

Onset of Nut Allergy in a Pediatric Cohort: Clinical and Molecular Patterns in the AFRUSEN Study

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■ Abstract

Background: Nut allergy is a growing problem, yet little is known about its onset in children.

Objective: To characterize the onset of nut allergy in children in southern Europe.

Methods: The study population comprised consecutive patients up to 14 years of age who visited allergy departments with an initial allergic reaction to peanut, tree nut, or seed. The allergy work-up included a clinical history, food challenge, skin prick testing, determination of whole-extract sIgE, and ImmunoCAP ISAC-112 assay.

Results: Of the 271 children included, 260 were first diagnosed with nut allergy at a mean age of 6.5 years and at a mean (SD) of 11.8 (21.2) months after the index reaction. The most common culprit nuts at onset were walnut (36.5%), peanut (28.5%), cashew (10.4%), hazelnut (8.5%), pistachio (5.4%), and almond (5%). Onset of peanut allergy was more frequent in children ≤6 years and walnut in those aged >6 years ($P=.032$). In 65% of cases, the allergic reaction occurred the first time the patient consumed the nut, and 35% of reactions were anaphylactic. Overall, polysensitization to nuts was detected by skin prick testing in 64.9% of patients, although this rate was lower among walnut-allergic children (54.7%) and peanut-allergic children (54.1%) ($P<.0001$). Sensitization to 2S albumins was predominant (75%), especially Jug r 1 (52.8%), whereas sensitization to lipid transfer proteins was less relevant (37%).

Conclusion: In the population we assessed, the onset of nut allergy occurred around 6 years of age, slightly later than that reported in English-speaking countries. Walnut was the main trigger, followed by peanut. 2S albumin storage proteins, especially Jug r 1, were the most relevant allergens. This study will help guide management and may contribute to preventive strategies in pediatric nut allergy.

Key words: Nut allergy. Walnut. Peanut. Allergy onset. Sensitization profile. Component-resolved diagnosis. Anaphylaxis. Food allergy.

■ Resumen

Antecedentes: La alergia a frutos secos es un problema creciente. Sin embargo, existe poca información relativa al inicio de su establecimiento en la población infantil.

Objetivos: Describir el debut de alergia a frutos secos en niños del sur de Europa.

Métodos: Se incluyeron pacientes de hasta 14 años que acudieron de forma consecutiva a la consulta de alergia debido a una reacción inicial con cacahuete, frutos secos o semillas. El estudio alergológico incluyó realización de historia clínica, provocación oral, prueba intraepidérmica (SPT), determinación de IgE específica para extracto completo y mediante ImmunoCAP ISAC-112.

Resultados: De los 271 niños incluidos, 260 se diagnosticaron de alergia a frutos secos por primera vez a los 6,5 años de media, habiendo tenido la reacción índice 11,8 ($\pm 21,2$ SD) meses antes. Los frutos secos responsables en el debut fueron nuez (36,5%), cacahuete (28,5%), anacardo (10,4%), avellana (8,5%), pistacho (5,4%) y almendra (5%). La instauración de la alergia a cacahuete fue más frecuente en niños ≤ 6 años y para nuez en > 6 años ($p=0,032$). En el 65% de los casos, la reacción alérgica sucedió en la primera vez en que el paciente consumía el fruto seco, y el 35% de las reacciones fueron anafilaxia. En conjunto, la polisensibilización a frutos secos se identificó en el 64,9% de los pacientes, aunque este porcentaje fue significativamente inferior en niños alérgicos a nuez (54,7%) y cacahuete (54,1%) ($p<0,0001$). La sensibilización a albúminas 2S fue predominante (75%), especialmente a Jug r 1 (52,8%), mientras que la identificación de LTP fue menos relevante (37%).

Conclusión: En nuestra población, el debut de alergia a frutos secos sucedió alrededor de los 6 años de edad, ligeramente más tardío al reportado en países anglosajones. La nuez fue el principal desencadenante, seguido de cacahuete, y las albúminas de almacenamiento 2S, especialmente Jug r 1, fueron los alérgenos más relevantes. Los hallazgos de este estudio podrán ayudar a guiar el manejo clínico y contribuirán a desarrollar estrategias preventivas en población alérgica.

Palabras clave: Alergia a fruto seco. Nuez. Cacahuete. Debut de alergia. Perfil de sensibilización. Diagnóstico por componentes. Anafilaxia. Alergia a alimentos.

Introduction

Tree nut, peanut, and seed allergy are potentially life-threatening diseases that have increased in prevalence over the last 2 decades, possibly owing to rising consumption [1,2]. These allergens are among the most common causes of acute allergic reactions to food and have been associated with fatal reactions, even when eaten in very small amounts or as hidden allergens [3]. The European Anaphylaxis Registry identified tree nuts and peanut as prevalent elicitors of anaphylaxis in children aged 2 to 17 years [4], and allergy to these foods is the most common risk factor for near-fatal anaphylaxis [5]. The quality of life of affected patients and their families is impaired by the threat of accidental ingestion. Consequently, patients require constant vigilance with respect to food choices [6,7], training on food allergen avoidance, recognition of anaphylaxis, and prompt use of adrenaline [8].

Nut allergy develops early in life and is rarely outgrown, often becoming a lifelong disease [9]. Published data indicate that the prevalence of tree nut allergy is between 0.05% and 4.9% and that of peanut allergy approximately 0.5%-2.5%, depending on the diagnostic method used [10-12], although information on various geographic areas and age groups is limited [10,13]. More importantly, data on the age of onset, culprit food, and allergen sensitization profiles outside the English-speaking world remain scarce.

Homology between nut proteins and cross-reactivity between their main allergens (ie, 2S albumins, 7S and 11S globulins, lipid transport proteins [LTPs], and PR-10) leads to frequent cosensitization in nut-allergic patients. However, cosensitization does not always translate into true concurrent allergy to different nuts [14]. Deeper knowledge of the pattern of sensitization to the allergens of peanuts, tree nuts, and seeds in each geographic area would lead to a more accurate and

specific diagnostic approach and more precise dietary guidance for patients allergic to these foods.

Given the paucity of reports on pediatric nut allergy, the Committee for Pediatric Allergy of the Spanish Society of Allergy and Clinical Immunology (SEAIC) carried out this prospective multicenter study (AFRUSEN [Spanish acronym]) to evaluate clinical and sensitization-related features in children (≤ 14 years) presenting an initial allergic reaction to peanut or tree nuts, among others. These foods share features such as type, preparation, consumption patterns, and allergenicity. Therefore, and for simplicity, throughout this paper we will use the term “nuts and seeds” to refer to peanut, tree nuts, and seeds collectively.

