concentrations were standardized for the statistical

analysis by subtracting the mean values of each variable and then dividing by the corresponding standard deviations. The sIgE values below the limit of detection (<0.10 KU/L) were assigned a value of 0.01; the sIgE values above the limit of detection (>100 KU/L) were assigned a value of 100. Missing data (codified in Table 1 with n.d.) were not considered in the statistical analysis. The standardized data of the patients classified as HA versus the patients classified as HS were analyzed by means of nonparametric Kruskal-Wallis one-way ANOVA, because of the non-normal distribution. The correlation between the standardized sIgE concentrations of the different allergens was calculated by means of Kendall's tau. All the analyses were performed using PAST software, version 2.17.[12]

A subgroup of the HA patients (patient nos. 23, 24, 25, 26, and 27), who were characterized by low rCor a 1, rCor a 8, rCor a 9, and rCor a 14 ImmunoCAP IgE values, ranging from 0.10 kU/L to 1.88 kU/L, were defined as low ImmunoCAP hazelnut-allergic (LIHA) patients.

The HS patients were characterized according to the presence of atopic dermatitis (AD) and/or other food allergy (FA) and/or other nut allergies (NA), with a positive PbP to hazelnut and/or positive sIgE to hazelnut, but negative to OFC.

The experimental controls were the sera of non-nut-allergic pediatric subjects (NNA) and of non-food-allergic pediatric subjects (NFA), used individually or pooled.

The study was approved by the local ethics committee of the Città della Salute e della Scienza (Turin, Italy) (approval no. 312, prot. no. 22050). The parents of all the patients gave written informed consent.

2.2 | Hazelnut water-soluble and OB-associated protein extraction

Raw hazelnuts (*Corylus avellana* cv. Tonda Gentile delle Langhe, TGL) were obtained from experimental fields, roasted at a laboratory scale (120°C for 30 min), and used for the OFC, PbP, and immunoblotting experiments. The water-soluble proteins were extracted according to Platteau et al.[13] The hazelnut OBs were isolated according to Cao et al.[14] and the OB-associated proteins were extracted according to Lamberti et al., 2020.[15]

2.3 | Protein separation by means of LDS-PAGE, 2-dimensional electrophoresis (2-DE), and immunoblotting analysis

The hazelnut water-soluble proteins were separated by means of LDS-PAGE. The OB-associated proteins were separated by means of both LDS-PAGE and 2-DE. Protein bands and spots were electro-transferred to PVDF membranes and incubated with the patient's serum. Immunoblotting was developed using an Alkaline Phosphatase Substrate Kit (Bio-Rad).

2.4 | Use of sera

Individual serum was used for 1D immunoblotting of the OB protein extracts, ELISA, and an immunoblotting inhibition assay with a raw OB protein extract. Pooled sera were used for the remaining experiments: 2D immunoblotting of the OB protein extracts, an immunoblotting inhibition assay with rCor a 15, and immunoblotting of the total water-soluble hazelnut protein extracts, due to the paucity of the available sera.

2.5 | Protein identification by means of MALDI-TOF/TOF and ESI-Q-TOF analyses

In-gel digestion was performed to identify the allergens contained in the bands and spots from the gel electrophoresis, as in Nebbia et al.,[16] and protein identification was performed by coupling the MALDI-TOF/TOF[17] and ESI-Q-TOF-MS techniques.[15] The spectra were searched against the NCBI *C. avellana* database implemented with two new oleosin sequences derived from both the *C. avellana* cv Jefferson genome[18] and *C. avellana* cv TGL.[19]

2.6 | Expression and purification of recombinant Cor a 15

In order to establish whether the newly discovered oleosin was an allergen, it was produced as a recombinant protein. Its complete coding sequence, obtained by means of RNA retrotranscription, was cloned in a pGEM-T Easy vector (Promega, Madison, WI, The USA) and sent to PRIMM (Milan, Italy) for protein expression and purification. The full-length allergen (rCor a 15) and the C-terminal fragment (rCor a 15 C-term, from aa 102 to aa 169) were used for the blot inhibition studies, and only the full-length rCor a 15 was used for ELISA.

2.7 | Quantification of Cor a 15-specific IgEs by means of direct ELISA test

IgEs specific to rCor a 15 were quantified as described in Hilger et al.,[20] with some modifications. Briefly, patient sera (10 HA, 3 LIHA, 2 HS, 9 NNA, and 9 NFA patients) were transferred to 96-well plates previously coated with 0.1 μg/ml of rCor a 15. In order to obtain a semi-quantification of hazelnut-allergic patient IgE against rCor a 15, the serum of a patient with a known titer of IgE specific to α-lactalbumin (as determined by ImmunoCAP) was used as the reference standard. The reference serum was chosen since it contained IgE that was completely unrelated to the allergy that was the subject of this study. The reference serum was serially diluted in duplicate and added to α-lactalbumin-coated wells. The obtained standard curve allowed us to calculate the IgE titers specific to rCor a 15 for each patient's serum.

TABLE 1 Clinical characterization of the patient cohort (N=37)

Panel A: first patient recruitment														
									Hazelnut PbP (ø mm)		IgE ImmunoCAP (KU/l)			
ID	Patient classification	Sex	Age (y)	FHA	AD	FA	PA	NA	Raw	Roasted	Hazelnut	rCor a 1	rCor a 8	rCor a 9
1	HA	M	15	Yes	Yes	Yes	No	No	6	6	75.80	58.80	1.02	19.20
2	HA	M	5	Yes	Yes	No	Yes	No	13	5	13.70	0.10	0.25	9.80
3	HA	M	11	Yes	No	Yes	No	No	12	6	51.70	72.30	<0.10	4.95
4	HA	F	7	Yes	Yes	Yes	Yes	Yes	10	10	28.90	<0.10	<0.10	27.40
5	HA	M	8	Yes	Yes	Yes	No	Yes	9	8	73.00	64.30	19.20	11.20
6	HA	F	12	Yes	Yes	Yes	Yes	Yes	9	8	84.20	<0.10	<0.10	73.40
7	HA	M	7	Yes	No	Yes	Yes	Yes	6	4	55.90	<0.10	0.54	39.30
8	HA	F	7	Yes	Yes	Yes	No	Yes	5	5	9.55	<0.10	<0.10	19.90
9	HA	M	8	Yes	Yes	Yes	Yes	Yes	8	5	16.60	11.50	<0.10	6.85
10	HA	M	12	Yes	No	No	Yes	Yes	10	14	>100	29.10	0.11	>100
11	HA	M	11	No	No	Yes	Yes	No	3	0	30.70	0.42	36.80	0.69
12	HA	F	16	Yes	Yes	Yes	No	Yes	10	8	41.90	<0.10	<0.10	34.70
13	HA	M	10	Yes	Yes	Yes	Yes	Yes	10	8	7.45	4.33	<0.10	1.92
14	HA	M	6	Yes	Yes	No	No	No	6	7.5	3.75	<0.10	<0.10	<0.10
15	HA	M	10	Yes	Yes	No	Yes	Yes	7	6.5	38.20	59.70	0.75	0.22
16	HA	M	10	Yes	Yes	No	Yes	Yes	8	4.5	69.00	0.31	>100	29.10
17	HA	M	14	Yes	Yes	Yes	Yes	Yes	7	8	>100	17.40	2.30	56.90
18	HA	M	4	Yes	Yes	No	No	No	5	15	20.10	<0.10	0.73	8.57
19	HA	F	13	n.d.	Yes	Yes	Yes	Yes	11	7.5	>100	0.12	<0.10	>100
20	HA	M	14	Yes	Yes	Yes	Yes	Yes	10	7	3.62	<0.10	0.49	9.18
21	HA	F	9	Yes	Yes	Yes	Yes	No	3	6	48.00	2.73	29.80	9.68
22	HA	M	2	Yes	Yes	Yes	No	Yes	3	0	2.75	0.40	<0.10	n.d.
23	LIHA	M	11	Yes	No	Yes	No	Yes	5	5	0.76	<0.10	1.78	0.13
24	LIHA	M	12	Yes	Yes	Yes	Yes	Yes	12	7	1.29	0.55	0.13	<0.10
25	LIHA	M	16	Yes	Yes	Yes	Yes	No	7	5	0.71	<0.10	<0.10	0.36
26	LIHA	M	5	n.d.	n.d.	Yes	No	No	5	6	1.59	1.88	<0.10	<0.10
27	LIHA	F	5	n.d.	n.d.	No	Yes	Yes	5	0	0.45	<0.10	<0.10	1.13
28	HS	F	7	Yes	Yes	Yes	No	Yes	3	5	7.76	<0.10	6.50	0.32
29	HS	M	6	Yes	Yes	Yes	No	No	5	5	24.70	38.10	0.85	<0.10
30	HS	F	6	No	Yes	No	Yes	Yes	4.5	0	78.00	>100	1.37	1.74
31	HS	M	17	Yes	Yes	Yes	Yes	No	10	10	7.42	7.53	<0.10	0.71
32	HS	F	12	Yes	No	Yes	Yes	No	4.5	0	47.40	58.70	<0.10	0.18
33	HS	M	11	Yes	Yes	Yes	Yes	Yes	3	0	0.65	<0.10	<0.10	<0.10
34	HS	M	11	Yes	No	No	Yes	Yes	5	0	15.20	<0.10	14.60	<0.10
35	HS	M	9	Yes	Yes	Yes	No	Yes	5	4	6.44	<0.10	0.18	1.00
36	HS	M	7	Yes	No	Yes	No	No	12	9	6.14	<0.10	<0.10	0.65
37	HS	M	7	Yes	Yes	No	Yes	Yes	5	0	54.90	<0.10	<0.10	79.00

Abbreviations: A, angioedema; AD, atopic dermatitis; AN, anaphylaxis; AP, abdominal pains; AS, asthma; C, cough; ER, erythema; F, female; FA, other food allergy; FHA, family history of atopy; H, hoarseness; HA, hazelnut-allergic patient; HS, hazelnut-sensitized patient; HY, hypotension; LAE, labial edema; LE, laryngeal edema; LIHA, low ImmunoCAP hazelnut-allergic patient; M, male; NA, other nut allergy; NS, nasal symptoms; OAS, oral allergy syndrome; OS, ocular symptoms; PA, pollen allergy; PI, pharyngeal itching; R, respiratory problems; U, urticaria; V, vomiting.

