Molecular diagnosis of peanut and legume allergy

Nicolaos Nicolaou and Adnan Custovic

The University of Manchester, Manchester Academic Health Science Centre, NIHR Translational Research Facility in Respiratory Medicine, University Hospital of South Manchester NHS Foundation Trust, Manchester, UK

Correspondence to Nicolaos Nicolaou, MD, PhD, University of Manchester, University Hospital of South Manchester NHS Foundation Trust, Manchester M23 9LT, UK
Tel: +44 161 291 2869; fax: +44 161 291 5730; e-mail: nic.nicolaou@gmail.com

Current Opinion in Allergy and Clinical Immunology 2011, 11:222–228

Purpose of review

To review and discuss recent studies on molecular diagnosis of peanut and other legume allergy.

Recent findings

Studies from the UK and France suggest that quantification of Ara h 2-specific IgE may accurately discriminate peanut allergy from tolerance. However, the pattern of allergenic component recognition in peanut-sensitized patients from different populations or geographical areas varies, reflecting different pollen and dietary exposures. In the USA, peanut-allergic patients are commonly sensitized to Ara h 1–3, in Spain to Ara h 9 and in Sweden to Ara h 8. Patients with soybean allergy sensitized to Gly m 5 or Gly m 6 allergens may be at greater risk of experiencing severe allergic reactions.

Summary

Accurate diagnosis of peanut and legume allergy is challenging and essential. Measurement of IgE response to specific allergenic molecules may be more useful in predicting the presence and severity of clinical allergy than currently used skin or blood tests based on whole extracts. However, given the heterogeneity in component recognition patterns observed in different geographical areas, further studies are essential to identify and confirm potentially useful molecular diagnostic and prognostic markers. Until such markers are confirmed and replicated in different age groups, oral food challenge (OFC) remains the gold standard for accurate diagnosis.

Keywords

component-resolved diagnosis, legumes, molecular diagnosis, peanut

Curr Opin Allergy Clin Immunol 11:222–228
© 2011 Wolters Kluwer Health | Lippincott Williams & Wilkins
1528-4050

Introduction

Members of the legume family including peanut, soy, lentils, beans, pea and lupin are a relatively cheap and excellent source of high-biological-value proteins and other nutrients. They are widely used around the world, constitute an important part of healthy and vegan diets, and are common ingredients in many processed food products. For the majority of people legumes represent an excellent food source; however, some individuals may experience allergic reactions even with minimal exposure. Allergy to peanut and other legumes is becoming a significant health problem particularly in westernised countries [1,2•,3•,4]. Legume-induced allergic reactions can be potentially severe and sometimes fatal [5], and quality of life of patients and their families is highly impaired [6].

Given the impact of peanut and legume allergy, accurate diagnosis is critically important. The double-blind placebo-controlled food challenge (DBPCFC) is the gold standard for diagnosing food allergy [7]. However, the procedure is time-consuming, expensive and patients may be at risk of severe reaction. In daily clinical practice, diagnosis is usually based on a suggestive clinical history supported by positive skin prick test (SPT) or detection of specific IgE (sIgE) in serum. However, the utility of currently used tests (SPTs and sIgE in-vitro assays) in confirming the diagnosis of food allergy has been questioned [8]. These tests generally have low specificity, and a positive test identifies sensitization, which may suggest, but does not unequivocally confirm clinical reactivity upon exposure (i.e. allergy).

Cut-off points related to the size of skin test responses and sIgE levels which confer high probability of clinical reactivity have been proposed for some allergenic foods, but these decision points may not be applicable to every population or setting [9]. Furthermore, traditional tests do not predict the severity of allergic reactions.

One of the reasons why current tests perform relatively poorly in differentiating asymptomatic sensitization from true allergic reactions may be consequent to the fact that they are mostly based on crude natural extracts which contain both allergenic and nonallergenic molecules, with some of these molecules crossreacting with homologous proteins from other sources (e.g. pollen) [10].

