

Peanut Allergy in Spanish Children: Comparative Profile of Peanut Allergy versus Tolerance

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Keywords

Ara h 1 · Ara h 2 · Ara h 3 · Ara h 9 · Component-resolved diagnosis · Peanut allergy · Peanut tolerance · Pru p 3

Abstract

Background: Peanut storage proteins (Ara h 1, Ara h 2, and Ara h 3) have been described as the major peanut allergens in children, although not all peanut-sensitized individuals have clinical reactivity after exposure. **Objectives:** We studied the sensitization profile of peanut-allergic and peanut-tolerant children in a pediatric cohort. **Methods:** The clinical features and sensitization profile to the peanut storage proteins Ara h 9 and Pru p 3 were compared between peanut-allergic and peanut-tolerant children using component-resolved diagnostics. **Results:** Thirty-three peanut-sensitized children were included: 22 allergic and 11 tolerant patients. Seventy-two percent of the peanut-allergic children were sensitized to at least one peanut storage protein. The rates of sensitization to Ara h 1, Ara h 2, and Ara h 3 were 63.6, 68.1, and 68.1%, respectively, among the peanut-allergic children and 27.2, 18.1, and 45.4% among the peanut-tolerant children. IgE from the sera of 18% of the peanut-allergic patients recognized Ara h 9, whereas no sensitization to Ara h 9 was

detected in the peanut-tolerant children. A total of 59% of the peanut-allergic and 27% of the peanut-tolerant children were sensitized to Pru p 3. Sensitization to Ara h 1 and Ara h 2 was more frequent among the peanut-allergic children ($p < 0.05$). Although the levels of specific IgE against peanut storage proteins were higher in peanut allergy, there were not statistically significantly different from the levels in peanut tolerance, probably due to the small number of patients included. **Conclusions:** In our population, the peanut-allergic children were mainly sensitized to peanut storage proteins, and Ara h 2 sensitization allows a more accurate diagnosis of clinical reactivity to peanuts. More than half of the peanut-allergic patients were sensitized to peach Pru p 3, and 50% of them had fruit allergy at the time of the study.

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Introduction

Peanuts constitute an important and increasing cause of food allergy in children, affecting approximately 0.2–3% of the world's population [1, 2]. Accidental exposure

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to peanuts can cause serious and even fatal reactions that can occur early in life; symptoms may manifest as anything from mild oral allergy to severe anaphylactic reactions [2].

Peanut allergy may vary depending on the geographical regions studied, with differences in clinical expression and molecular patterns of recognition, which is related to exposure to other allergens present in different pollens, other vegetable proteins, and influences from culinary traditions [3].

Until now, different peanut proteins have been characterized (from Ara h 1 to Ara h 17) [4], with 9 of them related to peanut allergy. Of these, Ara h 1 and Ara h 3 belong to the cupin superfamily, while Ara h 2, Ara h 6, and Ara h 7 belong to the conglutins or prolamins. Ara h 8 is a member of the PR-10 proteins, relevant in patients with allergy to birch pollen, Ara h 9 is a nonspecific lipid transfer protein (nsLTP), and Ara h 5 is a profilin; all of them have a large number of closely related proteins in the plant kingdom. A high degree of thermal and digestive stability has been described for the seed storage proteins Ara h 1, 2, 3, 6, and 7, as well as for Ara h 9, which plays an important role in peanut allergy among patients from the Mediterranean region [5–7]. Lauer et al. [8] observed that Ara h 9 showed 62–68% amino acid sequence identity with Pru p 3, another LTP present in peach fruit, and demonstrated IgE antibody reactivity in 29 of 32 Spanish compared with 6 of 41 non-Mediterranean patients with peanut allergy.

Component-resolved diagnostics for peanut allergy might more rationally and discriminatively assess peanut sensitization profiles using individual-specific allergens instead of whole peanut extracts, which contain panallergens and nonallergenic proteins in addition to peanut allergens [9, 10]. Ara h 1, Ara h 2, and Ara h 3 are considered the major peanut allergens in pediatric populations, with many studies demonstrating a higher value of Ara h 2 as a predictor of peanut allergy [7, 10–20].

Methods

We studied the clinical features of a pediatric population with peanut allergy and compared the sensitization profiles of peanut-allergic and peanut-tolerant children using component-resolved diagnostics. For this purpose, we evaluated patients referred to our Pediatric Allergy Unit (Hospital Infantil Universitario Gregorio Marañón, Madrid, Spain) with a clinical history of reactions to peanuts or nuts. A detailed clinical history that included demographic characteristics, age at onset, characteristics of the reaction, plant-related food allergies, pollen sensitization, and the personal history of atopic diseases was taken.