				Reduced immunoblotting to RAW hazelnut OB		Reduced immunoblotting to ROASTED hazelnut OB	
rCor a 14	rBet v 1	Roasted OFC Symptoms	OFC symptoms Severity grading	14-kDa band	17-kDa band	14-kDa band	17-kDa band
67.80	55.80	OS, NS, V, ER, AP, LE, H	Severe	No	No	Yes	Yes
4.50	<0.10	OAS, LAE	Mild	Yes	Yes	Yes	Yes
5.43	<0.10	OAS, PI, AP	Moderate	No	Yes	No	Yes
39.30	<0.10	V, OAS, PI, ER	Moderate	No	No	Yes	Yes
26.10	<0.10	V, AS, LE	Severe	No	No	Yes	Yes
41.20	n.d.	AN	Severe	No	No	Yes	Yes
4.16	<0.10	V, AP, ER	Moderate	Yes	No	Yes	Yes
3.94	<0.10	OAS, AP, NS, C, H	Severe	Yes	No	Yes	Yes
4.76	9.80	OAS, C, ER	Moderate	Yes	Yes	Yes	Yes
>100	36.50	V, AP, U, LE, AS	Severe	No	Yes	Yes	Yes
0.27	4.44	ER	Mild	No	Yes	No	Yes
19.90	<0.10	OAS	Mild	Yes	Yes	Yes	Yes
1.49	4.40	OAS, PI, ER	Mild	No	Yes	Yes	Yes
4.67	<0.10	ER, U, AP, LAE	Moderate	No	Yes	Yes	Yes
<0.10	>100	U, PI	Mild	No	Yes	No	Yes
81.50	0.52	U, PI	Mild	No	Yes	No	Yes
74.50	57.60	U, PI	Mild	No	Yes	Yes	Yes
26.30	<0.10	AS, C, R	Severe	No	Yes	Yes	Yes
>100	<0.10	U	Mild	No	Yes	No	Yes
4.94	<0.10	LE, AP	Severe	No	Yes	No	Yes
34.30	n.d.	OAS	Mild	Yes	Yes	Yes	Yes
4.98	<0.10	LAE, U, LE	Severe	No	No	No	No
0.47	<0.10	OAS, PI, H	Severe	No	Yes	No	Yes
<0.10	<0.10	LAE, A, U	Mild	No	Yes	Yes	Yes
0.76	<0.10	LAE	Mild	No	Yes	Yes	Yes
0.70	4.98	LAE	Mild	No	Yes	No	Yes
0.19	<0.10	OS, U, C	Moderate	No	Yes	No	Yes
<0.10	<0.10	None		No	Yes	Yes	Yes
<0.10	<0.10	None		Yes	Yes	Yes	Yes
0.69	>100	None		No	Yes	Yes	Yes
0.14	15.80	None		No	Yes	Yes	Yes
<0.10	67.30	None		No	Yes	Yes	Yes
0.30	<0.10	None		No	Yes	Yes	Yes
<0.10	<0.10	None		No	Yes	Yes	Yes
0.91	<0.10	None		No	Yes	Yes	Yes
1.37	<0.10	None		No	No	No	Yes
6.40	n.d.	None		No	Yes	no	Yes

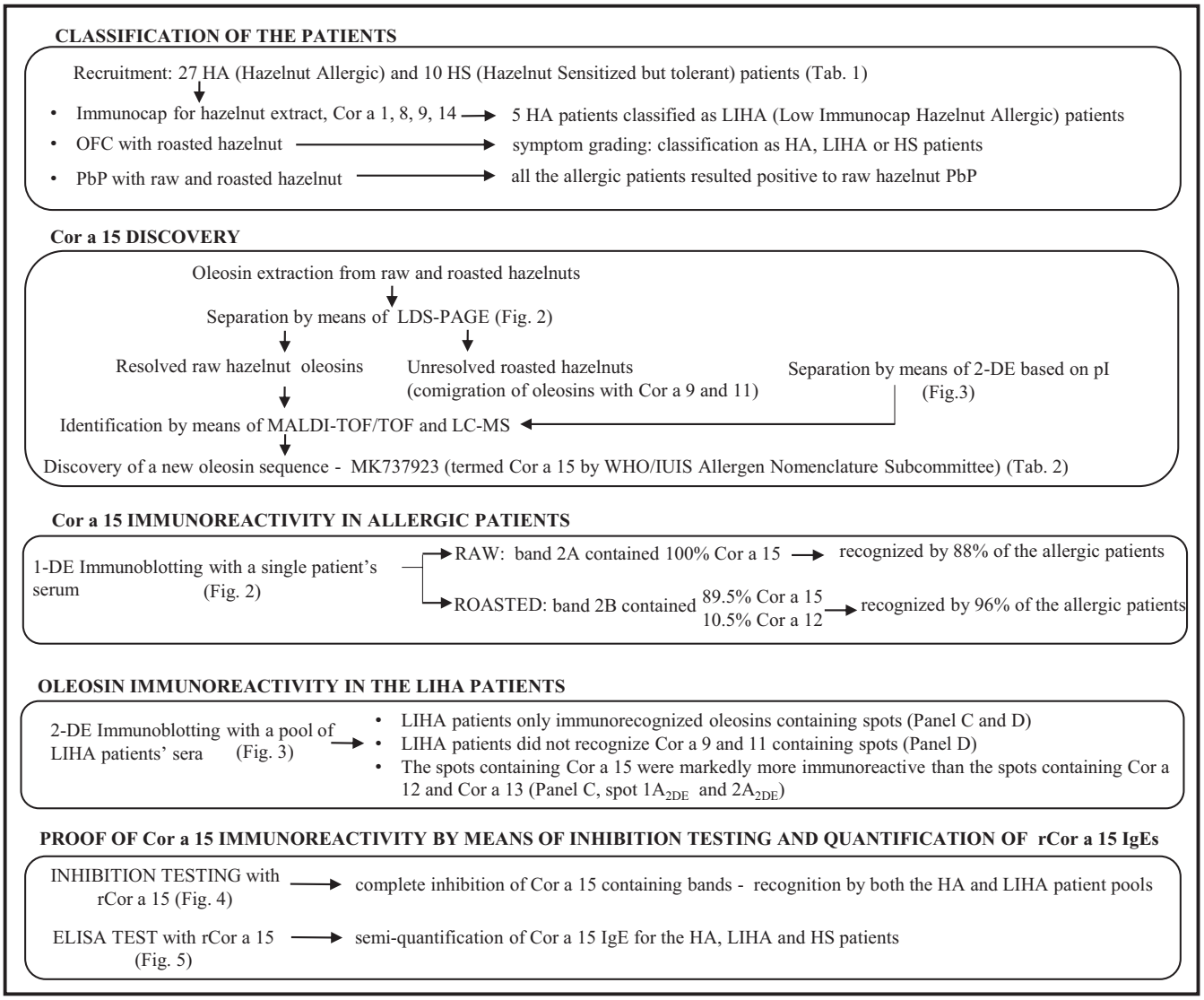

FIGURE 1 Workflow of the experiments

3 | RESULTS

A flowchart of the experiments and a summary of the main results are given in Figure 1.

3.1 | Study population

The main characteristics of the pediatric subjects included in the study are summarized in Table 1. All the patients tested with PbP resulted positive to raw hazelnuts: 24/27 HA patients and 6/10 HS patients showed a wheal diameter ≥ 5 mm. Three HA patients out of 27 and 5/10 HS patients showed negative PbP results when tested for roasted hazelnuts.

The median scores of the sIgE of rCor a 1, rCor a 8, rCor a 9, and rCor a 14 for the HA patients were 0.31 (IQR 0.01–11.50), 0.11 (IQR 0.01–1.02), 9.18 (IQR 0.36–29.10), and 4.94 (IQR 0.76–39.30), respectively. The median scores of the sIgE of rCor a 1, rCor a 8, rCor a 9, and rCor a 14 for the HS patients were 0.01 (IQR 0.01–38.10), 0.10 (IQR 0.01–1.37), 0.49 (IQR 0.01–1.00), and 0.22 (IQR 0.01–0.91), respectively. The HA and HS patients differed significantly as far as the rCor a 9 and rCor a 14 values are concerned ($p_{Cora9} = 0.004$, $p_{Cora14} = 0.002$), while Cor a 1 and Cor a 8 did not discriminate the HA from the HS patients ($p_{Cora1} = 0.75$, $p_{Cora8}=0.98$). Furthermore, the rCor a 9 and rCor a 14 values were directly correlated ($r^2 = 0.67$; $p < .001$). Seventeen patients (12/27 HA patients and 5/10 HS patients) also suffered from a pollen allergy (PA). Finally, 17/27 HA patients and 6/10 HS patients were allergic to other tree nuts (NA). It was not possible to establish a correlation between the severity grading and oleosin immunorecognition because of the limited number of LIHA patients.

3.2 | Discovery of two new hazelnut oleosins

The OB-associated proteins were separated (Figures 2 and 3) and identified (Table 2, Table S1, and Table S2), and two new oleosin-like proteins were found (Figure S1). Their sequences were submitted to

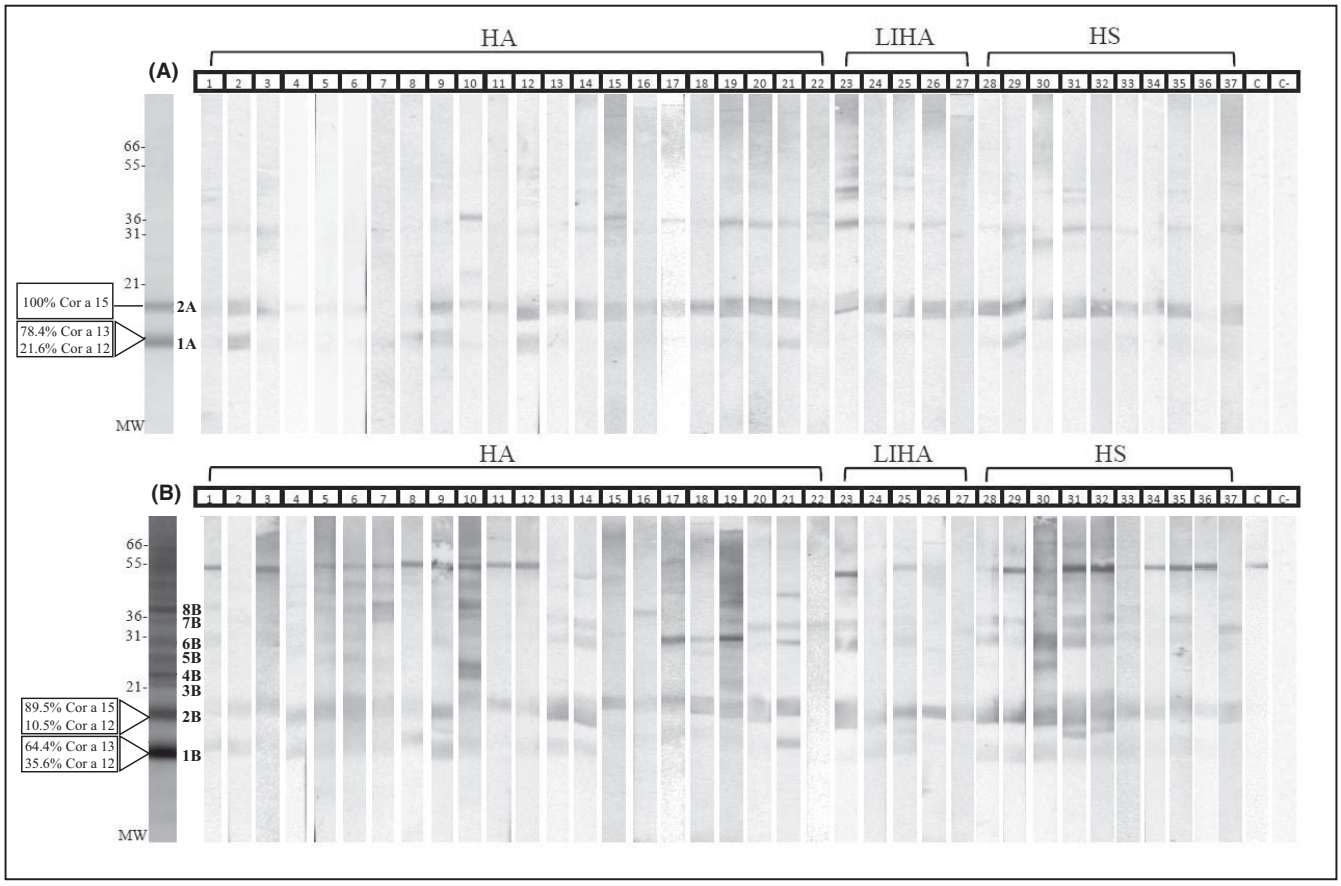

FIGURE 2 For each panel: first lane, stained LDS-PAGE of raw (A) and roasted (B) OB-associated protein extracts; lanes 1 to 37: immunoblotting with individual patient's serum. C, a pool of five sera of non-nut-allergic (NNA) pediatric subjects was used as a control. CII, secondary antibody control. MW, molecular weight; HA, hazelnut-allergic patients; LIHA, low ImmunoCAP hazelnut-allergic patients; HS, hazelnut-sensitized patients

GenBank under accession numbers MK737923 and MK737924. The MK737923 sequence showed 56% similarity with Cor a 12 and 41% with Cor a 13. The MK737924 sequence showed 63% similarity with Cor a 12 and 76% with Cor a 13 (Figure S2).