 DOI:10.1097/ACI.0b013e32834656c2

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

A classical example of crossreactive structures with limited clinical relevance is that of crossreactive carbohydrate determinants (CCDs) which have been implicated in clinically irrelevant 'sensitization' to peanut among grass pollen-sensitized patients [11•,12].

Recent advances in molecular biology and biochemistry have led to isolation, characterization and recombinant production of many allergenic proteins, as well as the synthesis of IgE epitope-emulating peptides for individual food allergens, including members of the legume family [13•,14•,15••,16–20]. These molecules are increasingly used within the context of molecular diagnosis [21•] or component-resolved diagnosis (CRD) [10] to facilitate more accurate diagnosis of food allergy. This has been aided by the advances in microarray technology, which have allowed simultaneous measurement of IgE antibodies to a large number of allergenic components using minute amounts of sera and antigens [22].

Using allergenic components or synthetic epitopes in diagnostic tests, a detailed analysis of a patient's sensitization profile can be determined and association between the pattern of such sIgE responses and clinically relevant aspects of the allergic disease investigated [10,21•]. This may help identification of disease-eliciting molecules, components implicated in crossreactivity, and molecules associated with disease severity. Immunotherapy with the clinically relevant specific components to which a patient is sensitized could then be undertaken.

Key points

- Recent studies employing a molecular diagnostic approach suggest that measurement of IgE response to specific allergenic components may be more useful in predicting the presence (e.g. Ara h 2 for peanut) and severity (e.g. Gly m 5 and Gly m 6 for soybean) of clinical allergy than currently used skin or blood tests based on whole extracts.
- Given the heterogeneity in component recognition patterns observed in different populations and geographical areas, further studies are of critical importance to identify and confirm potentially clinically useful molecular diagnostic and prognostic markers of peanut and legume allergy.

Allergenic proteins

A thorough knowledge of allergenic proteins is essential for the understanding of molecular diagnosis of peanut and legume allergy.

Peanut allergens

By December 2010, 11 peanut allergens have been recognized by the International Union of Immunological Societies (IUIS) Nomenclature Subcommittee [23] (Table 1). Ara h 1 and Ara h 2 were identified and characterized in the early 1990s [24] and are the most extensively studied. Ara h 1–3 are considered the major peanut allergens [25], and recent reports suggest an important role for Ara h 6 [26].

Ara h 1 is a member of the 7/8S globulins (vicilins) of seed storage proteins that belongs to the cupin superfamily [27]. Ara h 2, 6 and 7 are members of the 2S albumins (conglutin) of seed storage proteins that belong to the prolamin superfamily [27]. Ara h 2 has high sequence homology with Ara h 6. As a result of their stability to heat and gastrointestinal digestion, many allergens of the prolamin superfamily are important and may account for severe allergic reactions [28]. Ara h 3 and 4 are nearly identical isoforms and are members of the 11S globulins (legumins/glycinins) of seed storage proteins that belong to the cupin superfamily [29].

Ara h 5 belongs to the profilin family. Profilins show high sequence homologies even from distantly related plants and are known pan-allergens involved in crossreactions between pollen and plan foods [27]. Cabanos *et al.* [13•] using homology modelling have recently identified specific epitopes which may be associated with Ara h 5 allergenicity and crossreactivity and suggested that Ara h 5 could be the allergen of choice for the diagnosis of profilin allergy.

Ara h 8 belongs to the pathogenesis-related protein family (PR)-10 and it is of relevance to peanut-allergic patients with birch pollen allergy because of the crossreactivity to the homologous Bet v 1 allergen [30]. Ara h 9 belongs to the nonspecific lipid transfer proteins (nsLTPs) that belong to the prolamin superfamily. Ara h 10 and 11 or oleosins have recently been recognized and their allergenic properties are largely unknown [19]. Allergenic oleosins were found in legumes, nuts, and seeds.

A number of factors related to the harvesting and processing of peanuts may affect the allergenic properties of peanut proteins. For example, roasting enhances the allergenicity of Ara h 1–3, whereas boiling or frying appears to reduce the allergenic properties of these molecules [31]. Ara h 5 and 8 are heat labile and cooking has an adverse effect on their allergenicity.

