

ORIGINAL ARTICLE

Peanut seed storage proteins are responsible for clinical reactivity in Spanish peanut-allergic children

María Pedrosa, Teresa Boyano-Martínez, María Carmen García-Ara, Teresa Caballero & Santiago Quirce

Department of Allergy, Hospital La Paz Health Research Institute (IdiPAZ), Madrid, Spain

To cite this article: Pedrosa M, Boyano-Martínez T, García-Ara MC, Caballero T, Quirce S. Peanut seed storage proteins are responsible for clinical reactivity in Spanish peanut-allergic children. *Pediatr Allergy Immunol* 2012; **00**.

Keywords

Ara h 1; Ara h 2; Ara h 3; component-resolved diagnosis; peanut allergy; non-specific lipid transfer protein.

Correspondence

María Pedrosa, MD, Allergy Dept. University Hospital La Paz, P Castellana 261, 28046 Madrid, Spain.
Tel.: +34 657655869
Fax: +34 917277050
E-mail: maria.pedrosa@idipaz.es

Accepted for publication 26 May 2012

DOI:10.1111/j.1399-3038.2012.01337.x

Abstract

Background: Seed storage proteins (SSP; Ara h 1, Ara h 2, Ara h 3) have been shown to be major peanut allergens, although recently, peanut lipid transfer protein has been reported to be an important allergen in the Mediterranean area. We sought to investigate the sensitization pattern to peanut SSP and vegetable pan-allergens in a group of peanut-allergic children compared with a peanut-tolerant group.

Methods: One hundred and twenty-three children who presented with food allergy were included in the study. Tolerance to peanut ingestion was assessed. Specific IgE was determined by ImmunoCAP, and microarray ISAC was performed. Sensitization frequencies and levels of specific IgE were compared between groups.

Results: Fifty-five of 123 children presented symptoms upon contact or ingestion. Frequency of sensitization to Ara h 1, Ara h 2, and Ara h 3 was 60.0%, 72.7%, and 43.6%, respectively, in the group of allergic children vs. 7.4%, 1.5%, and 7.4% in the group of tolerant children. Levels of specific IgE against Ara h 1, Ara h 2, and Ara h 3 were significantly higher in the allergic group ($p < 0.001$). The frequency of sensitization and the levels of specific IgE against Cor a 8 (36.4% vs. 16.2%) were significantly higher in the allergic children, whereas no significant differences were found for Pru p 3. No differences were seen for other pan-allergens. Patients sensitized to SSP, regardless of sensitization to nsLTP, were allergic rather than tolerant.

Conclusion: In our population, peanut-allergic children were mainly sensitive to SSP. A few patients were also sensitive to some nsLTPs. No differences were shown in other pan-allergens.

Peanut allergy affects approximately 1% of the population worldwide (1–2). As peanuts are a common legume, accidental exposures are common and may be life-threatening (3).

Many advances have been made in molecular biology regarding allergenic proteins (4–12). Microarray technology has allowed the measurement of specific IgE (sIgE) antibodies to a large number of allergenic components simultaneously (13). Ara h 1, Ara h 2, and Ara h 3 are considered the major peanut allergens (14). Ara h 1 and Ara h 3 are vicilin and legumin seed storage proteins (SSP; 4–8, 9, 15), while Ara h 2 is a conglutinin (6–8, 15). Ara h 8 is a member of the pathogenesis-related protein family (PR-10), which is relevant to patients with birch pollen allergy (12). Ara h 9 is a non-specific lipid transfer protein (nsLTP) and has recently been reported to be an important allergen in the Mediterranean area (11, 12, 16, 17) to the detriment of SSP. In the afore-

mentioned studies, the majority of patients allergic to peanuts were also allergic to peaches, whose major allergen is known to be Pru p 3 (19).

In our study, we sought to investigate, by means of microarray, whether these differences were confirmed in an unselected population of children allergic to peanuts.