3.3 | IgE reactivity to hazelnut OB-associated proteins and discovery of Cor a 15

We hypothesized that other allergens, not included in the ImmunoCAP assay, could account for the positivity of the LIHA patients to OFC. We thus performed immunoblotting to evaluate the immunoreactivity of the single patients to the OB-associated proteins. Two oleosin-containing bands were found at 14 and 17 kDa (Figure 2).

One hundred percent of the allergic patients recognized at least one oleosin-containing band (Table 1). Thirty-three percent recognized the 14-kDa band, containing both Cor a 12 and Cor a 13 (band 1A in Figure 2, Table 2). The 17-kDa band, which exclusively contains the MK737923 oleosin (band 2A in Figure 2 and Table 2), was the most recognized one and was immunoreactive for 88% of the allergic patients. The MK737923 oleosin was therefore submitted as an allergen candidate to the WHO/IUIS Allergen Nomenclature Subcommittee, who approved it as a new hazelnut allergen and termed it Cor a 15. The three hazelnut oleosins were also immunorecognized by the HS patients, with a similar frequency to that of the allergic patients (Table 1 and Figure 2).

In order to verify that the LIHA patients did not recognize any other water-soluble allergens than those found negative by ImmunoCAP, a total hazelnut water-soluble protein extract was tested by immunoblotting against the pooled sera of the LIHA patients (nos. 23, 24, and 25) and against the pooled sera of the HA patients (nos. 1 to 14). The IgEs of the LIHA patients did not bind with any protein band, while several protein bands were recognized by the HA pool (Figure S3).

3.4 | Effect of roasting on the hazelnut oleosin profile

We further assessed whether the hazelnut OB protein profile was affected by processing, since hazelnuts are usually consumed roasted. In addition to the 14-kDa and 17-kDa bands, higher MW bands appeared (Figure 2, panel B). The 14-kDa band, containing a mixture of Cor a 12 and Cor a 13 (band 1B in Figure 2 and Table 2),

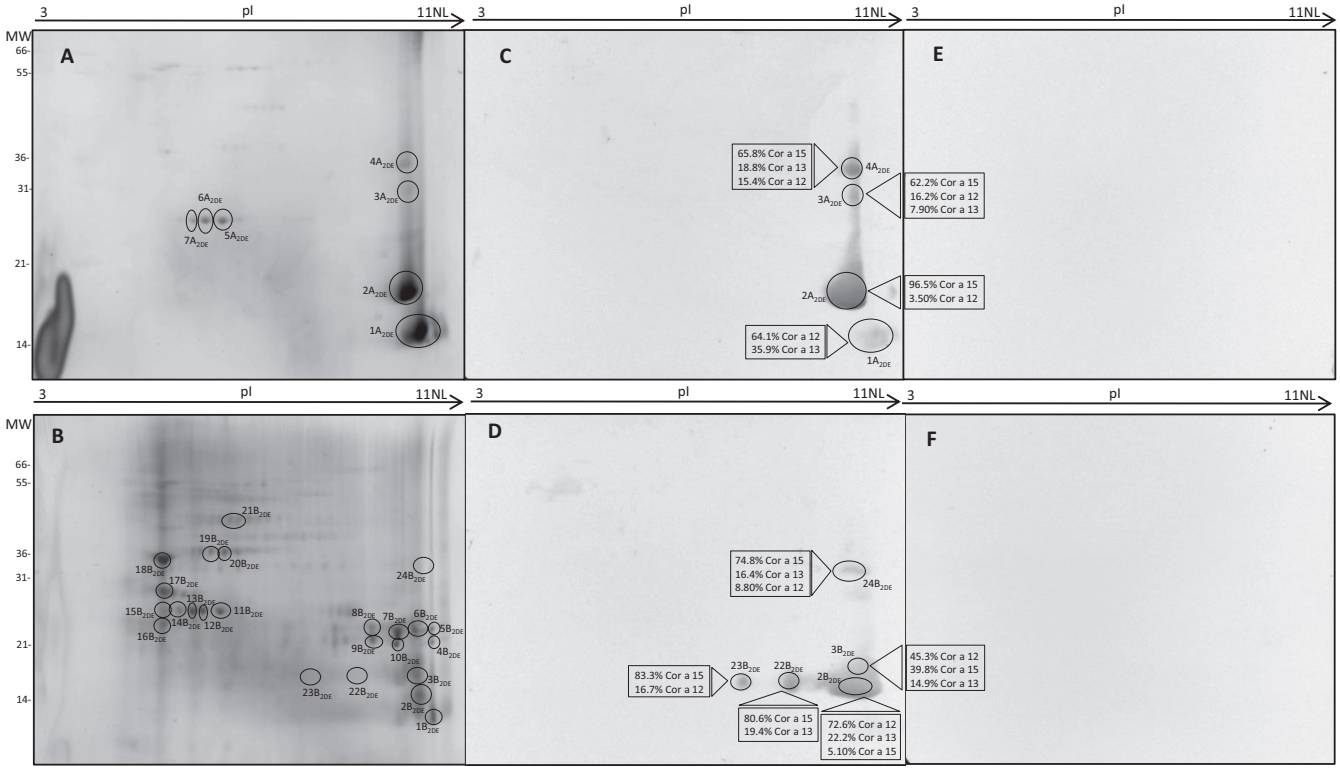

FIGURE 3 2-DE and immunoblotting of the OB-associated protein extracts. 2-DE of raw (A) and roasted (B) hazelnut OB protein extracts. Immunoblotting of raw (C) and roasted (D) hazelnut OB protein extracts with the pool of sera of the LIHA patients (nos. 23, 24, and 25). Immunoblotting of raw (E) and roasted (F) hazelnut OB protein extracts with a pool of five sera of non-nut allergic (NNA) pediatric subjects. MW, molecular weight; pI, isoelectric point; NL, non-linear

was recognized by 62% of the allergic patients (Table 1). The 17-kDa band, containing Cor a 15 with traces of Cor a 12 (band 2B in Figure 2 and Table 2), was again the most recognized band (96% of the allergic children) (Table 1). The bands around 20–40 kDa, generated by roasting, were recognized by almost all the patients. In addition to oleosins, these bands also contained Cor a 9 and/or Cor a 11 soluble hazelnut allergens (bands 3B to 8B in Figure 2, Table 2). To discriminate whether the LIHA patients' sera immunoreacted to Cor a 9 and Cor a 11 or against oleosins, a 2DE separation of the OB-associated proteins was performed, exploiting the different isoelectric points of these allergens (Figure 3, panels A and B). Most of the spots in the alkaline zone (right part) of the 2DE map were identified as oleosins, or as the basic subunit of Cor a 9, while those in the acidic zone were found to contain acidic subunits of Cor a 9 and Cor a 11 (Table 2).

In spite of the different 2DE protein profiles of the raw and roasted OB proteins (Figure 3, Panel A and B), the immunoreactivity profiles were very similar (Figure 3, Panels C and D) and exclusively localized in the alkaline region.

3.5 | Hazelnut oleosins are major allergens for LIHA patients and Cor a 15 is the most frequently recognized oleosin

Most reactive spots on 2DE separation of raw and roasted extracts contained co-migrating oleosins exclusively: Cor a 12, Cor a 13, and Cor a 15 (Figure 3, Table 2). Interestingly, the $1A_{2DE}$ and $2A_{2DE}$ spots, which showed a similar densitometric intensity in the 2DE gel, resulted in a highly different immunoreactivity. Spot $2A_{2DE}$, which contained 96.5% of Cor a 15, was much more immunoreactive than the $1A_{2DE}$ spot, which exclusively contained Cor a 12 and 13 (Figure 3, Panels A and C, and Table 2).

In order to verify whether Cor a 15 was the most frequently recognized oleosin, full-length Cor a 15 and its C-term fragment (from aa 102 to aa 168) were expressed and purified (Figure 4, panels A and C). The C-terminal fragment was expressed because it contains a similar peptide (11 aa out of 14 aa) to the most immunoreactive IgE epitope in Ara h 15[4] (Figure S4). The addition of full-length rCor a 15 to both the LIHA and HA pooled patients' sera completely inhibited the recognition of the 17-kDa band exclusively containing Cor a 15 (Figure 4, panel B). The same amount of rCor a 15 C-terminal did not result in the complete inhibition of the immunoreactivity of the sera (Figure 4, panel D). Moreover, the reverse was also true: the hazelnut oleosins present in the OB extract were able to inhibit the Cor a 15 immunorecognition by the patients' sera (Figure 4, panel E).