Allergens in other legumes

Although several allergenic molecules have been described in other legumes, relatively few are listed by the IUIS Nomenclature Subcommittee [23] (Soy: Gly m 1–6, Lentil: Len c 1–3, Pea: Pis s 1 and 2, Green bean: Pha v 3, Mung bean: Vir g 1 and Lupin: Lup an 1, Table 1). The knowledge on these allergenic components and their relevance to clinical allergy is limited.

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

Table 1 Peanut and other legume allergens

Allergenic protein	Plant family (biochemical name)	Superfamily	MW[a] (kDa)
Peanut (*Arachis hypogaea*)			
Ara h 1	7/8S globulins (vicilins) of seed storage proteins	Cupin	64
Ara h 2	2S albumins (conglutin) of seed storage proteins	Prolamin	17
Ara h 3	11S globulins (legumins) of seed storage proteins	Cupin	60
Ara h 4	11S globulins (legumins) of seed storage proteins	Cupin	37
Ara h 5	Profilin (Bet v 2-like)	Profilin	15
Ara h 6	2S albumins (conglutin) of seed storage proteins	Prolamin	15
Ara h 7	2S albumins (conglutin) of seed storage proteins	Prolamin	15
Ara h 8	Bet v 1 family of pathogenesis-related proteins (PR-10, Bet v 1-like)	Pathogenesis-related proteins	17
Ara h 9	nsLTPs	Prolamin	9.8
Ara h 10	Oleosin plant lipid storage bodies	Oleosin	16
Ara h 11	Oleosin plant lipid storage bodies	Oleosin	14
Soybean (*Glycine max*)			
Gly m 1	Hydrophobic protein from soybean, nsLTPs	Prolamin	7
Gly m 2	Defensin		8
Gly m 3	Profilin	Profilin	14
Gly m 4	Bet v 1 family of pathogenesis-related proteins (PR-10, Bet v 1-like)	Pathogenesis-related proteins	17
Gly m 5	7S globulin, beta-conglycinin (vicilin)	Cupin	
Gly m 6	11S globulin, glycinin (legumin)	Cupin	
Lentil (*Lens culinaris*)			
Len c 1	Gamma-vicilin subunit	Cupin	47
Len c 2	Seed-specific biotinylated protein		66
Len c 3	nsLTP type 1	Prolamin	9
Pea (*Pisum sativum*)			
Pis s 1	Vicilin	Cupin	44
Pis s 2	Convicilin	Cupin	63
Lupin (*Lupinus anqustifolius*)			
Lup an 1	Beta-conglutin (vicilin)	Cupin	55–61
Green bean (*Phaseolus vulgaris*)			
Pha v 3	nsLTP type 1	Prolamin	8.8–9.0
Mung bean (*Vigna radiata*)			
Vig r 1	Bet v 1 family of pathogenesis related (PR-10) proteins	Pathogenesis-related proteins	16

nsLTP, nonspecific lipid transfer protein. Information in the table derived from the Allergen Nomenclature, the *Allergome* and the *AllFam* databases (www.allergen.org, www.allergome.org and www.meduniwien.ac.at/allergens/allfam/, respectively).
[a] MW (kDa), molecular weight in kiloDalton.

Soybean allergens Gly m 1 and Gly m 2 are components of the soybean hull and have been associated with respiratory symptoms through inhalation of soy particles [1]. Gly m 3 is a profilin and is considered a minor allergen. Gly m 4 is Bet v 1 homologue, belongs to the PR-10 family and is a common allergen in patients with birch pollen and soy allergy [32,33]. Gly m 5 (β-conglycinin) and Gly m 6 (glycinin) are 7S and 11S globulins (cupin superfamily) and are major soybean storage proteins.