Methods

Study population

A total of 123 children who consulted to the Allergy Clinic at University Hospital La Paz in Madrid, Spain, with suspected food allergy were included in the study. Patients were considered allergic if they had at least two convincing reactions in their history (skin rash, urticaria, angio-edema,

rhinitis, cough, vomiting and abdominal pain, and wheezing) within the first hour after exposure and a positive skin prick test (SPT) and/or sIgE. Patients were considered to be tolerant if they tolerated peanut ingestion on a regular basis.

In vivo and *in vitro* tests

SPTs were performed with raw peanut extract 10 mg/ml (ALK-Abelló, Madrid, Spain), and positive (histamine 10 mg/ml) and negative (saline solution) controls.

Total IgE and sIgE for peanut extract were measured with the ImmunoCAP system FEIA (Phadia AB, Uppsala, Sweden) in venous blood samples. Specific IgE determination to allergenic components was performed using microarray platform ImmunoCAP-ISAC 103 (Phadia Multiplexing Diagnostics GmbH, Wien, Austria and Phadia AB).

The study was approved by the local ethics committee. Informed consent was obtained from parents.

Statistical analysis

The analysis was performed using the statistical package SPSS 11.0 for Windows (SPSS Inc, Chicago, IL, USA). Frequencies were compared using the chi-squared test and Fisher's exact test. Qualitative variables are expressed as absolute numbers and percentages. Quantitative variables are summarized as medians, interquartile ranges, and minimum–maximum ranges. Quantitative variables were compared with the Mann–Whitney *U*-test as they were non-normally distributed.

Results

Patient characteristics

Patients were classified as peanut allergic (*n* = 55) and peanut tolerant (*n* = 68), and characteristics are shown in Table 1.

Table 1 Characteristics of the patients grouped into peanut-allergic vs. tolerant

	Allergic (<i>n</i> = 55)	Tolerant (<i>n</i> = 68)	<i>p</i>
Age (yr) (mean ± s.d.)	8.16 ± 3.52	9 ± 4.16	0.238
Gender (male), <i>n</i> (%)	34 (61.8)	43 (63.2)	1.00
Current AD, <i>n</i> (%)	10 (18.2)	15 (22.1)	0.657
Allergy to animal food, <i>n</i> (%)	30 (54.5)	38 (55.9)	1.00
Tropical fruits allergy, <i>n</i> (%)	12 (21.8)	25 (36.8)	0.079
Kiwi, <i>n</i> (%)	8 (14.5)	13 (19.1)	0.631
Banana, <i>n</i> (%)	5 (9.1)	8 (11.8)	0.771
Rosaceae fruits allergy, <i>n</i> (%)	8 (14.5)	19 (27.9)	0.084
Peach, <i>n</i> (%)	8 (14.5)	15 (22.1)	0.355
Apple, <i>n</i> (%)	2 (3.6)	3 (4.4)	1.00
Legume allergy, <i>n</i> (%)	12 (21.8)	10 (14.7)	0.349
Nuts allergy, <i>n</i> (%)	38 (69.1)	28 (41.2)	0.003
Allergic rhinitis, <i>n</i> (%)	28 (50.9)	29 (42.6)	0.371
Allergic asthma, <i>n</i> (%)	19 (34.5)	25 (36.8)	0.851

s.d., standard deviation; AD, atopic dermatitis, bold values: statistically significant.

No differences were found regarding allergy to any plant-derived food except other nuts, which was significantly more frequent in the group of allergic patients. Of note is that similar frequencies of sensitization were found for fruits of the *Rosaceae* family.

Sensitization to peanut allergens

Allergic patients had higher levels of specific IgE to complete peanut extract (median 8.55 kU/l; IQR: 3.09–70.4) than those who tolerated peanut extract (median 1.18 kU/l; IQR: 0.51–4.20) (*p* < 0.001).

Allergic children were mainly sensitive to Ara h 2 (72.7%), followed by Ara h 1 (60%) and Ara h 3 (43.6%). Ara h 8 was only recognized by 7.3% of allergic patients. Data on frequency of sensitization to allergens are shown in Table 2. Interestingly, the number of tolerant patients who reacted to Ara h 1, Ara h 2, or Ara h 3 was very low. Differences were statistically significant in all cases (*p* < 0.001). No differences were found for CCD, profilins, or PR-10 allergens. No differences were found in Pru p 3 or in Par j 2, but there were differences for Cor a 8 and Art v 3.