3.6 | All the patients showed specific IgEs toward Cor a 15

In order to obtain a semi-quantification of the patients' IgEs that were specific to Cor a 15 in the different subgroups of patients

TABLE 2 1-DE and 2-DE protein identification

No band	Entry (NCBI)	Protein name	MW_{exp}/MW_{theor}	Protein score	No of matching peptides	Protein coverage (%)	Molar fraction (%)
1-DE protein identification							
Raw hazelnut							
1A	AAO65960	Cor a 13	14,000/14,723	776	7	36.6	78.4
	AAO67349	Cor a 12	14,000/16,745	455	4	26.9	21.6
2A	MK737923	Cor a 15	17,000/17,695	1045	7	45.9	100
Roasted hazelnut							
1B	AAO65960	Cor a 13	14,000/14,723	1231	6	38.9	64.4
	AAO67349	Cor a 12	14,000/16,745	624	6	34.8	35.6
2B	MK737923	Cor a 15	17,000/17,695	1080	8	45.9	89.5
	AAO67349	Cor a 12	17,000/16,745	140	3	21.7	10.5
3B	AHA36627	Cor a 9	22,000/59,200	1853	14	31.2	69.5
	AAO65960	Cor a 13	22,000/14,723	203	4	26.9	30.6
4B	AHA36627	Cor a 9	23,000/59,200	2268	17	28.4	100
5B	AAO65960	Cor a 13	27,000/14,723	456	4	29.1	47
	AAO67349	Cor a 12	27,000/16,745	378	4	26.9	38.3
	AHA36627	Cor a 9	27,000/59,200	433	7	16.2	14.7
6B	MK737923	Cor a 15	29,000/17,695	293	5	36.6	40.6
	AAO65960	Cor a 13	29,000/14,723	401	4	29.1	27.2
	AAO67349	Cor a 12	29,000/16,745	410	4	26.9	22.2
	AHA36627	Cor a 9	29,000/59,200	539	8	18.6	10.1
7B	MK737923	Cor a 15	34,000/17,695	78	4	36.6	53.6
	AHA36627	Cor a 9	34,000/59,200	181	3	21.7	46.4
8B	AHA36627	Cor a 9	38,000/59,200	524	8	19.9	68.8
	AAO67349	Cor a 12	38,000/16,745	178	4	26.9	31.2

No band	Entry (NCBI)	Protein name	MWexp/MWtheor -PIexp/PItheor	Protein score	No of matching peptides	Protein coverage (%)	Molar fraction (%)
2-DE protein identification							
Raw hazelnut							
$1A_{2DE}$	AAO67349	Cor a 12	14,000/16,745-10.00/10.54	4430	8	38.8	64.1
	AAO65960	Cor a 13	14,000/14,723-10.00/9.98	3846	8	41.1	35.9
$2A_{2DE}$	MK737923	Cor a 15	17,000/17,695-10.00/9.56	3709	13	55.8	96.5
	AAO67349	Cor a 12	17,000/16,745-10.00/10.54	666	5	28.9	3.5
$3A_{2DE}$	MK737923	Cor a 15	29,000/17,695-10.00/9.56	889	8	49.6	62.2
	AAO67349	Cor a 12	29,000/16,745-10.00/9.98	404	4	26.9	16.2
	AAO65960	Cor a 13	29,000/14,723-10.00/10.54	563	4	29.1	13.7
	MK737924	Oleosin	29,000/15,790-10.00/9.43	93	3	18.7	7.9
$4A_{2DE}$	MK737923	Cor a 15	34,000/17,695-10.00/9.56	1298	8	45.9	65.8
	AAO65960	Cor a 13	34,000/14,723-10.00/9.98	524	4	29.1	18.8
	AAO67349	Cor a 12	34,000/16,745-10.00/10.54	312	3	21.7	15.4
$5A_{2DE}$	Not identified						
$6A_{2DE}$	Not identified						
$7A_{2DE}$	Not identified						
Roasted hazelnut							

(Continues)

TABLE 2 (Continued)

No band	Entry (NCBI)	Protein name	MWexp/MWtheor -PIexp/PItheor	Protein score	No of matching peptides	Protein coverage (%)	Molar fraction (%)
$1B_{2DE}$	AAO67349	Cor a 12	14,000/16,745-10.00/10.54	1679	7	35.5	81
	AAO65960	Cor a 13	14,000/14,723-10.00/9.98	1051	5	31.4	19.0
$2B_{2DE}$	AAO67349	Cor a 12	15,000/16,745-10.00/10.54	2155	7	39.4	72.6
	AAO65960	Cor a 13	15,000/14,723-10.00/9.98	1001	5	31.4	22.2
	MK737923	Cor a 15	15,000/17,695-10.00/9.56	134	3	24.8	5.1
$3B_{2DE}$	AAO67349	Cor a 12	17,000/16,745-10.00/9.56	920	6	34.2	45.3
	MK737923	Cor a 15	17,000/17,695-10.00/9.98	1054	7	45.3	39.8
	AAO65960	Cor a 13	17,000/14,723-10.00/10.54	962	4	29.1	14.9
$4B_{2DE}$	AHA36627	Cor a 9	22,000/59,200-10.50/8.93	1406	13	27.8	100
$5B_{2DE}$	Not identified						
$6B_{2DE}$	AHA36627	Cor a 9	23,000/59,200-10.00/8.93	4799	14	30.2	57.1
	MK737923	Cor a 15	23,000/17,695-10.00/9.10	132	5	36.6	27.0
	AAO65960	Cor a 13	23,000/14,723-10.00/9.56	273	3	34.3	15.9
$7B_{2DE}$	AHA36627	Cor a 9	23,000/59,200-9.00/8.93	6112	14	32.3	81.1
	MK737923	Cor a 15	23,000/17,695-9.00/9.56	124	4	36.6	18.9
$8B_{2DE}$	AHA36627	Cor a 9	23,000/59,200-8.50/8.93	3519	14	29	100
$9B_{2DE}$	AHA36627	Cor a 9	22,000/59,200-10.00/9.56	3333	18	29.4	84.5
	MK737923	Cor a 15	22,000/17,695-10.00/10.54	56	3	30.4	15.5
$10B_{2DE}$	AHA36627	Cor a 9	22,000/59,200-9.00/8.93	2197	13	27.4	75.1
	MK737923	Cor a 15	22,000/17,695-9.00/9.56	96	3	31	24.9
$11B_{2DE}$	Not identified						
$12B_{2DE}$	Not identified						
$13B_{2DE}$	Not identified						
$14B_{2DE}$	Not identified						
$15B_{2DE}$	AAL86739	Cor a 11	27,000/51,110-5.00/6.12	473	8	23.2	100
$16B_{2DE}$	AAL86739	Cor a 11	24,000/51,110-5.00/6.15	536	8	22.4	75.2
	AHA36627	Cor a 9	24,000/59,200-5.00/6.12	616	5	15.2	24.8
$17B_{2DE}$	AHA36627	Cor a 9	30,000/59,200-5.00/6.15	296	5	14.4	60,6
	AAL86739	Cor a 11	30,000/51,110-5.00/6.12	145	3	9.2	39,4
$18B_{2DE}$	AHA36627	Cor a 9	38,000/59,200-5.00/6.15	189	5	15.7	51.4
	AAL86739	Cora 11	38,000/51,110-5.00/6.12	112	5	9.4	48.6
$19B_{2DE}$	AHA36627	Cor a 9	38,000/59,200-5.70/6.15	1562	16	45.1	100
$20B_{2DE}$	AHA36627	Cor a 9	38,000/59,200-6.00/6.46	1702	17	40.1	90.8
	AAL86739	Cor a 11	38,000/51,110-6.00/6.12	122	5	13.2	9.2
$21B_{2DE}$	AAL86739	Cor a 11	45,000/51,110-6.20/6.12	4659	24	55.3	91.4
	AHA36627	Cor a 9	45,000/59,200-6.20/5.69	985	15	39	8.6
$22B_{2DE}$	MK737923	Cor a 15	17,000/17,695-8.00/9.56	803	6	45.3	80.6
	AAO65960	Cor a 13	17,000/14,723-8.00/9.98	174	3	28.4	19.4
$23B_{2DE}$	MK737923	Cor a 15	17,000/17,695-6.70/9.56	17741	7	44.6	83.3
	AAO67349	Cor a 12	17,000/16,745-6.70/10.54	16745	3	21.7	16.7
$24B_{2DE}$	MK737923	Cor a 15	36,000/17,695-10.00/9.56	1055	8	45.9	74.8
	AAO65960	Cor a 13	36,000/14,723-10.00/9.98	517	4	29.1	16.4
	AAO67349	Cor a 12	36,000/16,745-10.00/10.54	350	3	28.2	8.8

(Continues)

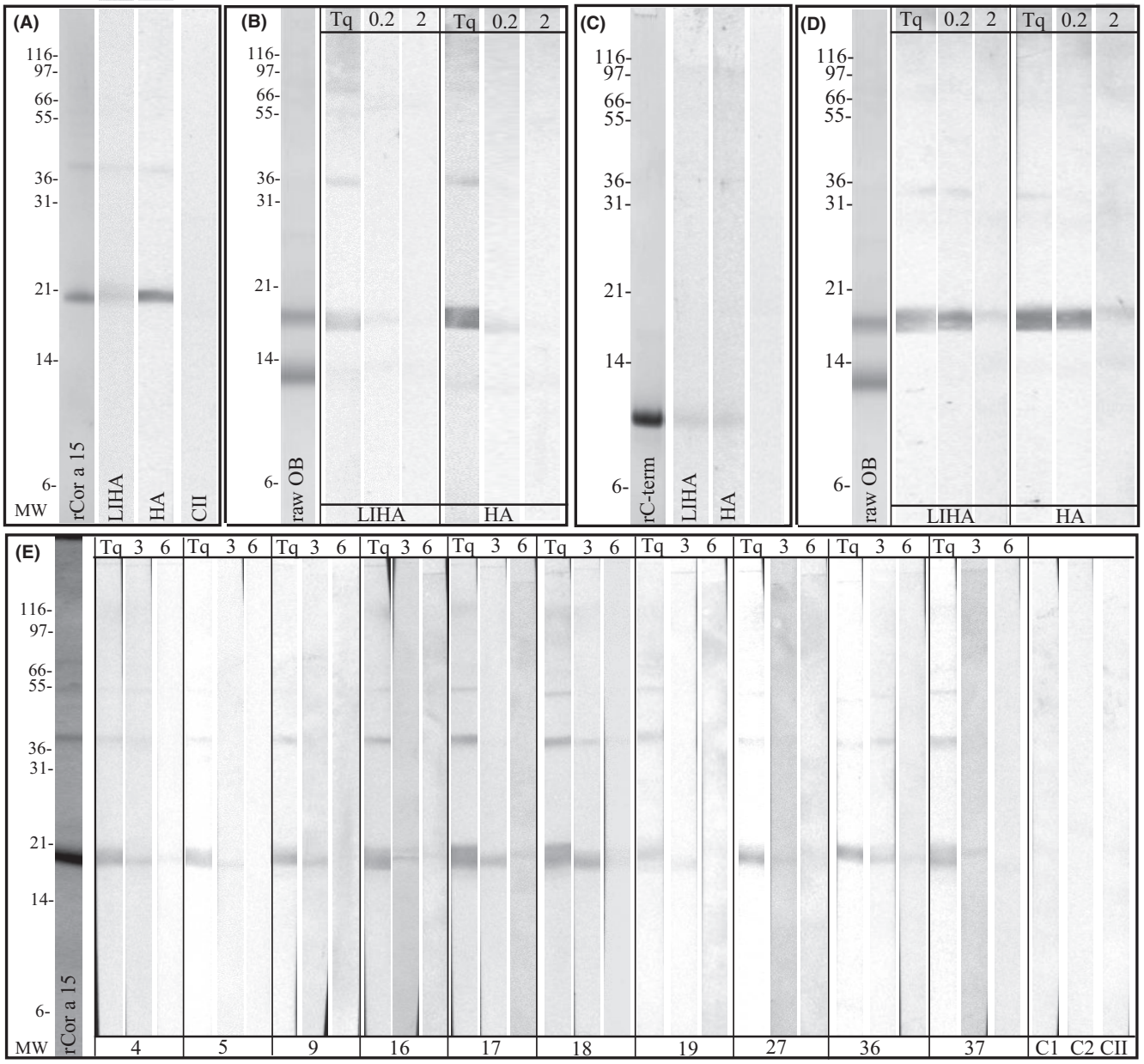

FIGURE 4 Immunoblotting inhibition assay. Panels A and C: LDS-PAGE of full-length rCor a 15 (rCor a 15) and rCor a 15 C-terminal (rC-term), respectively. Immunoblotting with pooled sera of the LIHA patients (25, 26 and 27) and HA patients (2, 10, and 13). CII: secondary antibody control; MW: molecular weight. Panels B and D: LDS-PAGE of raw hazelnut OB protein extracts (raw OB). Immunoblotting with the pooled sera of the LIHA patients (25, 26, and 27) and HA patients (2, 10, and 13), without (Tq) and with the addition of 0.2 and 2 μg of rCor a 15 and rCor a 15 C-terminal to the patients' sera, respectively. Panel E: Immunoblotting with the sera of 10 patients (nos. 4, 5, 9, 16, 17, 18, 19, 27, 36, and 37) without (Tq) and with the addition of 3 and 6 μg of raw OB protein extract to the patients' sera. C1: pool of five sera of non-nut-allergic (NNA) pediatric subjects; C2: pool of five sera of non-food-allergic (NFA) pediatric subjects

(HA, LIHA, and HS) and in the negative controls (NNA and NFA), the patients' sera were tested by means of an in-house direct ELISA test. The patients' sera all showed specific IgEs for rCor a 15 (Figure 5); however, the ELISA test did not differentiate between the HA, LIHA, and HS patients on the basis of Cor a 15 immunorecognition, as already demonstrated by the immunoblotting experiments. An aspecific reaction was found in 3/18 of the control sera.