The conglutins α (legumin-like) and β (vicilin-like) of seed storage globulins are the most abundant allergenic proteins in *Lupinus* species. Lup an 1, a β-conglutin is the major allergen identified in *L. angusitfolius* and shares sequence homology with Ara h 1. Recently, Guillamon *et al.* [14•] have described Lup-1 (a β-conglutin) and Lup-2 (an α-conglutin fraction) as major allergens in *L. albus* which show high-amino-acid sequence homology with the major allergens from peanut, pea lentil and soybean. These structural similarities may explain serological and clinical crossreactivity between lupin and other legumes. However, more studies are needed to clarify the complex phenomena of crossreactivity.

Zoccatelli *et al.* [34] have recently identified major allergen of green bean as a nonspecific LTP (Pha v 3).

Allergenic component sensitization profiles in peanut and legume allergy

The following section will review studies which ascertained the relationship between IgE responses to individual peanut allergenic components and peanut and legume allergy.

Peanut

In the last decade, a number of studies using individual peanut allergenic components have established associations between patients' sensitization profile and clinical allergy. Beyer *et al.* [35] using recombinant peptides from Ara h 1, 2 and 3 have demonstrated differences in epitope recognition between patients with symptomatic allergy compared with those who were sensitized, but tolerant. This suggested that determination of IgE-binding epitopes may provide an additional tool to diagnose peanut allergy [35]. Shreffler *et al.* [36] aimed to develop a peptide microarray-based immunoassay to map peanut epitopes. Although this method confirmed that Ara h 1, 2 and 3 were major peanut allergens and allowed parallel epitope analysis, it also revealed remarkable heterogeneity in the number and patterns of epitope recognition between individual patients. Of note, patients with IgE binding to a greater number of epitopes had a history of more severe

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

reactions [36]. Astier *et al.* [37] used SPT with individual recombinant peanut components (Ara h 1, 2, and 3) and found Ara h 2 to be dominant, with severity being related to corecognition of more than one components. Flinterman *et al.* [26] have shown that children with peanut allergy recognize predominantly Ara h 2 and Ara h 6, and that this pattern remained stable over time.

Recent studies

In a study from Singapore, Chiang *et al.* [38•] evaluated the specific IgE responses to the major peanut allergens Ara h 1–3 in 31 peanut-sensitized Asian children of whom 27 were considered peanut allergic. Reactivity to Ara h 1 and Ara h 2 was predictive of peanut allergy, and the authors suggested that Asian children with peanut sensitization have similar responses to major peanut allergens as children from North America and Europe. However, relatively small sample size and the fact that other peanut allergens were not investigated may not allow firm conclusions.

In contrast, Vereda *et al.* [39••] demonstrated markedly different clinical and immunologic patterns of peanut allergy in patients from three different geographical areas. Peanut-allergic patients (challenge or convincing history) were recruited in the USA ($n = 30$), Spain ($n = 50$) and Sweden ($n = 35$). American patients had the highest frequency of sensitization to Ara h 1, 2 and 3 (80, 90 and 56.7%, respectively) and the highest IgE levels to Ara h 1 and 2. Spanish patients were less often sensitized to Ara h 1–3 (30, 42 and 16%) but had highest rate of sensitization to Ara h 9 (60%), which in turn was rare in Sweden (14.3%) and the USA (7.7%). Patients from Sweden recognized Ara h 1–3 (62.9, 74.3 and 37.1%) more commonly than those from Spain, but not as often as those from the USA, and had highest frequency of sensitization to the Bet v 1 homologue Ara h 8 (65.7%). There were also differences in the number of peanut allergens recognized by the patients from different areas, with 60% of Americans recognizing three or more allergens, and the majority (54%) of Spanish patients only one allergen. American patients became allergic around 1 year of age, whereas Spanish and Swedish patients developed peanut allergy at the age of 2 years or later. American and Spanish patients tended to experience more severe reactions than those from Sweden. Many Spanish patients developed peanut allergy after becoming allergic to other plant-derived foods (e.g. peach, tree nuts and legumes). These results demonstrated heterogeneity in the clinical and immunological phenotype of peanut allergy in distinct geographical areas, which might reflect exposures to different environmental pollen and dietary traditions and emphasize the importance of molecular diagnostics in our understanding the puzzle of peanut allergy.