Table 2 Frequency of sensitization to seed storage proteins (SSP), profilins, PR-10, nsLTPs, CCD, and major pollen allergens

Family	Allergen	Allergic (<i>n</i> = 55)	Tolerants (<i>n</i> = 68)	<i>p</i>
SSP	Ara h 1	33 (60.0%)	5 (7.4%)	<0.001
	Ara h 2	40 (72.2%)	1 (1.5%)	<0.001
	Ara h 3	24 (43.6%)	5 (7.4%)	<0.001
	Cor a 9	14 (25.5%)	2 (2.9%)	<0.001
	Gly m 5	14 (25.5%)	5 (7.4%)	0.011
	Gly m 6	17 (30.9%)	3 (4.4%)	<0.001
PR-10	Ara h 8	4 (7.3%)	2 (2.9%)	0.406
	Act d 8	2 (3.6%)	2 (2.9%)	1.0
	Cor a 1	6 (10.9%)	10 (14.7%)	0.599
	Gly m 4	5 (9.1%)	4 (5.9%)	0.512
	Mal d 1	6 (10.9%)	5 (7.4%)	0.538
	Pru p 1	4 (7.3%)	8 (11.8%)	0.545
Profilins	Bet v 2	8 (14.5%)	13 (19.1%)	0.631
	Ole e 2	8 (14.5%)	7 (10.3%)	0.582
	Hev b 8	8 (14.5%)	9 (13.2%)	1.0
	Mer a 1	10 (18.2%)	12 (17.6%)	1.0
	Phl p 12	7 (12.7%)	10 (14.7%)	0.799
	Art v 3	17 (30.9%)	9 (13.2%)	0.025
nsLTPs	Cor a 8	20 (36.4%)	11 (16.2%)	0.013
	Par j 2	2 (3.6%)	1 (1.5%)	0.586
	Pru p 3	25 (45.5%)	25 (36.8%)	0.360
	Ana c 2	1 (1.8%)	0 (0.0%)	0.447
	Ole e 1	35 (63.6%)	36 (52.9%)	0.273
	Phl p 1	30 (54.5%)	36 (52.9%)	1.0
Others	Cor a 8–Pru p 3	26 (47.3%)	29 (42.6%)	0.716
	Ara h x	42 (76.4%)	6 (8.8%)	<0.001

Ara h x: Ara h 1 and/or Ara h 2 and/or Ara h 3, bold values: statistically significant.

Results of the levels of sIgE against the various allergens were consistent with those of the frequencies of sensitization. Ara h 1, Ara h 2, and Ara h 3 as well as other SSP were significantly higher in the group of peanut-allergic children compared with tolerant children ($p < 0.001$).

Similarly, no differences were found in IgE levels to profilins or PR-10, nor in Pru p 3 or Par j 2, whereas higher levels of IgE to Cor a 8 and Art v 3 were detected in the allergic group.

Up to 76.4% of the allergic patients were sensitive to at least one peanut SSP (named Ara h x), whereas they were only recognized by 8.8% of the tolerant children. When we consider positivity to any of the plant food nsLTPs (Cor a 8 and/or Pru p 3), differences in the frequency of sensitization were not significant between groups (Table 2).

Correlation between symptoms and allergen-specific IgE

The most frequently reported symptom was urticaria and/or angio-edema (52.7%), followed by oral allergy syndrome (OAS) (38.2%), vomiting and/or abdominal pain (27.3%), and dyspnoea and wheezing (23.6%).

Interestingly, almost one-quarter of our patients (13 patients, 23.6%) reported anaphylaxis after peanut ingestion. Seven patients (12.7%) reported symptoms after inhalation/contact.