The primary objective of our study was to determine which nuts most frequently trigger nut allergy and age at diagnosis. Our secondary objectives were to describe pre-existing atopic diseases, clinical manifestations, sensitization to all nuts, and molecular recognition patterns in nut allergy.

Methods

Study Design

The Nut Allergy Onset in Children Study (AFRUSEN) is a prospective multicenter study sponsored by the SEAIC and led by a task force formed within the SEAIC Children’s Allergy Committee. The protocol underwent previous ethics committee review (online supplement). All data were stored in a custom-designed online database that allowed comprehensive registry management and subsequent data analysis. All data were anonymized.

Participants

This study population included consecutive patients aged 0 to 14 years who attended a participating allergy outpatient

clinic for the first time with complaints suggestive of IgE-mediated allergic reaction to peanut, tree nuts (almond, hazelnut, chestnut, pistachio, cashew, pine nut, walnut), and/or seeds (sunflower, pumpkin, and sesame) between April 2014 and November 2015 and whose parents or guardians signed a written informed consent document. The index nut was the first nut to trigger symptoms in the patient's life. The index reaction was the first reaction that led the patient to visit an allergy clinic. Patients were excluded in cases of previous diagnosis of nut allergy and/or severe atopic dermatitis, active chronic urticaria, dermatographism, or any other baseline disease for which diagnostic testing is contraindicated.

Patient allergic background and reactions to nuts were recorded (see Methods in online supplement). Anaphylaxis was used as a post hoc variable in accordance with the definition of Muraro et al [15]. Following the diagnostic procedures, the index nut triggering disease onset was established for all patients.

Some patients were also evaluated for secondary allergies, either to nuts causing other reactions after the index nut or nut allergies identified along the course of this study. During determination of both index and secondary nut allergies, participants were classified as allergic if any of the following applied: (i) a solid history of allergy and positive sensitization test (skin prick test [SPT] and/or specific serum IgE [sIgE]); (ii) a compatible clinical history and positive oral food challenge (OFC) result; or (iii) a positive OFC in sensitized patients who had not come into previous contact with the nut studied. Only those patients with a negative OFC result were classified as not allergic.

Interventions

Skin prick tests

SPTs were performed for pollen (*Phleum*, *Olea*, *Platanus*, *Cupressus*, birch, ragweed, *Parietaria*, *Salsola*), nuts (walnut, peanut, hazelnut, pistachio, almond, cashew, pine nut, chestnut), seeds (sunflower, pumpkin, sesame), peach peel (Pru p 3, LTP-marker), and palm tree (Pho d 2, profilin-marker). Extracts from the same batch and lancets were provided by ALK-Abelló (see online supplement).

Serum analysis

The patients' serum samples were tested to determine sIgE to 112 allergen components. Whole nut sIgE was measured only if the researcher deemed it necessary. Both assays were performed according to the manufacturer's instructions (ImmunoCAP ISAC-112 and ImmunoCAP, Thermo Fisher Scientific). The cut-off value for positive determinations was set at ≥ 0.1 ISU-E for ISAC and >0.35 kU_A/L for ImmunoCAP.

Oral food challenge

OFC was carried out in an open fashion and only performed in selected cases at the attending physician's discretion and according to published guidelines [16].

Statistical Analysis

No formal sample size calculation was undertaken, as there was no expected primary outcome; therefore, the size

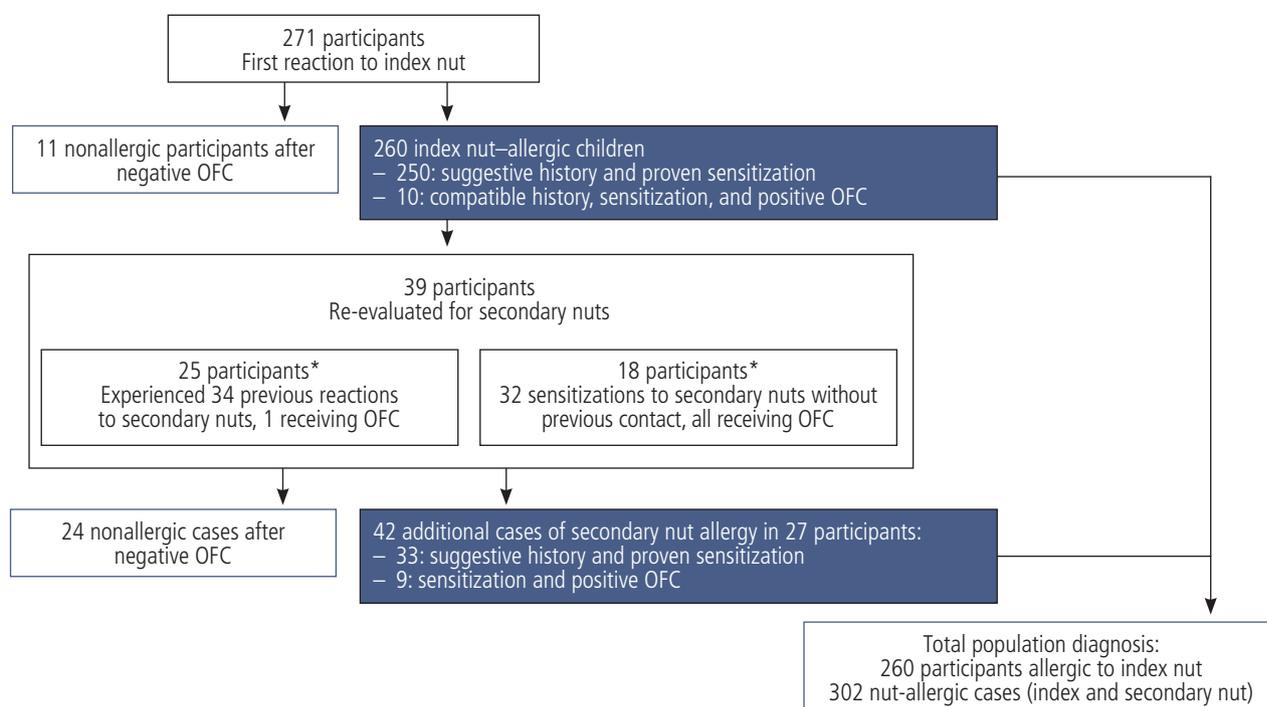


Figure 1. CONSORT-like diagram of diagnostic criteria. Onset of nut allergy was detected in 260/271 patients. Additionally, in 25 patients, 34 reactions to other nuts occurred before the patient sought care, and in 18 patients, 32 sensitizations were detected during the course of the study and then evaluated. *Four patients were counted twice; they were assigned to these 2 groups owing to a report of a previous reaction and sensitization without previous contact. As a result, a total of 42 additional cases of secondary nut allergy were confirmed in 27 patients. OFC indicates oral food challenge.

of the sample was determined based on feasibility constraints. *P* values below .05 indicated statistical significance. The analysis was performed using IBM SPSS Statistics for Windows, Version 25 (IBM Corp.) (see online supplement for further information).