4 | DISCUSSION

With the aim of conducting an immune-allergological characterization of hazelnut oleosins in pediatric patients, we performed the isolation and identification of hazelnut OB-associated proteins. We were able to separate oleosins from soluble allergens and to identify, for the first time, a new allergen belonging to the oleosin family, termed Cor a 15 by WHO-IUIS. We then demonstrated the immunogenic power of

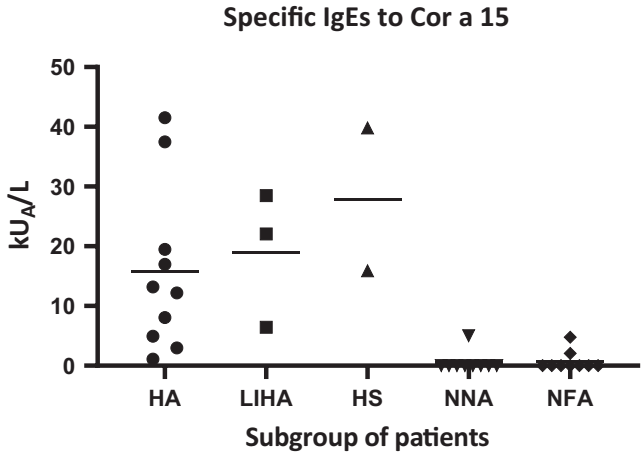

FIGURE 5 ELISA quantification of Cor a 15 IgEs in three subgroups of patients' sera (10 HA, 3 LIHA, and 2 HS). A line marks the mean kUA/L for each subgroup of patients. NNA control: 10 sera of non-nut allergic pediatric subjects; NFA control: 10 sera of non-food-allergic pediatric subjects

the newly reported Cor a 15 oleosin for most of our hazelnut-allergic pediatric patients. In addition, we succeeded in unambiguously ascribing the immunoreactivity of LIHA patients (characterized by low ImmunoCAP assay IgEs values and positive OFC and PbP) only to oleosins, thereby excluding the contribution of other allergens.

Regarding the IgE levels, the Cor a 9 and Cor a 14 IgE levels were clearly associated with the elicitation of the allergic reaction, according to Datema et al.,[21] and were useful to discriminate between the hazelnut-allergic and hazelnut-sensitized but tolerant patient groups. This was not found for the Cor a 1 and Cor a 8 IgE levels, for which a moderate diagnostic predictive value has been demonstrated.[22] The IgE levels of the newly discovered Cor a 15 seemed to have a similar behavior, as they were unable to discriminate between allergic and sensitized patients.

The evaluation of oleosin immunoreactivity for hazelnut-allergic patients was assessed by 1D and 2D immunoblotting, coupled with protein identification by means of mass spectrometry. The results indicated that all the HA patients immunorecognized at least one oleosin, although it was not possible to assess the role of the oleosins in eliciting the allergic reaction, as they were co-sensitized to genuine hazelnut allergens. On the contrary, we found that a subset of LIHA patients exclusively immunorecognized oleosins and, among them, mainly Cor a 15. Cor a 15 appeared to be the best candidate for the allergic reaction in this subgroup of patients. This evidence highlights the relevance of dosing IgEs against Cor a 15 in hazelnut allergy diagnosis. We also observed IgE reactivity to hazelnut oleosins in the HS patients, which could be ascribed to a cross-reactivity with other oleosins, at least for those patients with a confirmed allergy to other nuts.[23]

Cor a 15, which we exclusively identified in the 17-kDa band, was the most immunorecognized oleosin (88% of the allergic patients). This result was different from the results of Zuidmeer-Jongejan, who detected both Cor a 12 and Cor a 13 in a band at the same MW.[7] This is probably due to the lack of Cor a 15 gene sequence in the database at the time of the protein identification. We also identified Cor a 12 and 13 in a band at 14 KDa, with a recognition frequency of 33%, similar to what was reported in the EuroPrevall project for Cor a 12 in children (34%).[2]

As already demonstrated by Schwager et al.[4] for Ara h 15, we hypothesized that the epitope responsible the most for Cor a 15 immunorecognition is located at the C-terminal, since both the full-length rCor a 15 and rCor a 15 C-terminal were able to inhibit IgEs against the 17-kDa band containing only Cor a 15.

Our findings also show a resistance of oleosins to high temperatures, which resulted in an apparent enhancement of oleosin immunoreactivity after roasting, with a more evident effect on Cor a 12 and Cor a 13.[15] The immunorecognition of roasted Cor a 12 and Cor a 13 exceeded 60% for the allergic patients, while Cor a 15 reached 96%. Similarly, Schwager et al.[4] demonstrated an increase in oleosin immunoreactivity after roasting in-shell peanuts. Glycation after roasting may play a role in increasing the affinity and/or the accessibility of oleosins to IgEs.[24]

In conclusion, we have discovered a new hazelnut oleosin allergen, Cor a 15, which resulted to play a role in eliciting an allergic reaction in a subgroup of our pediatric patients that exclusively immunorecognized oleosins, thus confirming the relevance of including lipophilic allergens in the protein extracts used for diagnostic tests.

CONFLICT OF INTEREST

The authors have no conflict of interest.

AUTHOR CONTRIBUTION

Stefano Nebbia: Data curation (equal); Formal analysis (equal); Investigation (lead); Writing-original draft (equal). **Cristina Lamberti:** Investigation (lead); Methodology (lead); Supervision (equal); Writing-original draft (equal); Writing-review & editing (equal). **Simona Cirrincione:** Data curation (equal); Investigation (equal); Methodology (equal). **Alberto Acquadro:** Investigation (equal); Writing-review & editing (equal). **Simona Abbà:** Investigation (equal); Resources (equal). **Marina Ciuffo:** Investigation (equal); Writing-review & editing (equal). **Daniela Torello Marinoni:** Resources (equal); Writing-review & editing (equal). **Marcello Manfredi:** Investigation (equal); Writing-review & editing (equal). **Emilio Marengo:** Investigation (equal); Writing-review & editing (equal). **Roberta Calzedda:** Resources (equal); Writing-review & editing (equal). **Giovanna none Monti:** Conceptualization (equal); Methodology (equal); Resources (lead); Writing-review & editing (equal). **laura cavallarin:** Conceptualization (lead); Methodology (lead); Project administration (lead); Supervision (equal); Writing-original draft (equal); Writing-review & editing (lead). **Maria Gabriella Giuffrida:** Conceptualization (lead); Funding acquisition (equal); Methodology (lead); Supervision (lead); Writing-original draft (equal); Writing-review & editing (equal).

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/pai.13579.

ORCID

Laura Cavallarin https://orcid.org/0000-0001-7006-1683

REFERENCES

1. McWilliam V, Koplin J, Lodge C, et al. The prevalence of tree nut allergy: a systematic review. *Curr Allergy Asthma Rep*. 2015;15(9). https://doi.org/10.1007/s11882-015-0555-8.
2. Datema MR, Zuidmeer-Jongejan L, Asero R, et al. Hazelnut allergy across Europe dissected molecularly: a EuroPrevall outpatient clinic survey. *J Allergy Clin Immunol*. 2015;136:382–391.
3. Schwager C, Kull S, Krause S, et al. Development of a novel strategy to isolate lipophilic allergens (oleosins) from peanuts. *PLoS One*. 2015;10:1–15.
4. Schwager C, Kull S, Behrends J, et al. Peanut oleosins associated with severe peanut allergy—importance of lipophilic allergens for comprehensive allergy diagnostics. *J Allergy Clin Immunol*. 2017;140:1331–1338.e8.
5. Leduc V, Moneret-Vautrin DA, Tzen JTC, et al. Identification of oleosins as major allergens in sesame seed allergic patients. *Allergy Eur J Allergy Clin Immunol*. 2006;61:349–356.
6. Teodorowicz M, Terlouw RJ, Jansen A, et al. Immunological characterization of Dutch sesame seed-allergic patients. *Int Arch Allergy Immunol*. 2016;169:13–22.
7. Zuidmeer-Jongejan L, Fernández-Rivas M, Winter MGT, et al. Oil body-associated hazelnut allergens including oleosins are underrepresented in diagnostic extracts but associated with severe symptoms. *Clin Transl Allergy*. 2014;4:1–10.
8. Huang AHC. Plant lipid droplets and their associated proteins: potential for rapid advances. *Plant Physiol*. 2018;176:1894–1918.
9. Akkerdaas JH, Schocker F, Vieths S, et al. Cloning of oleosin, a putative new hazelnut allergen, using a hazelnut cDNA library. *Mol Nutr Food Res*. 2006;50:18–23.
10. Jappe U, Schwager C. Relevance of lipophilic allergens in food allergy diagnosis. *Curr Allergy Asthma Rep*. 2017;17:1–9.
11. Zhu J, Pouillot R, Kwegyir-Afful EK, et al. A retrospective analysis of allergic reaction severities and minimal eliciting doses for peanut, milk, egg, and soy oral food challenges. *Food Chem Toxicol*. 2015;80:92–100.
12. Hammer Ø, Harper DAT, Ryan PD. PAST: Paleontological statistics software package for education and data analysis. Palaeontol Electron; 2001:4.
13. Platteau CMF, Bridts CH, Daeseleire EA, et al. Comparison and functional evaluation of the allergenicity of different hazelnut (Corylus avellana) protein extracts. *Food Anal Methods*. 2010;3:382–388.
14. Cao Y, Zhao L, Ying Y, et al. The characterization of soybean oil body integral oleosin isoforms and the effects of alkaline pH on them. *Food Chem*. 2015;177:288–294.
15. Lamberti C, Nebbia S, Antoniazzi S, et al. Effect of hot air and infrared roasting on hazelnut allergenicity. *Food Chem*. 2021;342:128174.
16. Nebbia S, Lamberti C, Giorgis V, et al. The Cockroach Allergen-like protein is involved in primary respiratory and food allergy to yellow mealworm (Tenebrio molitor). *Clin Exp Allergy*. 2019;1:4.
17. Zava S, Barello C, Pessione A, et al. Mare's colostrum globules stimulate fibroblast growth in vitro: a biochemical study. *J Med Food*. 2009;12:836–845.
18. Rowley ER, VanBuren R, Bryant DW, et al. A draft genome and high-density genetic map of European hazelnut (*Corylus avellana* L.). *bioRxiv*. 2018;1:469015. https://doi.org/10.1101/469015.
19. Pavese V, Cavalet-Giorsa E, Barchi L, et al. Whole-genome assembly of Corylus avellana cv "Tonda Gentile delle Langhe" using linked-reads (10X Genomics). *G3-Genes Genom Genet*. 2021. http://dx.doi.org/10.1093/g3journal/jkab152
20. Hilger C, Bessot JC, Hutt N, et al. IgE-mediated anaphylaxis caused by bites of the pigeon tick Argas reflexus: Cloning and expression of the major allergen Arg r 1. *J Allergy Clin Immunol*. 2005;115:617–622.
21. Datema MR, van Ree R, Asero R, et al. Component-resolved diagnosis and beyond: Multivariable regression models to predict severity of hazelnut allergy. *Allergy Eur J Allergy Clin Immunol*. 2018;73:549–559.
22. Caffarelli C, Mastrorilli C, Santoro A, et al. Component-resolved diagnosis of hazelnut allergy in children. *Nutrients*. 2021;13:1–12.
23. Smeekens JM, Bagley K, Kulis M. Tree nut allergies: Allergen homology, cross-reactivity, and implications for therapy. *Clin Exp Allergy*. 2018;48:762–772.
24. Toda M, Hellwig M, Henle T, et al. Influence of the Maillard reaction on the allergenicity of food proteins and the development of allergic inflammation. *Curr Allergy Asthma Rep*. 2019;19:1–7.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Nebbia S, Lamberti C, Cirrincione S, et al. Oleosin Cor a 15 is a novel allergen for Italian hazelnut allergic children. *Pediatr Allergy Immunol*. 2021;00:1–13. https://doi.org/10.1111/pai.13579