In an attempt to correlate severity of symptoms with the pattern of allergen recognition, allergic children were subgrouped into those who presented with isolated OAS ($n = 8$), isolated urticaria and/or angio-edema ($n = 5$), anaphylaxis ($n = 13$), and those who presented symptoms upon contact and/or inhalation ($n = 7$). The last group was considered to have a severe form of the disease, as many of them presented bronchospasm. Frequencies and levels of sIgE against SSP and pfnsLTPs were analyzed. As ImmunoCAP-ISAC 103 does not include Ara h 9 on its panel, Pru p 3 and Cor a 8 were considered potential markers of sensitization to peanut nsLTP. Pru p 3 can be considered a marker of nsLTP sensitization, as it shows the highest inhibiting capacity over the rest of nsLTPs (19). Given that Cor a 8 has a similar sequence homology with Ara h 9 (45%) and with Pru p 3 (38%), as revealed by the sequence alignment tool of FASTA 3.45 (<http://fermi.utmb.edu/SDAP/index.html>), it was also considered to be a potential marker of Ara h 9 sensitization.

No significant differences were found in the frequency of sensitization to any of the allergens, either SSP or pfnsLTPs, between subgroups. Nevertheless, levels of Ara h 2 and Ara h 3 were significantly higher in the subgroup of patients who presented isolated symptoms after contact or inhalation ($p = 0.016$ and $p = 0.037$ respectively), compared with the rest of the patients.

Diagnostic performance of sIgE (ImmunoCAP-ISAC 103)

ROC characteristic curves were constructed for Ara h 1, Ara h 2, Ara h 3, Cor a 8, and Pru p 3 (Fig. 1). The areas under the curves (AUC) were 0.772 (95% CI: 0.684–0.861, $p < 0.001$) for Ara h 1; 0.861 (95% CI: 0.786–0.935, $p <$

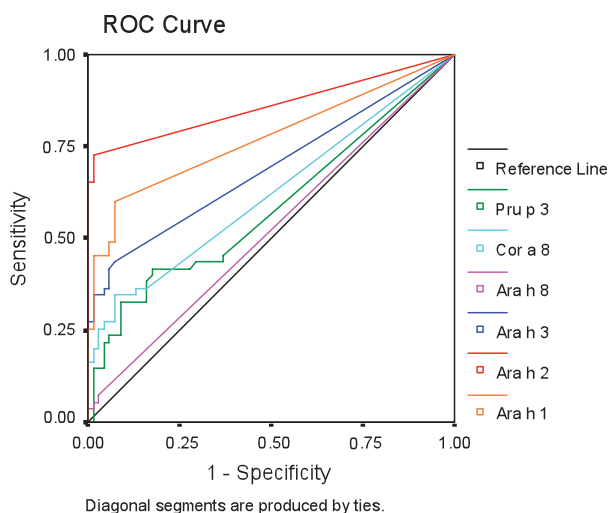


Figure 1 ROC curves for Ara h 1, Ara h 2, Ara h 3, Ara h 8, Cor a 8, and Pru p 3.

0.001) for Ara h 2; and 0.691 (95% CI: 0.594–0.789, $p < 0.001$) for Ara h 3. AUC for Ara h 8 and for Pru p 3 showed no statistical significance. Cor a 8 showed an AUC of 0.62 (95% CI: 0.518–0.72, $p = 0.023$).

Cutoff points with maximal sensitivity and specificity associated with or without tolerance to peanuts were calculated. The cutoff point for Ara h 2 was set at 0.145 ISU, showing a diagnostic accuracy of 83%. The PPV was 97% with a good NPV of 77%. Data for other allergens are shown in Table 3.

As ISAC is a semiquantitative analysis, we sought to investigate the diagnostic performance of Ara h x. The specificity was slightly lower (91%) than for Ara h 2, but it showed higher sensitivity (76%) and 85% diagnostic accuracy.

On the other hand, best cutoff point for nsLTPs showed poor sensitivity and specificity.

Sensitization to SSP vs. nsLTPs

Some patients who showed clinical reactivity were sensitized to both SSP and nsLTPs. To find out whether clinical reactivity was because of sensitization to nsLTPs or SSP, patients were subgrouped into four different categories: (i) having sIgE only to SSP; (ii) having sIgE only to pfnsLTPs; (iii) having sIgE to both nsLTP and SSP; and (iv) having no sIgE to any of them.