Peanut allergy was defined as a convincing history of allergic symptoms (oral allergy syndrome, urticaria, angioedema, and respiratory, cardiovascular, and/or gastrointestinal symptoms) appearing within 1 h after having eaten peanuts, as well as a positive skin prick test and a peanut sIgE level of ≥ 0.10 kU/L in serum confirmed by an oral challenge test (OCT). Sera from 33 children with an IgE-mediated reaction after having ingested peanuts and other nuts were consecutively collected between April and December 2014 at the Department of Allergy, Hospital Infantil Universitario Gregorio Marañón, Madrid, Spain. Specific IgE antibodies to peanut and recombinant components (Ara h 1, Ara h 2, and Ara h 3), Ara h 9, and Pru p 3 were quantified according to the manufacturer's instructions (Thermo Fisher Scientific, Uppsala, Sweden). Results ≥ 0.10 kU/L were considered positive.

Skin prick tests with commercial peanut extract were carried out, with histamine phosphate (10 mg/mL) and saline solution used as positive and negative controls, respectively. A reading was taken after 15 min and considered positive when the size of the swelling was ≥ 3 mm compared to the negative control.

Open OCTs were conducted, fractionating peanut doses, starting with half or one seed depending on the severity of the initial symptoms. The doses were doubled up to a cumulative dose of 15 seeds and administered at 30-min intervals until objective clinical manifestations were observed. Subjects with skin test positivity who tolerated the maximum dose of peanuts were classified as sensitized. Every patient was kept under surveillance for at least 2 h after the last dose had been administered or 2 h after the symptoms had totally subsided. An OCT was not done in cases of suspected serious systemic reactions due to the potential risk of eliciting a severe reaction.

Quantitative variables are expressed as median values (range) and the qualitative ones are described as frequencies (percentages). The χ^2 test was used for comparison of dichotomous variables between groups. The Mann-Whitney test was used where appropriate, and *p* values < 0.05 were considered significant. All statistical analyses were performed with SPSS software (version 18).

Results

Our study group consisted of 22 peanut-allergic patients and 11 peanut-sensitized children with peanut tolerance demonstrated through OCT, and these peanut-sensitized children were allergic to other nuts. The clinical characteristics of all peanut-sensitized subjects are summarized in Tables 1 and 2. No significant differences in demographic characteristics or asthma, pollinosis, eczema, or other plant-related food allergies were found between the two groups (Table 2). Total IgE and sIgE levels for the whole peanut extract and the components are shown in Tables 1 and 3.

Peanut Allergy

Sixteen of the 22 peanut-allergic children (72.7%) were sensitized to at least one peanut storage protein. The peanut-allergic children were mainly sensitized to Ara h 2 (68.1%) and Ara h 3 (68.1%), followed by Ara h 1 (63.6%).

Table 1. Individual levels of IgE (kU/L) for whole peanuts and specific peanut allergens, as well as clinical presentation in peanut-tolerant and peanut-allergic children