Results

Patient Characteristics

Nineteen researchers from 14 centers located in 6 geographic areas of Spain recruited 271 participants, of whom 94.5% were Caucasian and 56.5% were male. A total of 260 participants (95.9%) with a mean (SD) age of 6.5 years (3.5 years, range 6 months to 14 years) were diagnosed with nut or seed allergy; in these patients, the index reaction had occurred a mean of 11.4 (20.7) months previously, and the last contact with the index nut occurred a mean of 5.6 (11.5) months earlier. In 96.2% of patients, diagnosis was based on a compatible history and positive sensitization, and in 3.8%, diagnosis followed a positive OFC (Figure 1). At least 1 baseline atopic disease was present in 79.3% of the sample: 48.7% of all participants had atopic dermatitis, 42.5% asthma, and 17.4% allergy to a plant food (Table 1). Thirty-nine patients were diagnosed with a secondary nut allergy (Supplementary table 3), and 42 additional cases of allergy to secondary nuts were confirmed, thus raising the number of confirmed cases of nut allergy up to 302 (Figure 1, Supplementary table 1). According to the accounts of parents and guardians, 34.2% of participants had previously tolerated the triggering nut, and 34.2% had experienced more than 1 reaction with the same nut prior to seeking care.

Index Reaction

Index nut

The nuts most frequently implicated in the onset of allergy were as follows: walnut (36.5%), peanut (28.5%), cashew (10.4%), hazelnut (8.5%), pistachio (5.4%), and almond (5%). These 6 nuts were responsible for at least 10 cases each, affecting 245 patients. Sunflower and pumpkin seed, pine nut, and sesame were the offending food in $\leq 1.5\%$ of cases each (Table 1). The prevalence of each index nut allergy by age group can be seen in Figure 2, with peanut having a significantly earlier onset ($P=.016$); older patients were more likely to develop reactions to sunflower seed ($P=.016$) and walnut, thus indicating a clear, yet not statistically significant trend toward later onset.

Focusing on the 6 main nut allergies ($n=245$, Table 2), onset of peanut allergy was more frequent in children ≤ 6 years, and onset of walnut allergy was associated with age >6 years ($P=.032$). In this population, mite-triggered respiratory allergy was more frequent in walnut-allergic children than in the overall population (44.1% vs 31.8%, $P=.045$ [Supplementary table 2]).

Characteristics of the Index Reaction Among Allergic Children ($n=260$)

The index reaction occurred following oral, cutaneous, and respiratory exposure in 90%, 9.6%, and 0.4% of cases, respectively (see Supplementary table 4 for a detailed description of patients with skin and respiratory contact). Time to onset of symptoms was significantly shorter among allergic than nonallergic children: 65.4% vs 9.1% of reactions began

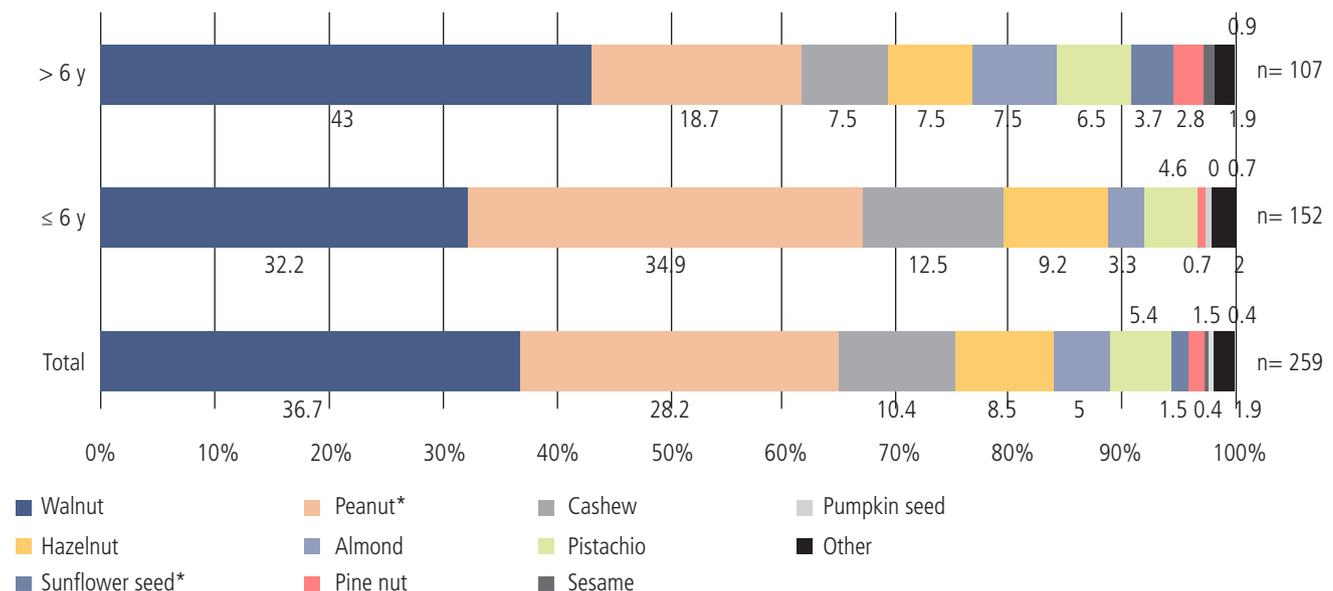


Figure 2. Index nut stratified by age group among allergic patients ($n=259$). The figure displays the prevalence of allergy by index nut for the whole population stratified by age (≤ 6 years vs >6 years). Among walnut-allergic children, there is a nonsignificant trend toward older age at onset. *Among peanut-allergic patients, onset occurred more frequently in younger children, whereas older patients were more likely to develop reactions to sunflower seed as the index nut ($P=.016$). The total number of cases in this figure is 259 instead of 260, as data on age were missing for 1 peanut-allergic patient.