Distribution in these groups was as shown in Table 4. Patients who reacted to SSP were mainly allergic, whether they reacted to pfnsLTP or not. Differences were statistically significant ($p < 0.001$).

Discussion

Our population of children has similar patterns of peanut allergen recognition as those from the USA (16) or the UK (20), revealing no differences as previously suggested (11, 16).

Table 3 Diagnostic performance of sIgE as measured by MA ISAC (ISU) cutoff points

	Ara h 1 >0.11	Ara h 2 >0.145	Ara h 3 >0.105	Ara h 8 >0.285	Cor a 8 >0.335	Pru p 3 >1.415	pfnsLTP positive	Ara h x positive
Sensitivity	60.0	63.6	40.0	7.3	34.5	41.8	47.3	76.4
Specificity	92.6	98.5	92.6	97.1	83.8	82.4	57.4	91.2
PPV	86.8	97.2	83.9	66.7	63.3	65.7	47.3	87.5
NPV	74.1	77.0	61.8	56.4	61.3	63.6	57.4	82.7
LR+	8.160	43.273	5.440	2.473	2.136	2.370	1.108	8.655
LR–	0.432	0.369	0.648	0.955	0.781	0.706	0.919	0.259
Accuracy	78.0	82.9	66.9	56.9	61.8	64.2	52.8	84.6

pfnsLTP, plant food nsLTP; Ara h x, Ara h 1 and/or Ara h 2 and/or Ara h 3; PPV, positive predictive value; NPV, negative predictive value; LR+, positive likelihood ratio; LR–, negative likelihood ratio.

Table 4 Distribution of children by sensitization pattern

	Ara h 1–3 (n = 27)	pfnsLTP (n = 34)	Ara h 1–3 + pfnsLTP (n = 21)	None (n = 38)
Allergic	23 (85.2%)	7 (20.6%)	19 (90.5%)	6 (14.6%)
Tolerant	4 (14.8%)	27 (79.4%)	2 (9.5%)	35 (85.4%)

$p < 0.001$.

One strong point of this study is that both the allergic and tolerant groups were homogeneous, above all, in the distribution of allergies to other plant foods. The frequency of sensitization to peaches is similar in both groups. This is important to avoid the possible bias of overestimating sensitization to some pan-allergens such as nsLTP. Similarly, the proportion of patients with allergic airway disease was similar in both groups, thus avoiding the possible bias of overestimating sensitization to profilins or other pan-allergens present in pollens.

Seventy-two percent of the patients reacted to Ara h 2, which was the most frequently recognized allergen, followed by Ara h 1 (60%) and Ara h 3 (43.6%). The percentage of patients who were sensitive to any of the three SSP rises up to 76.4%. These outcomes are in line with recent reports from Nicolaou (20) in the UK, in which they showed that Ara h 2 measured by ImmunoCAP was recognized by the majority of allergic patients and that peanut-allergic subjects tended to have higher levels of Ara h 1–3, as we also found. This study points out that Ara h 2 is a clinically useful tool in predicting peanut allergy. Other studies have also shown, in adults and children, the importance of Ara h 2 and the rest of peanut SSP (21–26) and that this pattern remains stable over time (26). Codreanu et al. (27) in France described 166 peanut-allergic patients of whom 96% recognized Ara h 2, 75% Ara h 1, 61% Ara h 3, and 40% Ara h 8, frequencies similar to ours. The control group of birch/grass pollen-sensitized peanut-tolerant patients showed a rate of sensitization to SSP below 20%, similar to our data (the sensitization rate to SSP was lower than 8%) (Table 1). The percentage of recognition in these studies was a bit higher than in ours, which may be because ISAC is less sensitive than ImmunoCAP.