	Peanut IgE	IgE Ara h 1	IgE Ara h 2	IgE Ara h 3	IgE Ara h 9	IgE Pru p 3	Symptoms
Monosensitized	4.05	0.0926	0.1649	0.2904	0.0695	0.0915	Anaphylaxis
	19.4	0.3695	0.1851	0.3842	0.0539	0.0802	Anaphylaxis
	350	0.5474	0.654	1.3941	0.0515	0.0944	Anaphylaxis
	0.50	0.1855	0.1529	0.1692	0.0508	0.5494	OAS
	100	1.0842	0.9397	1.0119	0.9758	0.095	Urticaria-angioedema
Polysensitized	1.58	0.0913	0.3458	0.0467	0.0571	0.0735	Urticaria
	76.9	0.3856	0.0503	0.7128	0.0934	0.5699	Urticaria
	30.5	0.2642	0.4492	0.3617	0.0791	0.2925	Urticaria-angioedema
	27	0.2508	0.2888	0.8001	0.0709	0.0947	Urticaria-angioedema
	7.48	0.3389	0.2902	0.6542	0.0724	0.2315	Urticaria-angioedema
	11.2	0.2614	0.2189	0.5287	0.0609	0.0966	Urticaria-angioedema
	1.19	0.0378	0.0424	0.0376	0.0488	0.3213	Urticaria-angioedema
	44	0.0546	0.0486	0.0516	0.0501	0.995	Urticaria-angioedema
	3.25	0.0686	0.0507	0.0596	0.0551	0.3283	Urticaria-angioedema
	100	1.2137	0.7494	0.9815	0.8654	0.2123	OAS
	11.2	0.2352	0.3261	0.4609	0.0598	0.0983	Anaphylaxis
	100	0.0368	0.0425	0.042	0.0448	0.1705	Anaphylaxis
	100	1.0627	1.4097	1.2362	1.3229	0.2805	Anaphylaxis
	100	1.2963	0.6631	1.5203	0.1391	0.6728	Anaphylaxis
	1.46	0.2664	0.1622	0.3143	0.1882	0.0904	Anaphylaxis
	11	0.0979	0.0571	0.0968	0.0748	0.2037	Urticaria-angioedema
	3.1	0.0815	0.0569	0.0692	0.063	0.2158	Urticaria-angioedema
Tolerant	0.16	0.092	0.0946	0.2056	0.058	0.0689	–
	11	0.3553	0.0527	0.2915	0.0986	0.0846	–
	0.15	0.0982	0.0635	0.2586	0.0578	0.0751	–
	0.15	0.0912	0.0994	0.268	0.0552	0.0716	–
	12	0.0637	0.1521	0.2075	0.0967	0.0863	–
	2.6	0.1934	0.2769	0.0984	0.0914	0.0632	–
	2.8	0.1542	0.0781	0.0861	0.0971	0.1623	–
	0.8	0.094	0.0988	0.0964	0.0876	0.4648	–
	2.15	0.0948	0.0532	0.074	0.0636	0.319	–
	2.24	0.091	0.0916	0.0633	0.0612	0.0987	–
	1.9	0.0702	0.0887	0.0997	0.0563	0.0845	–

OAS, oral allergy syndrome.

IgE from the sera of 18.1% ($n = 4$) of the peanut-allergic patients recognized Ara h 9 (Tables 1, 3). These 4 cases corresponded to older children. Thirteen peanut-allergic patients (59%) were sensitized to Pru p 3, 6 of them having fruit allergy (Table 2): 2 to peaches, 3 to kiwis, and 1 to bananas.

Seventeen of the 22 peanut-allergic children were also allergic to other nuts: hazelnuts (88%), walnuts (76%), almonds (47%), sunflower seeds (35%), cashew nuts (47%), and pistachios (35%) (“polysensitized”). The remaining 5 peanut-allergic children had no allergy or sensitization to other nuts (“monosensitized”). Six of the 17 polysensitized peanut-allergic patients had no sensitization to any

peanut storage proteins (Ara h 1, Ara h 2, and Ara h 3) or Ara h 9, but all of them were sensitized to Pru p 3 (Table 1). The data on the frequency of sensitization to the respective components are shown in Table 3.

Peanut Tolerance

There were 11 peanut-tolerant children. Interestingly, 63% ($n = 5$) of them were sensitized to peanut storage proteins, mainly to Ara h 3 (Table 3). Sensitization to Ara h 1 and Ara h 2 was less frequent at 27% ($n = 3$) and 18% ($n = 2$), respectively. None were sensitized to Ara h 9. Three patients (27.2%) were sensitized to Pru p 3, with 1 of them having peach allergy (Table 3).

Table 2. Clinical characteristics of the study population

Clinical characteristics	Peanut-allergic children (<i>n</i> = 22)	Peanut-tolerant children (<i>n</i> = 11)	Total (<i>n</i> = 33)	<i>p</i> value
Male	15 (68)	7 (63.6)	22 (66.7)	0.068
Current age, years	8.5 (5–13)	8 (4–15)	8 (5–15)	–
Age at peanut allergy onset, years	4 (2–9)	–	4 (2–9)	–
Atopic history	18 (81.8)	10 (90.9)	28 (84.8)	0.471
Rhinitis and/or asthma	15 (68.1)	7 (63.6)	22 (66.6)	0.679
Pollen allergy	10 (45.4)	7 (63.6)	17 (51.5)	0.062
Eczema/atopic dermatitis	7 (31.8)	5 (45)	12 (36.3)	0.324
Other plant-related food allergies	fruits: 6 (27) peaches: 2 kiwis: 3 pears: 1 bananas: 1 legumes: 1	1 (9) peaches: 1	7 (21)	0.258
<i>Symptoms caused by peanuts</i>				
Oral allergy syndrome	2 (9)	–	–	
Urticaria-angioedema	12 (54.5)	–	–	
Anaphylaxis	8 (36.3)	–	–	
Values are presented as <i>n</i> (%) or median (range).				