Table 1. Demographic and Baseline Characteristics of the Population (N=271)^a

Variable	Allergic participants, No. (%)	Nonallergic participants, No. (%)	Total, No./total participants for the variable (%)
Male sex	147 (56.5%)	6 (54.5%)	153/271 (56.5%)
Mean (SD) age at diagnosis, y	6.5 (3.5)	7.0 (3.7)	6.49 (3.4)
Mean (SD) time from IR to allergy evaluation, mo	11.8 (21.2)	3.3 (3.3)	11.4 (20.7)
Mean (SD) time from last contact with trigger nut to allergy evaluation, mo	5.8 (11.8)	2.2 (2.7)	5.6 (11.5)
Age distribution by ranges			
0-2 y	45 (17.4%)	1 (9.1%)	46/270 (17%)
3-5 y	89 (34.4%)	4 (36.4%)	93/270 (34.4%)
6-10 y	95 (36.7%)	4 (36.4%)	99/270 (36.7%)
11-14 y	30 (11.6%)	2 (18.2%)	32/270 (11.9%)
Personal history of allergy			
Atopic dermatitis	125 (48.8%)	7 (63.6%)	132/267 (48.7%)
Other food allergies	86 (33.7%)	4 (36.4%)	90/266 (33.8%)
Allergy to any plant food	45 (17.8%)	1 (9.1%)	46/264 (17.4%)
Allergic rhinitis	118 (46.3%)	7 (63.6%)	125/266 (47%)
Allergic asthma	110 (43%)	3 (30%)	113/266 (42.5%)
Drug allergy	4 (1.6%)	0	4/267 (1.5%)
Index reaction–triggering nut			
Walnut	95 (36.5%)	3 (27.3%)	98/271 (36.2%)
Peanut	74 (28.5%)	2 (18.2%)	76/271 (28%)
Cashew	27 (10.4%)	0	27/271 (10%)
Hazelnut	22 (8.5%)	2 (18.2%)	24/271 (8.9%)
Pistachio	14 (5.4%)	1 (9.1%)	15/271 (5.5%)
Almond	13 (5%)	2 (18.2%)	15/271 (5.5%)
Sunflower seed	4 (1.5%)	1 (9.4%)	5/271 (1.8%)
Pine nut	4 (1.5%)	0	4/271 (1.5%)
Pumpkin seed	1 (0.4%)	0	1/271 (0.4%)
Sesame	1 (0.4%)	0	1/271 (0.4%)
Chestnut	0	0	0
Other	5 (1.9%)	0	5/271 (1.8%)

Abbreviation: IR, index reaction.

^aThere were no significant differences between allergic and nonallergic participants. Of note, the total number of allergic and nonallergic participants is 260 and 11; however, the fact that information was missing for some variables means that there is a slight change in the total number of patients, as indicated for each variable.

within 5 minutes of nut exposure, respectively ($P < .0001$), this being the only difference between allergic and nonallergic children. The most common (46.9%) duration of reactions was from 1 to 2 hours.

Among allergic participants, the skin was the most frequently involved organ (76.2%), followed by the gastrointestinal tract (66.9%) and respiratory tract (29.2%) (Figure 3). The most common symptoms were urticaria (49.2%), oral allergy symptoms (48.7%), and angioedema (45.6%) (Figure 3).

Symptoms involving the cardiovascular system (0.8%) and nervous system (0.4%) were highly infrequent. Only erythema and pruritus as symptoms and skin and gastrointestinal organ involvement were more frequent ($P < .05$) among allergic children than children without allergy. No associations were found between specific symptoms or organs affected and individual nuts.

According to the attending physician, 41.9%, 46.2%, and 11.9% of participants had a mild, moderate, and severe index

Table 2. Characteristics of the Patients and Index Reaction Stratified for Nuts with at Least 10 Cases (n=245 Allergic Participants)^a

	Walnut (n=95)	Peanut (n=74)	Hazelnut (n=22)	Cashew (n=27)	Pistachio (n=14)	Almond (n=13)	Total (n=245)	P Value
Mean (SD) age at diagnosis, y	7.0 (3.5)	5.6* (3.2)	6.2 (3.4)	5.4* (3.4)	6.8 (3)	8.1* (3.1)	6.4 (3.4)	.019*
Participants ≤6 y, %	51.6 *	72.6 *	63.6	70.4	50.0	38.5	60.2	.032*
Male sex, %	62.1	55.4	45.5	63	50	46.2	57.1	.611
Index reaction characteristics								
Post hoc anaphylaxis criteria, %	31.6	29.7	40.9	40.7	28.6	15.4	31.8	.605
Time of onset								
<5 min, %	64.2	71.6	45.5	77.8	50.0	69.2	65.7	.185
5 to 20 min, %	31.6	17.6	40.9	18.5	35.7	15.4	26.1	
21 to 120 min, %	4.2	8.1	9.1	0.0	7.1	15.4	6.1	
>120 min, %	0	2.7	4.5	3.7	7.1	0	2.0	
Duration of the reaction								
30 to 60 min, %	37.9	47.9*	42.9	15.4*	30.8	25.0	37.9	.01*
61 to 120 min, %	48.4	41.1	33.3	65.4*	61.5	25.0	46.3	
>120 min, %	13.7	11.0	23.8	19.2	7.7	50.0*	15.8	
Sensitization patterns for nuts								
SPT positive to walnut, %	83.9*	36.5*	71.4	30.8*	35.7	50.0	57.9	<.0001*
SPT positive to peanut, %	31.2*	91.9*	52.4	42.3	64.3	38.5	55.2	<.0001*
SPT positive to hazelnut, %	38.7	36.5	100.0*	46.2	50.0	46.2	45.2	<.0001*
SPT positive to cashew, %	16.1*	16.2*	38.1	88.5*	71.4*	23.1	29.5	<.0001*
SPT positive to pistachio, %	19.4*	21.6*	33.3	73.1*	85.7*	30.8	31.5	<.0001*
SPT positive to almond, %	21.5*	29.7	33.3	30.8	35.7	84.6*	30.3	<.0001*
SPT peach peel (LTP marker), %	22.6	16.2	33.3	15.4	7.1	15.4	19.5	.371
SPT nPho d 2 (profilin marker), %	8.6	6.8	9.5	15.4	0.0	0.0	7.9	.461
SPT positive to 2 or more nuts, %	54.7	54.1	90.9*	85.2*	85.7*	92.3*	64.9	<.0001*
Median (range) index nut SPT, mm	5 (1-18)	8 (2-95)*	6 (1-14)	7 (1-28)	8 (1-18)	3 (3-9)*	6 (1-95)	<.0001*
Median (range) index nut whole extract sIgE values, kU _A /L	2.93 (0->100)	2.1 (0.14->100)	3.32 (0.13->100)	2.32 (0->100)	4.98 (0-95.7)	0.90 (0-7.2)	2.51 (0->100)	.125

Abbreviations: LTP, lipid transfer protein; SPT, skin prick test.