On the other hand, studies have highlighted the importance of nsLTP in peanut allergy, mainly in the Mediterranean area. Lauer et al. (11) reported the importance of peanut LTP in peanut allergy. Their study comprised 32 patients, 90% of whom recognized Ara h 9. Interestingly, these patients were all allergic to peaches. The sensitization pattern of the entire population sample was not investigated, but some patients were analyzed. The majority of them were sensitized to other peanut allergens such as Ara h 1 or Ara h 2 and only a few were mono-sensitized to Ara h 9. Similarly, in the study published by Vereda et al. (16), 60% of the patients studied were sensitized to peanut LTP, which may be related to the fact that 63.2% of the patients were previously allergic to other plant foods, including peaches. This co-sensitization may explain the high number of Ara h 9-reacting patients because of cross-reactivity with Pru p 3. Another approach was followed by Krause et al. (12), who studied patients allergic to peanuts and peaches. Nineteen of 42 peanut-allergic patients recognized Ara h 9, whereas only two of them recognized Ara h 1. Surprisingly, none of the patients recognized Ara h 2 or Ara h 3. Patients were classified as having peanut allergy with and without Pru p 3 sensitization and no peanut allergy, but Pru p 3 sensitization. In the first group, 16 of 25 patients recognized Ara h 9 (half of them had peanut allergy and the other half peanut and peach allergy); in the group of patients who were only sensitized to Pru p 3 without peanut allergy, six of 24 recognized Ara h 9. Interestingly, in the group of peanut-allergic patients with no sensitization to Pru p 3, only 3 of 17 recognized Ara h 9 (one had both peanut and peach allergy and the other two had only peanut allergy). This may be because Ara h 9 recognition was overestimated as result of cross-reactivity with Pru p 3. All these studies stress the importance of Ara h 9 in the

peanut-allergic population of the Mediterranean area. However, the results may have been overestimated, and the high proportion of subjects who react to Ara h 9 may be because of concomitant Pru p 3 sensitization, which also happens to be frequent in the Mediterranean area (28), as it is a predominant food allergen.

In our study, we found that the majority of children who reacted to SSP from peanuts were allergic regardless of whether they also recognized pfnsLTP (90.5% allergic vs. 9.5% tolerant) or not (85.2% allergic vs. 14.8% tolerant) ($p < 0.001$). This indicates that clinical reactivity is associated with sensitization to SSP rather than to pfnsLTP. Although ISAC lacks Ara h 9, we believe that this is a valid approach, because Pru p 3 can be considered a surrogate marker of sensitization to any nsLTP (<http://fermi.utmb.edu/SDAP/index.html>).

We did not find any differences in the frequency of recognition, nor in the level of sIgE to other pan-allergens. This may be explained because the distribution of pollinosis and allergy to other plant foods is homogeneous between both groups. Our results are in line with those published previously in a population from the UK (20), in which no differences were observed in the frequencies of sensitization to PR-10, nsLTP, polcalcin, or profilin.

It is worth noting that almost a quarter of our population presented with anaphylaxis upon ingestion. We found significantly higher levels of sIgE against Ara h 2 and Ara h 3 in the group who reported symptoms upon contact or inhalation. Patients with isolated OAS tended to have lower levels of sIgE against Ara h 2 when compared with the rest. Nevertheless, we did not find any difference in the group with anaphylaxis nor in the group with isolate urticaria and/or angio-edema. Conflicting data have been reported on this point. Flinterman et al. (26) did not find any correlation between severity and sensitization to any of the allergens studied, whereas Asarnoj et al. (29) found that those patients recognizing Ara h 2 and Ara h 1 or Ara h 3 were more likely to report respiratory symptoms after exposure than those who only reacted to Ara h 2. Differences may in fact exist, but we have not been able to find them, perhaps because of the small sample size in the subgroups.

In our study, we obtained good diagnostic performance of ISAC for predicting clinical allergy in patients with suspected