Figure S1

```
                                        *        20         *        40         *        60         *        80
Cor-a-15-TGL-MK737923         : MADYQHQQQHQRPADAFKGMFPEKGQAQVQGPSASKVIAVVTLLPLGGFLLLLAGLTFAGTLIGLALSTPLFVLCSPVLV :  80
Cor-a-15_Jefferson-gene_g5542 : MADYQHQQQHQRPTDAFKGMFPEKGQAQAQGPSASKVIAVVTLLPLGGFLLLLAGLTFAGTLIGLALSMPLFVLCSPVLV :  80
                                MADYQHQQQHQRP DAFKGMFPEKGQAQ QGPSASKVIAVVTLLPLGGFLLLLAGLTFAGTLIGLALS PLFVLCSPVLV

                                        *       100         *       120         *       140         *       160
Cor-a-15-TGL-MK737923         : PAAIVIGLAVTGFLTSGAFGITGISSLSWILKYLRGTSVPEQMEHAKRRAQDTAGHLGQKARETGQTVTGKGQEAGKTLE : 160
Cor-a-15_Jefferson-gene_g5542 : PAAIVIGLAVTGFLTSGAFGITGISSLSWILKYLRGTSVPEQMEHAKRRAQDTAGXXXXXXXXXXXXXXXXXXXXXXXX : 160
                                PAAIVIGLAVTGFLTSGAFGITGISSLSWILKYLRGTSVPEQMEHAKRRAQDTAG

                                        *       180         *       200         *
Cor-a-15-TGL-MK737923         : G-------------------------------------GRGEEKKT------- : 169
Cor-a-15 Jefferson-gene g5542 : XXXXXXXXXXXXXXXXXXXXXXXXXXXRARKLERLLKVGGARKRRHENDKKYN : 213
                                                                         G    44
```

```
                                          *        20         *        40         *        60         *        80
Oleosin-TGL-MK737924               : MCYPCKVATPRSPSNAAMRHPHRFSLSTPSISTTSPTIIIIIHALSQIKAMSSDQSRPVSQRLYDSAPSSRQTVKFLTA :  80
Oleosin-Jefferson-gene_g10916_upgr : ----------------------------------------------------MSSDQSRPVSQRLYDSAPSSRQTVKFLTA :  29
                                                                                          MSSDQSRPVSQRLYDSAPSSRQTVKFLTA

                                          *       100         *       120         *       140         *       160
Oleosin-TGL-MK737924               : VTVGATLLILSGLTLTGTVIALIMATPVLVLFSPILVPAGFVIFLVVAGFLFSGGCGVAALSALTWIYNYVTGKCPPGAE : 160
Oleosin-Jefferson-gene g10916 upgr : VTVGATLLILSGLTLTGTVIALIMATPVLVLFSPILVPAGFVIFLVVAGFLFSGGCGVAALSALTWIYNYVTGKCPPGAE : 109
                                     VTVGATLLILSGLTLTGTVIALIMATPVLVLFSPILVPAGFVIFLVVAGFLFSGGCGVAALSALTWIYNYVTGKCPPGAE

                                          *       180         *
Oleosin-TGL-MK737924               : KLDYARMRIADKARDMTERAKEYGNYVQHKAQEATQRA : 198
Oleosin-Jefferson-gene_g10916_upgr : KLDYARMRIADKARDMTERAKEYGNYVQHKAQEATQRA : 147
                                     KLDYARMRIADKARDMTERAKEYGNYVQHKAQEATQRA
```

Figure S2

```
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                
Cor a 15(MK737923)  M-ADYQHQQ-QH-QRPADAFKGMFPEKGQAQVQGPSASKVIAVVTLLPLGGFLLLLAGLTFAGTLIGLALSTPLFVLCSP
Oleosin (MK737924)  MSSDQSRPVSQRLYDS-----------------APSSRQTVKFLTAVTVGATLLILSGLTLTGTVIALIMATPVLVLFSP
Cor a 12(AAO67349)  M-ADRPQQLQVHPQRGHGHYEGGIK--NQ-RGGGPSAVKVMAVLAALPVGGTLLALAGLTLAGSVIGLLVTSPLFIIFSP
Cor a 13(AAO65960)  M-AEHPRQLQDPAHQ-------------------PRSHQVVKAATAATAGGSLLVLSGLILAGTVIALTLATPLFVIFSP
                    * ::  :                           * : :.:   :  . *. ** *:** ::*::*.* :::*:::: **

Cor a 15(MK737923)  VLVPAAIVIGLAVTGFLTSGAFGITGISSLSWILKYLRGT--SVPEQMEHAKRRAQDTAGHLGQKARETGQTVTGKGQEA
Oleosin (MK737924)  ILVPAGFVIFLVVAGFLFSGGCGVAALSALTWIYNYVTGKCPPGAEKLDYARMRIADKARDMTERAKEYGNYVQHKAQEA
Cor a 12(AAO67349)  VLVPAAIVVGLAVASFLSSGALGLTGLSSLSWVLNYLRCASQSLPREMDQAKRRMQDMAAFVGQKTREVGQEIQSRAQEG
Cor a 13(AAO65960)  VLVPAVITVSLIIMGFLASGGFGVAAVTVLSWIYRYVTGRHPPGADQLDHARMKLASKAREMKDRAEQFG----QQHVTG
                    :**** :.: * : .** **. *::.:: *:*: .*:     . . ::: *: :  . *  : ::::.: *     :   .

Cor a 15(MK737923)  GKTLEGGRGEEKKT  169 aa
Oleosin (MK737924)  TQR----------A  147 aa
Cor a 12(AAO67349)  RR-----------T  159 aa
Cor a 13(AAO65960)  SQG----------S  140 aa
                     :           :
```

Figure S3

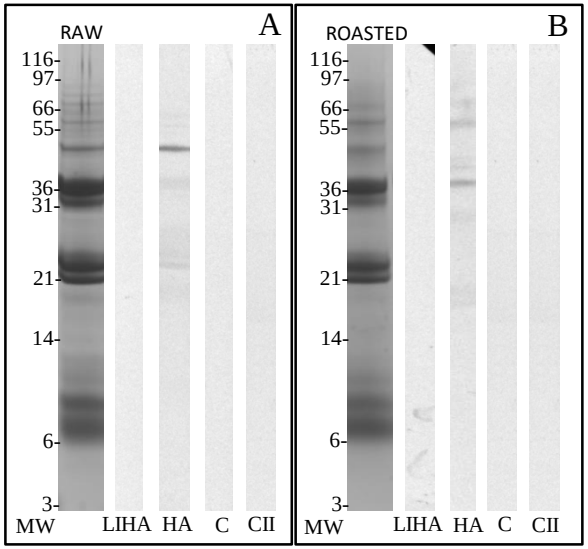
A
RAW
116-
97-
66-
55-
36-
31-
21-
14-
6-
3-
MW
LIHA
HA
C
CII
B
ROASTED
116-
97-
66-
55-
36-
31-
21-
14-
6-
3-
MW
LIHA
HA
C
CII