^aDetailed atopic background, index reaction characteristics, and sensitization profile for the most frequent onset-triggering nuts in the population. Significant differences ($P<.05$) between the value for a given nut with respect to the total are marked with an asterisk. Reported pollen sensitization reflects positivity in SPT and/or any ISAC allergen for each pollen source.

reaction. The post hoc analysis showed anaphylaxis to be present in 35% of the events ($n=91$). The level of correlation between physician-assessed severity and anaphylaxis by

post hoc analysis was high, with a Tau- β of 0.60 ($P<.0001$) (Supplementary figure 1). No associations were found between severity of anaphylaxis and individual nuts.

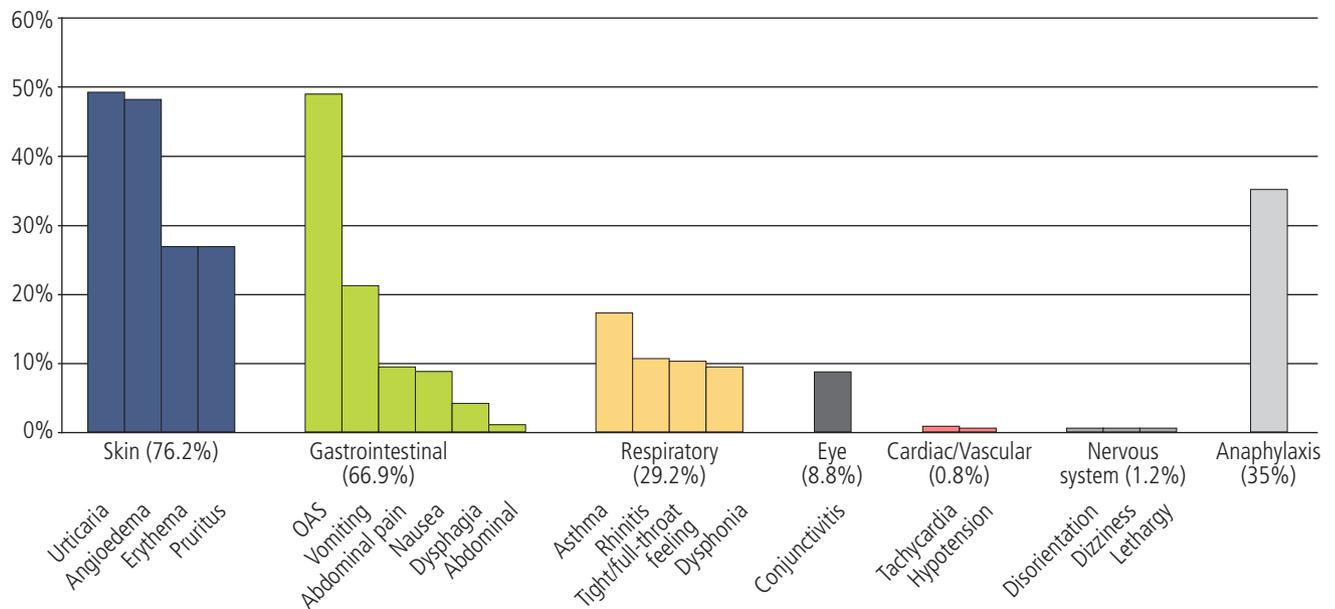


Figure 3. Clinical features of the index reaction among allergic patients. Symptoms reported to occur during index reactions, by organ and frequency. OAS indicates oral allergy syndrome.

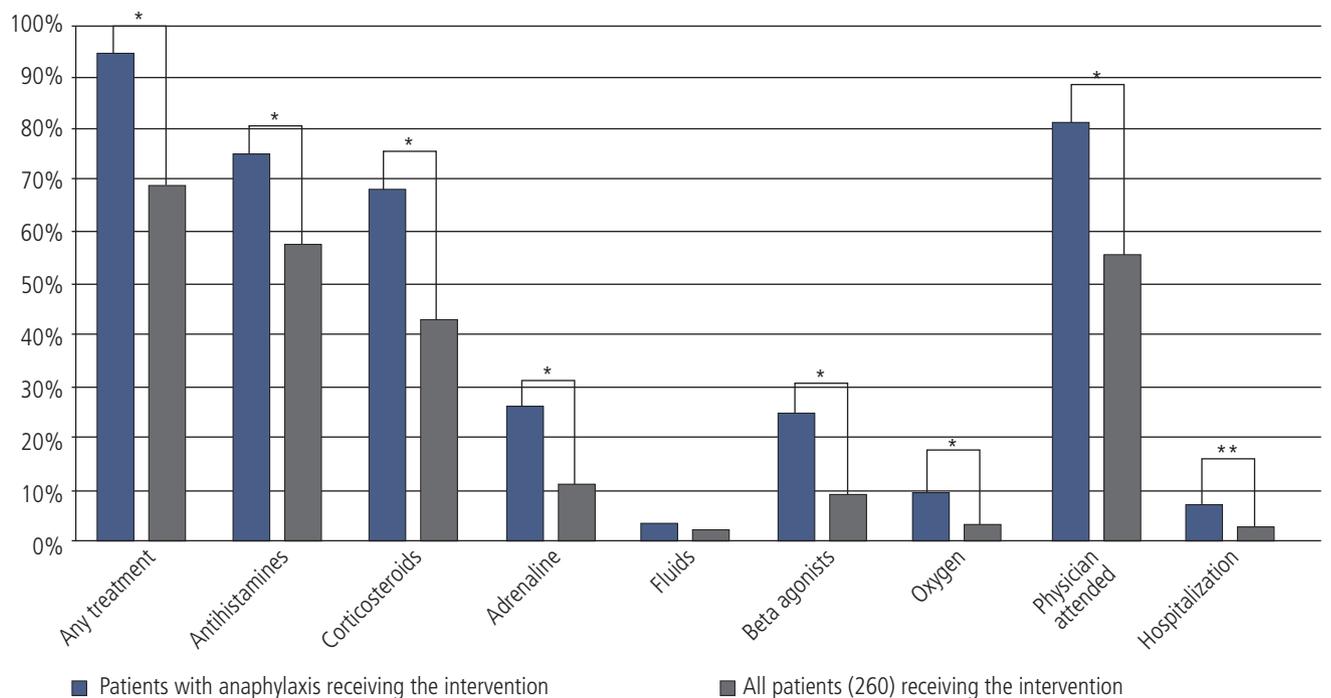


Figure 4. Treatment at the time of the index reaction ($n=260$) compared with treatment in patients who experienced anaphylaxis ($n=91$). This figure reflects drug therapy and other interventions applied in patients who developed anaphylaxis (blue) during the index reaction compared with the percentage of the whole population (gray) receiving the same treatment. Statistically significant differences were found between the percentage of anaphylaxis patients receiving the treatment and the percentage of the general population receiving the treatment.