Table 3. Levels of IgE for whole peanuts and peanut components

	Peanut-allergic children (<i>n</i> = 22)	Peanut tolerant children (<i>n</i> = 11)	<i>p</i> value
IgE total, IU/mL	390 (127–3,274)	296 (32.7–817)	
Peanut specific IgE, kU/L	100 (0.5–350)	11.5 (0.15–12)	0.001
Positive for peanuts	22 (100)	11 (100)	
Ara h 1 specific IgE, kU/L	0.25 (0.04–1.2)	0.09 (0.06–0.35)	0.13
Positive for Ara h 1	14 (63.6)	3 (27.2)	0.004
Ara h 2 specific IgE, kU/L	0.20 (0.05–1.4)	0.09 (0.05–0.27)	0.10
Positive for Ara h 2	15 (68.1)	2 (18.1)	0.001
Ara h 3 specific IgE, kU/L	0.38 (0.03–1.5)	0.09 (0.06–0.29)	0.08
Positive for Ara h 3	15 (68.1)	5 (45.4)	0.535
Ara h 9 specific IgE, kU/L	0.06 (0.04–1.3)	0.06 (0.05–0.09)	0.79
Positive for Ara h 9	4 (18.1)	0	0.101
Pru p 3 specific IgE, kU/L	0.20 (0.07–0.99)	0.11 (0.06–0.46)	0.056
Positive for Pru p 3	13 (59)	3 (27.2)	0.033
Values are presented as median (range) or <i>n</i> (%). Bold type denotes significance.			

Peanut Allergy versus Peanut Tolerance

The peanut-allergic children had higher levels of specific IgE to peanut extract (median 100 kU/L, range 0.5–350) than the children who tolerated peanuts (median 11.5 kU/L, range 0.15–12) ($p = 0.001$) (Table 3). The pres-

ence or absence of Ara h 2 (below/above the threshold of 0.10 kU/L) was compared by χ^2 test, and 68% of the peanut-allergic and 18% of the peanut-tolerant children were sensitized to it ($p = 0.001$). The median for Ara h 2 was 0.20 kU/L (range 0.05–1.4) and 0.09 kU/L (range 0.05–

0.27) among the peanut-allergic and the peanut-tolerant subjects, respectively. The median levels of Ara h 2 were higher in the peanut-allergic children than in the peanut-tolerant children, but no significant differences were found, probably due to the small sample size (Table 3). The peanut-allergic children without sensitization to Ara h 2 (7/22) were all sensitized to Pru p 3, but in none of them did we detect IgE antibodies to Ara h 9. We also found a greater amount of sensitization to Ara h 1 and Pru p 3 in the peanut-allergic group ($p < 0.05$) than among the peanut-tolerant children (Table 3). There were no differences in median levels or frequency of Ara h 3 between the peanut-allergic and the peanut-tolerant children.

Thirteen of the 22 peanut-allergic subjects (54%) showed simultaneous sensitization to Ara h 1, Ara h 2, and Ara h 3, and of these, 4 children also had sensitization to Ara h 9 and 6 children to Pru p 3. Eight peanut-allergic children (36.3%) developed anaphylaxis after peanut ingestion; 6 of them were sensitized to all peanut storage proteins (Ara h 1, Ara h 2, and Ara h 3). Of the peanut-tolerant children, none had multiple peanut storage protein sensitization.

Discussion and Conclusions

It is well known that peanut sensitization profiles are heterogeneous, with geographical differences in patterns of allergen recognition and clinical manifestations as well as in relationships to other related plant food and pollen inhalant allergens. Ara h 8 and Ara h 9 appear to be the main allergens in Central Europe and in Western/Southern Europe, respectively [3, 12, 21–23]. Sensitization to seed storage proteins starts at an early age and is rarely acquired in adolescence and adulthood [12, 13].

Previous studies have shown that Ara h 2 is the most important allergen for discriminating between peanut allergy and peanut tolerance [7, 10, 12–16, 18–20], and sensitization to it may predict the presence or absence of allergy. Measurement of antibodies to Ara h 2 in children with food allergy could decrease the need for OCTs and the risk taken with these procedures [12].