Figure S4

```
Cor a 15        MADYQHQQQHQRPADAFKGM-F----------P---E--KGQAQAQGPSA
Ara h 10.0101   MTDRTQ--PHTVQVHTTAGRFG-------DTAA-G-T---NRYPDRGPST
Ara_h 10.0102   MTDRTQ--PHAVQVHTTAGRFG-------DTAA-G-T---NRYADRGPST
Ara_h 11.0101   MAEALYY-------------------------GG--RQ-----RQEQPRS
Ara_h 11.0102   MAEALYY-------------------------GG--RQ-----RQDQPRS
Ara_h 14.0101   MATATDRAPHQVQVHTPTTQRVDVPRRGYDVSG-GGI--KTLLPERGPST
Ara_h 14.0102   MATATDRAPHQVQVHTPTTQRVDVQRRGYDVSG-GGV--KTFLPDRGPST
Ara_h 14.0103   MATATDRAPHQVQVHTPTTQRVDVPRRGYDVSG-GGI--KTLLPERGPST
Ara_h 15        MSDQTRTGYGGGGSY------G-------SSYGGGGTYGSSYGTSYDPST
Ses i 4         MADRDRPHPHQIQVHPQHPHRY----------E-GGV--KSLLPQKGPST
Ses i 5         MAEHYGQQQ---QTR-----------------AP--H------LQLQPRA
                *:                                            * :
Cor a 15            SKVIAVVTLLPLGGFLLLLAGLTFAGTLIGLALSTPLFVLCSPVLV
Ara h 10.0101       SKVIAVITGLPIGGTLLLFAGLALAGTLLGLAVTTPLFILFSPVIV
Ara_h 10.0102       SKVIAVITGLPIGGTLLLFAGLALAGTLLGLAVTTPLFILFSPVIV
Ara h 11.0101       TQLVKATTAVVAGGSLLILAGLVLAGTVIGLTTITPLFVIFSPVLV
Ara h 11.0102       TQLVKATTAVVAGGSLLILAGLVLAATVIGLTTITPLFVIFSPVLV
Ara h 14.0101       SQIIAVLVGVPTGGTLLLLSGLSLLGTIIGLAIATPVFTFFSPVIV
Ara h 14.0102       SQIIAVLVGVPTGGTLLLLSGLSLLGTIIGLAIATPVFTFFSPVIV
Ara h 14.0103       SQIIAVLVGVPTGGTLLLLSGLSLLGTIIGLAIATPVFIFFSPVIV
Ara h 15        NQPIRQAIKFMTASTIGVSFLILSGLILTGTVIGLIIATPLLVIFSPILV
Ses i 4             TQILAIITLLPISGTLLCLAGITLVGTLIGLAVATPVFVIFSPVLV
Ses i 5             QRVVKAATAVTAGGSLLVLSGLTLAGTVIALTIATPLLVIFSPVLV
                     : : . . :* ::*: : .*::.* **:: : **::*
Cor a 15        PAAIVIGLAVTGFLTSGAFGITGISSLSWILKYLRGTS---VPEQMEHAK
Ara h 10.0101   PAIIVVGLSVAGFLTSGACGLTGLSSFSWVMNYIRQTHGS-VPEQLEMAK
Ara h 10.0102   PATIVVGLSVAGFLTSGACGLTGLSSFSWVMNYIRQTHGS-VPEQLEMAK
Ara h 11.0101   PAVITVALLGLGFLASGGFGVAAITVLTWIYRYVTGKHPP-GANQLDTAR
Ara h 11.0102   PAVITVALLGLGFLASGGFGVAAITVLTWIYRYVTGKHPP-GANQLDTAR
Ara_h 14.0101   PAVVTIGLAVTGILTAGACGLTGLMSLSWMINFIRQVHGTTVPDQLDSVK
Ara h 14.0102   PAVVTIGLAVIGILTAGACGLTGLMSLSWMINFIRQVHGTTVPDQLDSAK
Ara h 14.0103   PAVVTIGLAVTGILTAGACGLTGLMSLSWMINFIRQVHGTTVPDQLDSVK
Ara h 15        PAAITLALAAGGFLFSGGCGVAAIAALSWLYSYVTGKHPA-GSDRLDYAK
Ses i 4         PAAILIAGAVTAFLTSGAFGLTGLSSLSWVLNSFRRAT---GQGPLEYAK
Ses i 5         PAVITIFLLGAGFLASGGFGVAALSVLSWIYRYLTGKHPP-GADQLESAK
                ** : : .:* :*. *::.: ::*: . :: .:
Cor a 15        RRAQDTAGHLGQKARETGQ-------TVTGKGQEAGKTLEGGRGEEKKT  169aa
Ara h 10.0101   HRMADVAGYVGQKTKDVGQKTKEVGQEIQTKAQDSKR-----------T  169aa
Ara h 10.0102   HRMADVAGYVGQKTKDVGQ-----------------------------  150aa
Ara h 11.0101   HKLMGKAREIK----DFGQ-------QQTSGAQA--------------S  137aa
Ara h 11.0102   HKLMSKAREIK----DYGQ-------QQTSGAQA--------------S  137aa
Ara h 14.0101   RRMADMADYVGQKTKDAGQ-------QIQTKAQDVKR------SS---S  176aa
Ara h 14.0102   RRMADMADYVGQKTKDAGQ-------EIQTKAQDVKR------SS---S  176aa
Ara h 14.0103   RRMADMADYVGQKTKDAGQ-------EIQTKAQDVKR------SS---S  176aa
Ara h 15        GVIADKARDVKDRAKDYAG-------A--GRAQEGTP------G----Y  166aa
Ses i 4         RGVQEGTLYVGEKTKQAGE-------AIKSTAKEGGREGT------ART  166aa
Ses i 5         TKLASKAREMKDRAEQFSQ-------QPVAGSQT--------------S  145aa
                      :  :     : .

                Cor a 15 DTAGHLGQKARETGQ
                Ara h 15 DKARDVKDRAKDYAG
                         *.* .: ::*:: .
```

Table S1: detailed list of the identified proteins from 1-DE, including the amino acid sequence of the detected peptides.

N° band	Entry (NCBI)	Protein name	Protein score	N° of matching peptides	Peptide sequences
Raw hazelnut					
1A	AAO65960	Cor a 13	776	7	QLQDPAHQPR HPPGADQLDHAR AEQFGQQHVTGSQGS DRAEQFGQQHVTGSQGS YVTGRHPPGADQLDHAR EMKDRAEQFGQQHVTGSQGS AREMKDRAEQFGQQHVTGSGS
	AAO67349	Cor a 12	455	4	CASQSLPR EVGQEIQSR MQDMAAFVGQK ADRPQQLQVHPR
2A	MK737923	Cor a 15	1045	7	ETGQTVTGK TLEGGRGEEK AQDTAGHLGQK GQAQVQGPSASK RAQDTAGHLGQK GTSVPEQMEHAK ADYQHQQQHQRPADAFK
Roasted hazelnut					
1B	AAO65960	Cor a 13	1231	6	QLQDPAHQPR HPPGADQLDHAR AEQFGQQHVTGSQGS DRAEQFGQQHVTGSQGS YVTGRHPPGADQLDHAR EMKDRAEQFGQQHVTGSQGS
	AAO67349	Cor a 12	624	6	CASQSLPR EVGQEIQSR GHGHYEGGIK MQDMAAFVGQK TREVGQEIQSR ADRPQQLQVHPQR
2B	MK737923	Cor a 15	1080	8	ETGQTVTGK TLEGGRGEEK AQDTAGHLGQK ARETGQTVTGK GQAQVQGPSASK RAQDTAGHLGQK GTSVPEQMEHAK ADYQHQQQHQRPADAFK
	AAO67349	Cor a 12	140	3	EVGQEIQSR MQDMAAFVGQK ADRPQQLQVHPQR
3B	AHA36627	Cor a 9	1853	14	QETTLAR LMENIGSR WLQLSAER ADIYTEQVGR TNDNAQISPLAGR SRADIYTEQVGR

					AESEGFEWVAFK INTVNSNTLPVLR RAESEGFEWVAFK QGQVLTIPQNFAVAK ALPDDVLANAFQISR VQVVDDNGNTVFDDELR ALPDDVLANAFQISREEAR TIEPNGLLLPQYSNAPELIYIER
	AAO65960	Cor a 13	203	4	CASQSLPR EVGQEIQSR MQDMAAFVGQK ADRPQQLQVHPQR
4B	AHA36627	Cor a 9	2268	17	QETTLAR LMENIGSR WLQLSAER ADIYTEQVGR YNRQETTLAR TNDNAQISPLAGR SRADIYTEQVGR AESEGFEWVAFK INTVNSNTLPVLR RAESEGFEWVAFK QGQVLTIPQNFAVAK ALPDDVLANAFQISR VQVVDDNGNTVFDDELR ALPDDVLANAFQISREEAR ARVQVVDDNGNTVFDDELR TIEPNGLLLPQYSNAPELIYIER VQVVDDNGNTVFDDELRQGQVLTIPQNFAVAK
5B	AAO65960	Cor a 13	456	4	QLQDPAHQPR HPPGADQLDHAR AEQFGQQHVTGSQGS DRAEQFGQQHVTGSQGS
	AAO67349	Cor a 12	378	4	CASQSLPR EVGQEIQSR MQDMAAFVGQK ADRPQQLQVHPQR
	AHA36627	Cor a 9	433	7	LMENIGSR LNALEPTNR ADIYTEQVGR INTVNSNTLPVLR QGQVLTIPQNFAVAK ALPDDVLANAFQISR VQVVDDNGNTVFDDELR
6B	MK737923	Cor a 15	293	5	ETGQTVTGK TLEGGRGEEK AQDTAGHLGQK GTSVPEQMEHAK ADYQHQQQHQRPADAFK
	AAO65960	Cor a 13	401	4	HPPGADQLDHAR AEQFGQQHVTGSQGS DRAEQFGQQHVTGSQGS ETGQTVTGK
	AAO67349	Cor a 12	410	4	CASQSLPR EVGQEIQSR MQDMAAFVGQK ADRPQQLQVHPQR
	AHA36627	Cor a 9	539	8	QETTLAR

					LMENIGSR TNDNAQISPLAGR AESEGFEWVAFK INTVNSNTLPVLR QGQVLTIPQNFAVAK ALPDDVLANAFQISR VQVVDDNGNTVFDDELR
7B	MK737923	Cor a 15	78	4	ETGQTVTGK TLEGGRGEEK AQDTAGHLGQK ADYQHQQQHQRPADAFK
	AHA36627	Cor a 9	181	3	EVGQEIQSR MQDMAAFVGQK ADRPQQLQVHPQR
8B	AHA36627	Cor a 9	524	8	SRQEWER LNALEPTNR QGQQQFGQR HFGECNLDR INTVNSNTLPVLR ALPDDVLANAFQISR HFYLAGNPDDEHQR TIEPNGLLLPQYSNAPELIYIER
	AAO67349	Cor a 12	178	4	CASQSLPR EVGQEIQSR MQDMAAFVGQK ADRPQQLQVHPQR

Table S2: detailed list of the identified proteins from 2-DE, including the amino acid sequence of the detected peptides.

N° Spot	Entry (NCBI)	Protein name	Protein score	N° of matching peptides	Peptides sequence
Raw hazelnut					
$1A_{2DE}$	AAO67349	Cor a 12	4430	8	CASQSLPR EVGQEIQSR GHGHYEGGIK MQDMAAFVGQK TREVGQEIQSR RMQDMAAFVGQK EVGQEIQSRAQEGR ADRPQQLQVHPQR
	AAO65960	Cor a 13	3846	8	AREMKDR MKLASKAR QLQDPAHQPR HPPGADQLDHAR AEQFGQQHVTGSQGS DRAEQFGQQHVTGSQGS YVTGRHPPGADQLDHAR EMKDRAEQFGQQHVTGSQGS
$2A_{2DE}$	MK737923	Cor a 15	3709	11	ETGQTVTGK TLEGGRGEEK AQDTAGHLGQK GQAQVQGPSASK RAQDTAGHLGQK GTSVPEQMEHAK AQDTAGHLGQKAR YLRGTSVPEQMEHAK GMFPEKGQAQVQGPSASK ADYQHQQQHQRPADAFK ADYQHQQQHQRPADAFKGMFPEK
	AAO67349	Cor a 12	666	5	EVGQEIQSR GHGHYEGGIK MQDMAAFVGQK RMQDMAAFVGQK ADRPQQLQVHPQR
$3A_{2DE}$	MK737923	Cor a 15	889	8	ETGQTVTGK TLEGGRGEEK AQDTAGHLGQK ARETGQTVTGK GQAQVQGPSASK RAQDTAGHLGQK GTSVPEQMEHAK ADYQHQQQHQRPADAFK
	AAO67349	Cor a 12	404	4	QLQDPAHQPR HPPGADQLDHAR AEQFGQQHVTGSQGS DRAEQFGQQHVTGSQGS
	AAO65960	Cor a 13	563	4	CASQSLPR EVGQEIQSR MQDMAAFVGQK ADRPQQLQVHPQR
	MK737924	Oleosin	93	3	AQEATQR