* $P<.001$

** $P=.021$

The index reaction subsided without treatment in 31% of allergic participants. The patient visited the emergency department in 54.4% of cases and was hospitalized in 3%. Antihistamines, oral corticosteroids, adrenaline, and β -agonists were used in 57.4%, 43%, 11.2%, and 9.2% of patients, respectively, although use of these drugs was significantly more frequent in cases of anaphylaxis (Figure 4). Nevertheless, even in cases of anaphylaxis, the use of adrenaline was very infrequent (26.2% of all anaphylactic reactions).

Diagnostic Tests

Skin prick tests

More than half of the allergic population had positive SPT results to walnut (57.9%) and peanut (55.2%), followed by hazelnut, cashew, pistachio, and almond (Table 2). Polysensitization to nut was detected in 64.9% of patients; older children (>6 years) were polysensitized more often (75.9% vs 59.9%, $P=.011$). Polysensitization to nuts was more frequent among patients who were allergic to hazelnut, almond, cashew, and pistachio (85.2% to 92.3%, $P<.0001$) than in the overall population (64.9%). One remarkable difference concerns the significantly lower percentage of walnut-allergic patients who were sensitized to peanut and vice versa (Table 2); this finding suggests the role of both these foods as primary sensitizers, particularly given the low percentage of sensitization to other nuts among patients initially selected owing to their sensitivity to walnut or peanut and further evaluated for other sensitizations using SPT (Supplementary figure 2).

Interestingly, the only statistically significant receiver operating characteristic (ROC) curve for the risk of anaphylaxis (Supplementary Figure 3) was for peanut SPT wheal size, with

an area under the curve of 0.758 (95%CI, 0.638-0.879) and an optimal cut-off size of 8.5 mm for anaphylaxis (OR, 5.5 [95%CI, 1.8-16.5]; $P=.002$).

For the whole population, a positive SPT result to at least 1 pollen was identified in 52.9%, with *Phleum* (30.2%) and *Parietaria* (5.7%) pollen being the most and least common sensitizers. Around 45.9% of patients with a positive SPT result to pollen had no respiratory symptoms.

Whole Extract Serum Specific IgE

Whole extract sIgE tests were available for most index nuts in allergic patients (222 out of 260, Table 2). We assessed the value of whole extract serum sIgE as a marker of specific symptoms during the index reaction, a more severe reaction, or anaphylaxis, although no correlation was found.

Serum Molecular Diagnosis

A molecular diagnosis was available for 96.2% of allergic patients ($n=250/260$). Allergens from the 2S albumin family were the most prevalent ($n=187$; 74.8%); results were positive for Jug r 1 in 52.8% of the population, followed by Ara h 2 (37.6%) and Ara h 6 (36.8%). The allergens with $\geq 50\%$ recognition among walnut-allergic children were Jug r 1, Ara h 2, and Ara h 6 for peanut, Jug r 1 and Pru p 3 for hazelnut, and Jug r 1 and Gly m 6 for pistachio (Figure 5). Only 9.6% of the population was sensitized to profilin (Hev b 8).

The 2S albumin family (Ara h 2, Jug r 1, Ara h 6, and Ses i 1) and 11S globulin family (Ara h 3, Cor a 9, and Ana o 2) were also evaluated for cross-sensitization. Among patients sensitized to 2S albumins, Ara h 2 and Ara h 6 showed a high degree of cross-sensitization ($\approx 90\%$), although this was lower for Jug r 1 ($<50\%$) (Figure 6A). For patients recognizing at

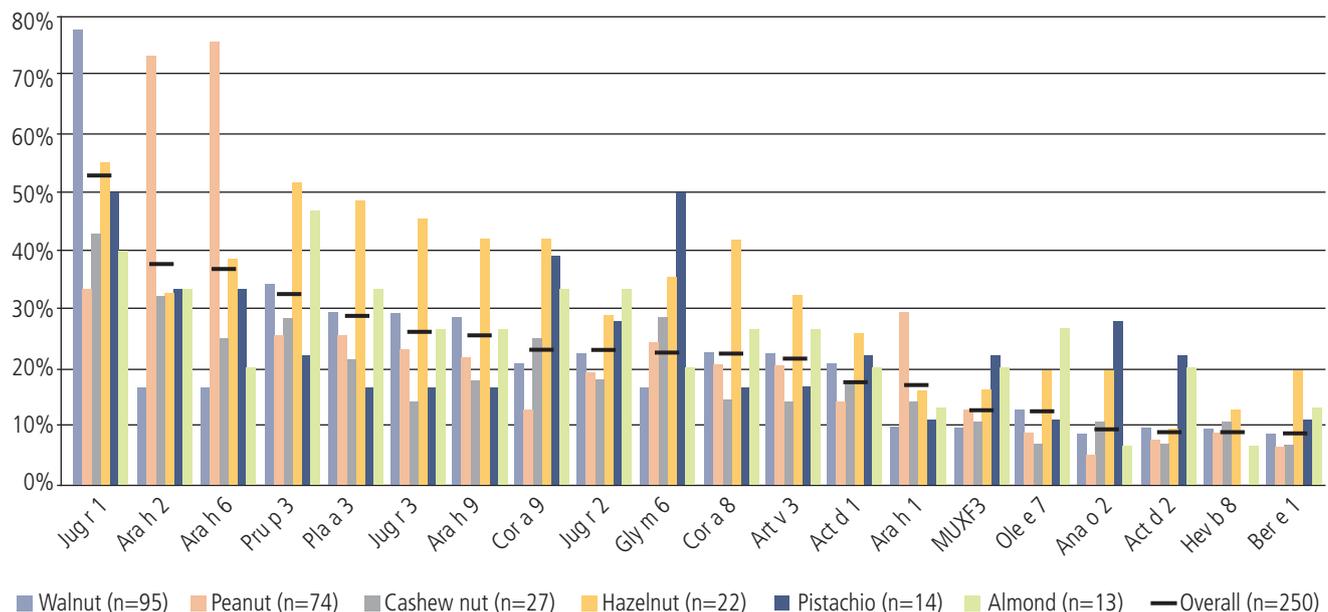


Figure 5. Molecular patterns of recognition (sIgE) for the 20 most relevant allergens among allergic patients. The graph shows the 20 most recognized vegetable allergens. Black lines indicate the percentage of recognition for the given allergen among the 250 allergic patients for whom ISAC results were available; colored bars represent the percentage of recognition for the allergen for each group of children allergic to the nut in question.