peanut allergy. Component-resolved diagnosis of peanut allergy has shown to be useful in clinical practice. Previous reports have underscored the importance of allergen profiling in peanut allergy. Nicolaou et al. (30) showed high sensitivity (100%) and specificity (96.08%) for sIgE against Ara h 2 as measured by ImmunoCAP. A cutoff point of 0.35 kU/l had a diagnostic accuracy of 97.50%. Similarly, Codreanu et al. (30) showed sensitization to Ara h 2 as the best predictor for clinical allergy with a cutoff level of 0.23 kU/l (sensitivity: 93%, specificity: 96%). In our study, a diagnostic accuracy of 82.9% was set for Ara h 2 > 0.145 ISU (sensitivity: 63.6%, specificity: 98.5%). Nevertheless, the best diagnostic performance was for the variable Ara h x, which showed a sensitivity of 76.4%, specificity of 91.2%, and diagnostic accuracy of 84.6%. We found ISAC to be a useful and convenient tool in the diagnosis of peanut allergy. It requires small amounts of blood and provides information not only on peanut allergens but also on a wide range of allergens, thus outlining a profile of sensitization to pan-allergens, which is very useful for clinicians. Moreover, MA ISAC has the advantage of being commercially available, which means it is easier to reproduce the results and compare different populations.

In conclusion, although peanut nsLTP may play a role in some allergic patients and cannot be ignored in component-resolved diagnosis of peanut allergy, the majority of children with peanut allergy, even those in the Mediterranean area, react to SSP.

Conflict of interest

The authors declare that they do not have any potential conflicts of interest.

Author contributions

All authors have contributed to the design of the study, data collection, writing, and review of the manuscript.

Funding

This study was self-funded.

References

1. Sampson HA. Clinical practice. Peanut allergy. *N Engl J Med* 2002; **346**: 1294–9.
2. Burks AW. Peanut allergy. *Lancet* 2008; **371**: 1538–46.
3. Yu JW, Kagan R, Verreault N, et al. Accidental ingestions in children with peanut allergy. *J Allergy Clin Immunol* 2006; **118**: 466–72.
4. Burks AW, Williams LW, Helm RM, Connaughton C, Cockrell G, O'Brien T. Identification of a major peanut allergen, Ara h I, in patients with atopic dermatitis and positive peanut challenges. *J Allergy Clin Immunol* 1991; **88**: 172–9.
5. Burks AW, Cockrell G, Stanley JS, Helm RM, Bannon GA. Recombinant peanut allergen Ara h I expression and IgE binding in patients with peanut hypersensitivity. *J Clin Invest* 1995; **96**: 1715–21.
6. De Jong EC, van Zijverden M, Spanhaak S, Koppelman SJ, Pellegrini H, Penninks AH. Identification and partial characterization of multiple major allergens in peanut proteins. *Clin Exp Allergy* 1998; **28**: 743–51.
7. Burks AW, Williams LW, Connaughton C, Cockrell G, O'Brien T, Helm RM. Identification and characterization of a second major peanut allergen, Ara h II, with the use of sera of patients with atopic dermatitis and positive peanut challenge. *J Allergy Clin Immunol* 1992; **90**: 962–9.
8. Kleber-Janke T, Cramer R, Appenzeller U, Schlaak M, Becker WM. Selective cloning of peanut allergens, including profilin and 2S albumins, by phage display technology. *Int Arch Allergy Immunol* 1999; **119**: 265–74.