					LYDSAPSSR SSDQSRPVSQR
$4A_{2DE}$	MK737923	Cor a 15	1298	8	ETGQTVTGK TLEGGRGEEK AQDTAGHLGQK ARETGQTVTGK GQAQVQGPSASK RAQDTAGHLGQK GTSVPEQMEHAK ADYQHQQQHQRPADAFK
	AAO65960	Cor a 13	524	4	QLQDPAHQPR HPPGADQLDHAR AEQFGQQHVTGSQGS DRAEQFGQQHVTGSQGS
	AAO67349	Cor a 12	312	3	EVGQEIQSR MQDMAAFVGQK ADRPQQLQVHPQR
$5A_{2DE}$	not identified				
$6A_{2DE}$	not identified				
$7A_{2DE}$	not identified				
Roasted hazelnut					
$1B_{2DE}$	AAO67349	Cor a 12	1679	7	CASQSLPR EVGQEIQSR GHGHYEGGIK MQDMAAFVGQK TREVGQEIQSR RMQDMAAFVGQK ADRPQQLQVHPQR
	AAO65960	Cor a 13	1051	5	QLQDPAHQPR HPPGADQLDHAR AEQFGQQHVTGSQGS AEQFGQQHVTGSQGS DRAEQFGQQHVTGSQGS
$2B_{2DE}$	AAO67349	Cor a 12	2155	7	GGGPSAVK CASQSLPR EVGQEIQSR GHGHYEGGIK MQDMAAFVGQK RMQDMAAFVGQK ADRPQQLQVHPQR
	AAO65960	Cor a 13	1001	5	QLQDPAHQPR HPPGADQLDHAR AEQFGQQHVTGSQGS DRAEQFGQQHVTGSQGS EMKDRAEQFGQQHVTGSQGS
	MK737923	Cor a 15	134	3	AQDTAGHLGQK GTSVPEQMEHAK ADYQHQQQHQRPADAFK
$3B_{2DE}$	AAO67349	Cor a 12	920	6	CASQSLPR EVGQEIQSR GHGHYEGGIK MQDMAAFVGQK RMQDMAAFVGQK ADRPQQLQVHPQR
	MK737923	Cor a 15	1054	7	ETGQTVTGK TLEGGRGEEK AQDTAGHLGQK ARETGQTVTGK GQAQVQGPSASK

					GTSVPEQMEHAK ADYQHQQQHQRPADAFK
	AAO65960	Cor a 13	962	4	QLQDPAHQPR HPPGADQLDHAR AEQFGQQHVTGSQGS DRAEQFGQQHVTGSQGS
$4B_{2DE}$	AHA36627	Cor a 9	1406	13	QETTLAR LMENIGSR WLQLSAER ADIYTEQVGR TNDNAQISPLAGR SRADIYTEQVGR AESEGFEWVAFK INTVNSNTLPVLR RAESEGFEWVAFK QGQVLTIPQNFAVAK ALPDDVLANAFQISR VQVVDDNGNTVFDDELR EGLYVPHWNLNAHSVVYAIR
$5B_{2DE}$	not identified				
$6B_{2DE}$	AHA36627	Cor a 9	4799	14	QETTLAR LMENIGSR WLQLSAER ADIYTEQVGR TNDNAQISPLAGR ESEQERERQR SRADIYTEQVGR INTVNSNTLPVLR RAESEGFEWVAFK QGQVLTIPQNFAVAK ALPDDVLANAFQISR VQVVDDNGNTVFDDELR ALPDDVLANAFQISREEAR EGLYVPHWNLNAHSVVYAIR
	MK737923	Cor a 15	132	5	ETGQTVTGK TLEGGRGEEK AQDTAGHLGQK GTSVPEQMEHAK ADYQHQQQHQRPADAFK
	AAO65960	Cor a 13	273	3	QLQDPAHQPR HPPGADQLDHAR AEQFGQQHVTGSQGS
$7B_{2DE}$	AHA36627	Cor a 9	6112	14	QETTLAR RSESEGR LMENIGSR WLQLSAER ADIYTEQVGR TNDNAQISPLAGR SRADIYTEQVGR AESEGFEWVAFK INTVNSNTLPVLR RAESEGFEWVAFK QGQVLTIPQNFAVAK ALPDDVLANAFQISR VQVVDDNGNTVFDDELR EGLYVPHWNLNAHSVVYAIR
	MK737923	Cor a 15	124	4	ETGQTVTGK TLEGGRGEEK AQDTAGHLGQK ADYQHQQQHQRPADAFK

$8B_{2DE}$	AHA36627	Cor a 9	3519	14	QETTLAR LMENIGSR WLQLSAER ADIYTEQVGR TNDNAQISPLAGR SRADIYTEQVGR AESEGFEWVAFK INTVNSNTLPVLR RAESEGFEWVAFK QGQVLTIPQNFAVAK ALPDDVLANAFQISR QGQVLTIPQNFAVAK VQVVDDNGNTVFDDELR EGLYVPHWNLNAHSVVYAIR
$9B_{2DE}$	AHA36627	Cor a 9	3333	18	QETTLAR LMENIGSR WLQLSAER ADIYTEQVGR YNRQETTLAR TNDNAQISPLAGR SRADIYTEQVGR AESEGFEWVAFK INTVNSNTLPVLR RAESEGFEWVAFK WLQLSAERGDLQR QGQVLTIPQNFAVAK ALPDDVLANAFQISR QGQVLTIPQNFAVAK VQVVDDNGNTVFDDELR ARVQVVDDNGNTVFDDELR EGLYVPHWNLNAHSVVYAIR VQVVDDNGNTVFDDELRQGQVLTIPQNFAVAK
	MK737923	Cor a 15	56	3	ETGQTVTGK AQDTAGHLGQK GQAQVQGPSASK ADYQHQQQHQRPADAFK
$10B_{2DE}$	AHA36627	Cor a 9	2197	13	QETTLAR LMENIGSR WLQLSAER ADIYTEQVGR TNDNAQISPLAGR SRADIYTEQVGR AESEGFEWVAFK INTVNSNTLPVLR RAESEGFEWVAFK QGQVLTIPQNFAVAK ALPDDVLANAFQISR VQVVDDNGNTVFDDELR EGLYVPHWNLNAHSVVYAIR
	MK737923	Cor a 15	96	3	ETGQTVTGK GTSVPEQMEHAK ADYQHQQQHQRPADAFK
$11B_{2DE}$	not identified				
$12B_{2DE}$	not identified				
$13B_{2DE}$	not identified				
$14B_{2DE}$	not identified				
$15B_{2DE}$	AAL86739	Cor a 11	473	8	LNALEPTNR INTVNSNTLPVLR TIEPNGLLLPQYSNAPELIYIER

$16B_{2DE}$	AAL86739	Cor a 11	536	8	ATITMVR LLSGIENFR VQVLENFTK ALSQHEEGPPR IPAGTPVYMINR AFSWEVLEAALK ESFNVEHGDIIR ILQPVSAPGHFEAFYGAGGEDPESFYR
	AHA36627	Cor a 9	616	5	LNALEPTNR QGQQQFGQR HFYLAGNPDDEHQR TIEPNGLLLPQYSNAPELIYIER IEAEAGQIESWDHNDQQFQCAGVAVIR
$17B_{2DE}$	AHA36627	Cor a 9	296	5	LNALEPTNR QGQQQFGQR ADIYTEQVGR HFYLAGNPDDEHQR TIEPNGLLLPQYSNAPELIYIER
	AAL86739	Cor a 11	145	3	LLSGIENFR VQVLENFTK AFSWEVLEAALK
$18B_{2DE}$	AHA36627	Cor a 9	189	5	SRQEWER LNALEPTNR QGQQQFGQR HFYLAGNPDDEHQR TIEPNGLLLPQYSNAPELIYIER
	AAL86739	Cora 11	112	5	LLSGIENFR VQVLENFTK IPAGTPVYMINR AFSWEVLEAALK ESFNVEHGDIIR
$19B_{2DE}$	AHA36627	Cor a 9	1562	16	SRQEWER WLQLSAER LNALEPTNR LQMVRPER QGQQQFGQR HFGECNLDR ADIYTEQVGR AESEGFEWVAFK INTVNSNTLPVLR ALPDDVLANAFQISR HFYLAGNPDDEHQR VQVVDDNGNTVFDDELR TIEPNGLLLPQYSNAPELIYIER RTIEPNGLLLPQYSNAPELIYIER GITGVLFPGCPETFEDPQQQSQQGQGQGQSQR GKEGEQEQQGEGNNVFSGFDAEFLADAFNVDVDTAR
$20B_{2DE}$	AHA36627	Cor a 9	1702	17	LQSNQDK NIVKVEGR RLQSNQDK SRQEWER WLQLSAER LQMVRPER

					LNALEPTNR QGQQQFGQR HFGECNLDR ADIYTEQVGR INTVNSNTLPVLR ALPDDVLANAFQISR HFYLAGNPDDEHQR TIEPNGLLLPQYSNAPELIYIER RTIEPNGLLLPQYSNAPELIYIER IEAEAGQIESWDHNDQQFQCAGVAVIR GITGVLFPGCPETFEDPQQQSQQGQGQGQSQR
	AAL86739	Cor a 11	122	5	ELAFNLPSR LLSGIENFR GNIVNEFER VQVLENFTK AFSWEVLEAALK
$21B_{2DE}$	AAL86739	Cor a 11	4659	24	QQEEGGR ATITMVR ELAFNLPSR VQVLENFTK ELAFNLPSR LLSGIENFR GNIVNEFER VQVLENFTK HPSQSNQFGR GSMAGPYYNSR ALSQHEEGPPR LYEAHPDDHK VQVLENFTKR DENEKLFIVK IPAGTPVYMINR AFSWEVLEAALK ESFNVEHGDIIR NQDQAFFFPGPNK IWPFGGESSGPINLLHK IFKNQDQAFFFPGPNK EQEENPYVFQDEHFESR IPAGTPVYMINRDENEKLFIVK ILQPVSAPGHFEAFYGAGGEDPESFYR LAILEANPHTFISPAHFDAELVLFVAK
	AHA36627	Cor a 9	985	15	LMENIGSR WLQLSAER LNALEPTNR QGQQQFGQR ADIYTEQVGR TNDNAQISPLAGR AESEGFEWVAFK

					INTVNSNTLPVLR RAESEGFEWVAFK QGQVLTIPQNFAVAK ALPDDVLANAFQISR HFYLAGNPDDEHQR VQVVDDNGNTVFDDELR TIEPNGLLLPQYSNAPELIYIER IEAEAGQIESWDHNDQQFQCAGVAVIR
$22B_{2DE}$	MK737923	Cor a 15	803	6	ETGQTVTGK TLEGGRGEEK AQDTAGHLGQK GQAQVQGPSASK GTSVPEQMEHAK ADYQHQQQHQRPADAFK
	AAO65960	Cor a 13	174	3	QLQDPAHQPR AEQFGQQHVTGSQGS DRAEQFGQQHVTGSQGS
$23B_{2DE}$	MK737923	Cor a 15	17741	7	ETGQTVTGK TLEGGRGEEK AQDTAGHLGQK GQAQVQGPSASK RAQDTAGHLGQK GTSVPEQMEHAK ADYQHQQQHQRPADAFK
	AAO67349	Cor a 12	16745	3	EVGQEIQSR MQDMAAFVGQK ADRPQQLQVHPQR
$24B_{2DE}$	MK737923	Cor a 15	1055	8	ETGQTVTGK TLEGGRGEEK AQDTAGHLGQK ARETGQTVTGK GQAQVQGPSASK RAQDTAGHLGQK GTSVPEQMEHAK ADYQHQQQHQRPADAFK
	AAO65960	Cor a 13	517	4	QLQDPAHQPR HPPGADQLDHAR AEQFGQQHVTGSQGS DRAEQFGQQHVTGSQGS
	AAO67349	Cor a 12	350	3	EVGQEIQSR MQDMAAFVGQK ADRPQQLQVHPQR