Codreanu et al. [24] reported for a French pediatric population that 96% of the peanut-allergic children recognized Ara h 2, followed by Ara h 1 (75%), Ara h 3 (61%), and Ara h 8 (40%). Nevertheless, Vereda et al. [3] described different patterns among Spanish, Swedish, and American children, with sensitization to Ara h 9 occurring in 60% of the Spanish patients, followed by Ara h 2 (42%), Ara h 1 (30%), and Ara h 3 (16%). In another Span-

ish study, Pedrosa et al. [14] reported Ara h 2 in 72% of their peanut-allergic subjects, followed by Ara h 1 (60%) and Ara h 3 (43.6%). Although both studies were made in the same city, Ara h 9 was not included in the study by Pedrosa et al. [14]. In this study, Pru p 3 and Cor a 8 were considered potential markers of sensitization to peanut nsLTP, and it was found that 36.4 and 45.5% of the peanut-allergic children had sensitization to Cor a 8 and Pru p 3, respectively.

The differences are related to the geographical location of the children and their age, since in the Pedrosa group, mostly adolescents were included. A study also conducted on a pediatric population from Madrid showed that as children allergic to peanuts were growing older, sensitization to Ara h 2 was decreasing with an increase in sensitization to Ara h 9 and Pru p 3 [13]. When compared to these two Spanish studies on pediatric populations [3, 14], our results are more similar to the work previously published by Pedrosa et al. [14]. We found that the frequency of sensitization to Ara h 2 was higher in the allergic group ($p < 0.05$), but with no differences in median levels, probably because of the small number of patients. The main limitation of our study is that the sample was too small to extrapolate reliable conclusions for the general population.

In our study group, 27% of the peanut-allergic patients had no sensitization to peanut storage proteins or to Ara h 9, but they were sensitized to peach Pru p 3. Ara h 6 could not be tested, but it might have shown relevance to the peanut-allergic children without sensitization to storage proteins. These data suggest that our cases could be primarily sensitized to Pru p 3 and that the response to Ara h 9 was due to cross-reactivity, as has in fact been reported in another study carried out in Spain [15]. In adult populations from the Mediterranean region, the most frequent peanut allergen described is Ara h 9 [3, 7, 21], and significant cross-reactivity has been described with peach Pru p 3 [7, 8, 12, 25], which seems to be the primary sensitizer.

Our patients had peanut allergy from an early age, but despite the high frequency of Pru p 3 sensitization among the peanut-allergic children (59%), no prevalent fruit allergy was observed. We speculate that Pru p 3 sensitization in our population could possibly precede the appearance of fruit allergy, which is highly prevalent in Mediterranean countries – whether by primary sensitization to Pru p 3 or cosensitization with Ara h 9 has been analyzed in other studies [3, 12, 21, 22].

We found a greater number of peanut-tolerant children sensitized to peanut storage proteins than previous-

ly published – most frequently to Ara h 3, which was recognized by 45% of the children; thus, Ara h 3 did not allow us to differentiate peanut-allergic from peanut-tolerant children. Although many peanut-tolerant patients may be sensitized to peanut storage proteins, Ara h 2 allows a more accurate prediction of a clinical response to peanuts. In previous studies, sensitization to multiple peanut storage proteins has been linked to a higher probability of symptoms [11, 12, 24]. In the present study, similar results were found, and more than half of the peanut-allergic children presented sensitization to multiple peanut storage proteins. Therefore, sensitization to multiple components could be helpful in detecting allergic patients.

In summary, we observed that clinical reactivity to peanuts was associated with sensitization to peanut storage proteins rather than nsLTP in our pediatric population. Although panallergens like Pru p 3 may play an important role in peanut-allergic patients, they occur at later ages when children have become older [13]. Ara h 2 allows us to make a more accurate diagnosis, which could help avoid greater risks. We would like to emphasize that the majority of patients sensitized to Pru p 3 had no sen-

sitization to Ara h 9 at the time of the study, but they could have it later and might also develop fruit allergy in the future.

Statement of Ethics

The authors declare that no patient data appear in this article and that no experiments were performed on humans or animals for this investigation. The study was explained to all participants and their parents, and written informed consent was obtained. This study was approved by the Research Ethics Committee of Gregorio Marañón Hospital, Madrid (No. ALE_[ALE-AAV]).

Disclosure Statement

The authors do not have any potential conflicts of interests to declare.

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