9. Eigenmann PA, Burks AW, Bannon GA, Sampson HA. Identification of unique peanut and soy allergens in sera adsorbed with cross-reacting antibodies. *J Allergy Clin Immunol* 1996; **98**: 969–78.
10. Mittag D, Akkedaas J, Ballmer-Weber BK, et al. Ara h 8, a Bet v 1-homologous allergen from peanut, is a major allergen in patients with combined birch pollen and peanut allergy. *J Allergy Clin Immunol* 2004; **114**: 1410–7.
11. Lauer I, Dueringer N, Pokoj S, et al. The non-specific lipid transfer protein, Ara h 9, is an important allergen in peanut. *Clin Exp Allergy* 2009; **39**: 1427–37.
12. Krause S, Reese G, Randow S, et al. Lipid transfer protein (Ara h 9) as a new peanut allergen relevant for a Mediterranean allergic population. *J Allergy Clin Immunol* 2009; **124**: 771–8.
13. Ferrer M, Sanz ML, Sastre J, et al. Molecular diagnosis in allergology: application of the microarray technique. *J Invest Allergol Clin Immunol* 2009; **19** (Suppl. 1): 19–24.
14. Burks W, Sampson HA, Bannon GA. Peanut allergens. *Allergy* 1998; **53**: 725–30.
15. Radauer C, Bublin M, Wagner S, Mari A, Breiteneder H. Allergens are distributed into few protein families and possess a restricted number of biochemical functions. *J Allergy Clin Immunol* 2008; **121**: 847–52.
16. Vereda A, van Hage M, Ahlstedt S, et al. Peanut allergy: clinical and immunologic differences among patients from 3 different geographic regions. *J Allergy Clin Immunol* 2011; **127**: 603–7.
17. Romano A, Fernández-Rivas M, Caringi M, Amato S, Mistrello G, Asero R. Allergy to peanut lipid transfer protein (LTP): frequency and cross reactivity between peanut and peach LTP. *Eur Ann Allergy Clin Immunol* 2009; **41**: 106–11.
18. Pastorello EA, Farioli L, Pravettoni V, et al. The major allergen of peach (*Prunus persica*) is a lipid transfer protein. *J Allergy Clin Immunol* 1999; **103**: 520–6.
19. Hartz C, Lauer I, del Mar San Miguel Moncin M, et al. Comparison of IgE-binding capacity, cross-reactivity and biological potency of allergenic non-specific lipid transfer proteins from peach, cherry and hazelnut. *Int Arch Allergy Immunol* 2010; **153**: 335–46.
20. Nicolaou N, Poorafshar M, Murray C, et al. Allergy or tolerance in children sensitized to peanut: prevalence and differentiation using component-resolved diagnostics. *J Allergy Clin Immunol* 2010; **125**: 191–7.
21. Koppelman SJ, Wensing M, Ertmann M, Knulst AC, Knol EF. Relevance of Ara h 1, Ara h 2 and Ara h 3 in peanut allergic patients, as determined by immunoglobulin E Western blotting, basophil-histamine release and intracutaneous testing: Ara h 2 is the most important peanut allergen. *Clin Exp Allergy* 2004; **34**: 583–90.
22. Astier C, Morisset M, Roiter O, et al. Predictive value of skin prick test using recombinant allergens for diagnosis of peanut allergy. *J Allergy Clin Immunol* 2006; **118**: 250–6.
23. Palmer GW, Dibbern DA, Burks AW, et al. Comparative potency of Ara h 1 and Ara h 2 in immunochemical and functional assays of allergenicity. *Clin Immunol* 2005; **115**: 302–12.
24. Beyer K, Ellman-Grunther L, Järvinen KM, Wood RA, Hourihane J, Sampson HA. Measurement of peptide-specific IgE as an additional tool in identifying patients with clinical reactivity to peanuts. *J Allergy Clin Immunol* 2003; **112**: 202–7.
25. Shreffler WG, Beyer K, Chu TH, Burks AW, Sampson HA. Microarray immunoassay: association of clinical history, in vitro IgE function, and heterogeneity of allergic peanut epitopes. *J Allergy Clin Immunol* 2004; **113**: 776–82.
26. Flinterman AE, van Hoffen E, den Hartog Jager CF, et al. Children with peanut allergy recognize predominantly Ara h 2 and Ara h 6, which remains stable over time. *Clin Exp Allergy* 2007; **37**: 1221–8.
27. Codreanu F, Collignon O, Roitel O, et al. A novel immunoassay using recombinant allergens simplifies peanut allergy diagnosis. *Int Arch Allergy Immunol* 2011; **154**: 216–26.
28. Fernández-Rivas M, González-Mancebo E, Rodríguez-Pérez R, et al. Clinically relevant peach allergy is related to peach lipid transfer protein, Pru p 3, in the Spanish population. *J Allergy Clin Immunol* 2003; **112**: 789–95.
29. Asarnoj A, Movérare R, Ostblom E, et al. IgE to peanut allergen components: relation to peanut symptoms and pollen sensitization in 8-year-olds. *Allergy* 2010; **65**: 1189–95.
30. Nicolaou N, Murray C, Belgrave D, Poorafshar M, Simpson A, Custovic A. Quantification of specific IgE to whole peanut extract and peanut components in prediction of peanut allergy. *J Allergy Clin Immunol* 2011; **127**: 684–5.