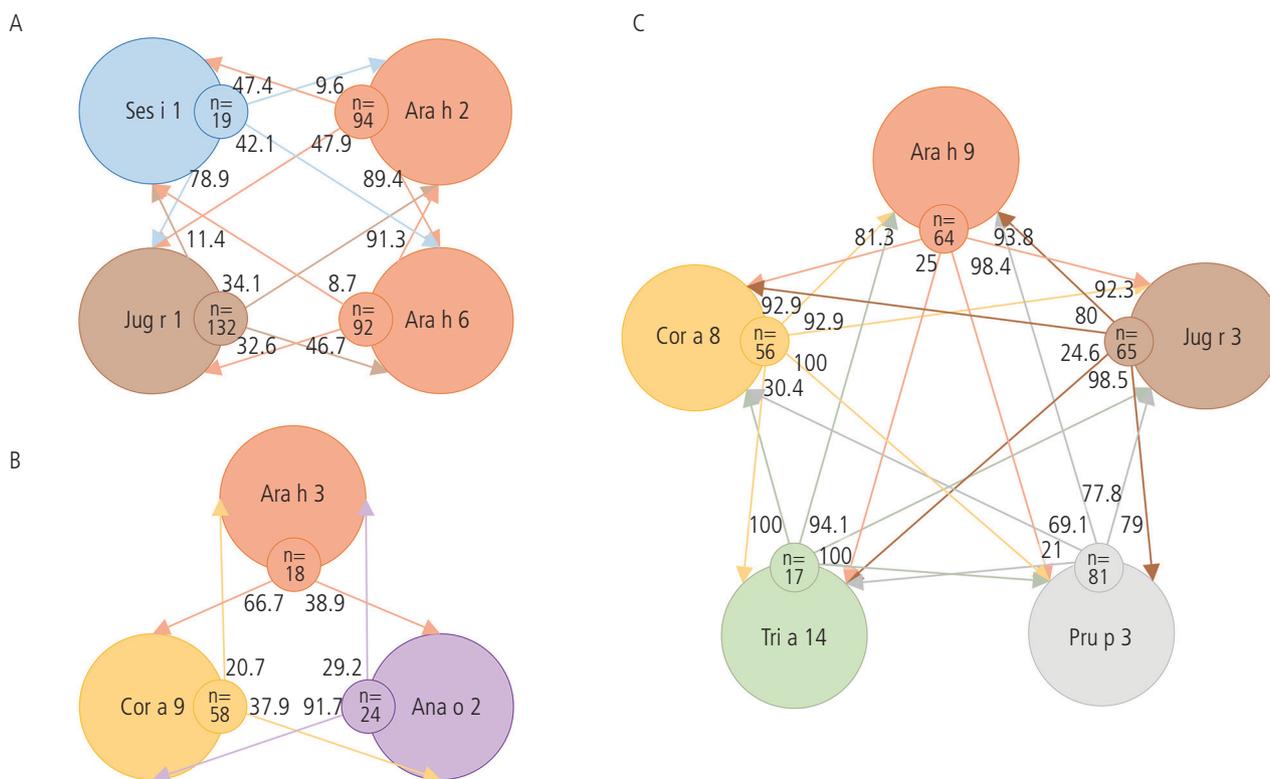


Figure 6. Two-way cross-sensitivity diagram for patients sensitive to at least one 2S albumin, and/or 11S globulin, and/or LTP from vegetables available in ISAC-112 assays. A, 2S albumins; B, 11S globulins. C, LTPs. Arrows pointing away from each allergen indicate patients with initial positivity for the pertinent allergen (n, in circle), and the number beside each outgoing arrow represents the percentage of these patients who also had a positive result for the allergen at the end of the arrow. By way of example, in Figure 6C, 64 patients recognized Ara h 9, and of these, 81.3% were also positive for Cor a 8, 25% for Tri a 14, 98.4% for Pru p 3, and 93.8% for Jug r 3. All associations were significant ($P < .0001$). A and B depict the same information for the 2S and 11S families, respectively.

least 1 member of the 11S globulin family ($n=66$; 26.4%), recognition by patients sensitized to the other members of the family was highest for Cor a 9 (66.7% and 91.7% among Ara h 3- and Ana o 2-sensitized children) (Figure 6B).

The nonspecific LTP family was the second most recognized ($n=92$; 36.8%). Pru p 3, the peach LTP, was the most relevant panallergen (32.4% positivity) among all allergic patients. The cross-sensitivity between LTPs present in foods available for the ISAC assay (Figure 6C) shows that Pru p 3 and Tri a 14 are the molecules most and least frequently recognized by other LTP-sensitized patients (mean, 99.2% vs 25.2%, respectively). On the other hand, for patients sensitized to Tri a 14 ($n=17$) and Pru p 3 ($n=81$), the percentage of recognition of other LTPs was the highest and lowest, respectively (97.1% and 61.7%).

Controlled Food Challenges

In total, 21 OFCs were performed to clarify index reactions to nut, and 33 OFCs were conducted to diagnose secondary nut allergy; in total, 35.2% (19/54) were positive. The only significant differences found between the OFC and index reaction symptoms were less frequent skin involvement (52.6% vs 76.2%, $P=.030$) and a lower rate of anaphylaxis (10.5% vs

35%, $P=.041$) during the challenges (Online Supplementary figure 4).

Discussion

In this prospective multicenter pediatric study, we found that Spanish children are first diagnosed with nut allergy at a mean age of 6.5 years, with onset of symptoms at a mean of 11.8 (21.2) months earlier, predominantly caused by walnut followed by peanut. Symptoms can be severe on first contact, and a third of patients present with anaphylaxis. Polysensitization to several nuts was recorded in more than half of the patients, and molecular diagnosis pointed to the 2S albumin family of the seed storage proteins, especially Jug r 1, as the most frequent sensitizers.

In our population of 260 allergic children, 51.8% of patients were younger than 6 years, although only 17% were younger than 2 years. A recent study [17] based on similar data from 3 European centers described a significantly earlier onset of nut allergy onset in London (4.5 years) than in Geneva (6 years) and Valencia (7.3 years). This slight trend toward earlier onset of nut/peanut allergy in English-speaking countries than in other regions had been reported previously in an international

cohort of 115 peanut-allergic patients, where the median age of onset was 1.4 years in US children and 4 years in Spanish and Swedish participants [18]. These differences may be related to cultural variations in diet and practices for introducing nuts to the diet.

We found that walnut (36.5%), peanut (28.5%), and cashew (10.4%) were the most frequent triggering nuts and that onset of peanut allergy was significantly more frequent before age 6 years, while onset of walnut allergy was more frequent in children over age 6 years ($P=.032$). In the study by Brough et al [17], the Spanish center also reported walnut as the most frequent nut, while in the UK and Switzerland, the most common culprit was peanut. Additionally, studies from Australia showed that tree nut allergy and allergy to cashew in particular, is uncommon in the first years of life [19]. However, at age 6 years, the prevalence of cashew allergy is similar to that of peanut allergy, thereby lending further validity to the influence of geography on sensitization patterns. As in our case, Brough et al, reported that allergy to seeds and sesame was nearly negligible in children.

In the current cohort, the index reaction occurred at the first contact in 66% of cases, and the reaction was moderate to severe in around 60% of patients. Thirty-five percent of cases were classified as anaphylaxis, and only 26.2% of these patients received adrenaline; these rates are consistent with those described in the European Anaphylaxis Registry [4], where nuts were also frequent elicitors and adrenaline was used in around 25% of cases in recent years. An 8-mm cut-off value for SPT to peanut was previously described as a good predictor of clinically relevant allergy to peanut [20]; in our cohort, this value was more reflective of risk of anaphylaxis.

Cosensitization was evaluated by means of SPT and molecular diagnosis. Polysensitization to 2 or more nuts based on SPT was detected in 65% of the children studied, with older patients (>6 years) polysensitized more often than their younger peers, thus confirming a trend towards increasing sensitization over time [19] and, therefore, a greater likelihood of nut allergy with age [17]. Cosensitization was significantly less frequent for the most common sensitizing nuts (walnut, 36.5%; peanut, 31.2%) compared with their total percentage of cosensitization (approximately 55%, $P<.0001$), thus suggesting an absence of association between these allergies. In contrast, the association between cashew and pistachio based on SPT was the most frequent (>70%), consistent with previous reports [17,21,22].

The most relevant allergen family was 2S albumins, as 75% of the population was sensitized to 1 family member (53% for Jug r 1, 37% for both Ara h 2 and Ara h 6). The high mutual recognition observed between Ara h 2 and Ara h 6 ($\approx 90\%$) was also previously reported [23]. However, the strikingly low degree of cosensitization of Jug r 1-sensitized patients who were sensitized to Ara h 2 and Ara h 6 (34% and 32%) may be attributable to their low amino acid sequence identities ($\approx 22\%$) [24], thus supporting the hypothesis that this is a true and independent primary sensitization to Jug r 1 and Ara h 2/6 rather than mere cross-sensitization. The role of panallergens in the study population was minor, the most frequent being LTP (37%).

In the subgroup of walnut-allergic children, Jug r 1 (77%) was the most relevant allergen, whilst Jug r 3 (LTP) and Jug r 2 behaved as minor allergens, with positive results for only 29% and 22% of patients, respectively. Although our study population could be expected to be mostly sensitized to LTP, as in Mediterranean walnut-allergic adults [25], the recognition patterns of our cohort resembled those of a recent international walnut-allergic cluster, where Jug r 1 was dominant (75%) in patients younger than 14 years [26].

The predominance of LTP has been described [18] in a population comprising 50 Spanish peanut-allergic children, with 60% and 42% recognition of rAra h 9 and rAra h 2, respectively. However, other reports have shown different patterns that are age-dependent. Garcia-Blanca et al [27] studied a large group of peanut-allergic patients ($n=250$, age ≤ 20 years), in which the authors also described the expected predominance of the response to Ara h 9 among older patients, albeit with an interesting change in pattern toward predominance of Ara h 2 among younger participants. In the peanut-allergic population in our study, 76% and 73% of patients had a positive test result for Ara h 6 and 2, and only 22% were Ara h 9-positive. These percentages are in line with those described by Pedrosa et al [28] in 55 peanut-allergic infants, 72% of whom were sensitized to Ara h 2 and fewer (45%) to LTPs. Of note, sensitization to LTP was predominantly driven by sensitization to Pru p 3, as this was the most widely recognized allergen of the family.

Hazelnut-allergic patients were most commonly sensitized to albumin 2S (Jug r 1, 55%). This finding may be due either to genuine sensitization to the hazelnut 2S albumin (Cor a 14, not available in ISAC-112) and its cross-reactivity to Jug r 1 or to the influence of clinically relevant walnut allergy, which was the case in half of the hazelnut-allergic patients (data not shown). As previously described in the Mediterranean area, LTPs were relevant allergens (52% sensitization), but Cor a 1 (Bet v 1 homolog, 9% sensitization) was not, in contrast with central and northern Europe [29], where hazelnut allergy is a Cor a 1-driven phenomenon.

The present study is limited by the fact that, since it was designed to describe the onset of nut allergy and not to measure prevalence, not all patients underwent OFC. However, current guidelines [11,30] support using a combination of clinical presentation with proven sensitization for diagnosis. Additionally, use of a flexible protocol without compulsory challenge facilitated inclusion, enabling the main study objective to be achieved. On the other hand, a strength of the present study is the geographic variety of the study patients, with representation throughout Spain. An additional merit stems from its prospective design, including a common protocol that called for in-depth molecular diagnosis.

The results presented here indicate that allergy to nuts, including peanut, tree nuts, and seeds, usually presents at around age 6 years and can cause severe reactions. Walnut and peanut are the main triggers at onset of nut allergy, with peanut being the earliest. Sensitization to the 2S storage proteins Jug r 1, Ara h 2, and Ara h 6 was mostly acquired during childhood, while the role of LTPs was less relevant.

This study provides insight into various clinical and sensitization-related aspects that must be kept in mind when

managing pediatric patients with suspected nut allergy. Our relevant findings may guide early preventive strategies in infants at risk.

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Conflicts of Interest

Dr Ibáñez reports the following: grants from Aimmune Therapeutics during the conduct of the study; grants from National Instituto Salud Carlos III; personal fees from Faes Farma, Merck, LETI, ROXALL, and CIRCASSIA outside the submitted work.

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