In the Swedish BAMSE birth cohort, Asarnoj *et al.* [40••] investigated using microarrays the IgE reactivity to peanut allergen components in school-age children in relation to pollen sensitization and reported peanut allergy. The pattern of components recognition differed in the four groups with distinct peanut and birch pollen combination sensitizations (sensitized to peanut, but not birch; sensitized to both peanut and birch; sensitized to birch, but not peanut; not sensitized to peanut or birch). Children with concomitant sensitization to peanut and birch reported fewer symptoms to peanuts than children with sensitization to peanut only; peanut allergy was more common and more severe in those with reactivity to Ara h 1–3, than Ara h 8 and pollen sensitization.

Codreanu *et al.* [41••] measured IgE reactivity to peanut extract and recombinant peanut components in patients from two hospitals in France (166 challenge-confirmed peanut-allergic patients, 61 birch and/or grass pollen-sensitized peanut-tolerant patients and 10 healthy controls). In both centres, all peanut-allergic patients were sensitized to peanut extract, 96% to Ara h 2, 92% to Ara h 6, 75% to Ara h 1, 61% to Ara h 3, 43% to Ara h 7, and 40% to Ara h 8. The majority of pollen-sensitized peanut-tolerant patients had IgE directed towards peanut extract (79%) and Ara h 8 (69%), whereas detection of other peanut allergens was below 20%. Measurement of Ara h 2 sIgE was the best predictor of peanut allergy, with a cut-off level of 0.23 kU/l conferring 93% sensitivity and 96% specificity. However, specific IgE to Ara h 2 was not detectable in seven (4%) peanut-allergic patients (six of these could be diagnosed by measurement of sIgE Ara h 1, 3, or 6). At the cut-off level of 0.1 kU/l both Ara h 2 and Ara h 6 had 98% sensitivity and 85%specificity. At the same cut-off level, IgE to peanut extract had 100% sensitivity but considerably low specificity (21%).

In the UK study [42••], on the basis of the outcome of OFC, we have recently shown that the majority of children sensitized to whole peanut extract do not have peanut allergy. Although approximately 10% of 8-year-old children in our population-based birth cohort were sensitized to peanut using traditional tests (SPT and/or sIgE to whole peanut extract), only approximately 2% had peanut allergy (~8% were sensitized, but peanut-tolerant). Using microarray CRD amongst peanut-sensitized children, we demonstrated marked differences in the component sensitization profile between peanut-allergic and peanut-tolerant patients. Peanut-allergic patients tended to have higher major peanut components Ara h 1–3, whereas the peanut-tolerant patients had higher values to CCD and grass components Phl p 1, Phl p 4 and Phl p 5b. The groups did not differ for Ara h 8, Bet v 1, Pru p 3, Phl p 7 and Phl p 12. Ara h 2 offered the best discrimination between peanut-allergic and peanut-tolerant patients. In a follow-up study [43•], we compared

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

the diagnostic performance of sIgE levels to the whole extract and different peanut components (Ara h 1, 2, 3, 8 and 9) using standard ImmunoCAP method. sIgE to Ara h 2 had the highest accuracy in differentiating between peanut-allergic and peanut-tolerant children, with cut-off of 0.35 kU_A/l of Ara h 2 IgE conferring 100% sensitivity and 96.1% specificity. Using this cut-off point, 97.5% of patients were correctly classified (importantly, all children with peanut allergy were given the correct classification). The optimal cut-off quantification point for the whole peanut sIgE had lower accuracy in discriminating peanut allergy from tolerance (sensitivity 87.6%, specificity 75.9%).

Flinterman *et al.* [44•] used the CRD approach to investigate T-cell responses to crude peanut extract (CPE) and purified peanut components Ara h 1–3 and six in 18 peanut-allergic, seven peanut-sensitized tolerant and 11 nonatopic controls. Primary peripheral blood mononuclear cells and short-time T-cell lines were stimulated with CPE and peanut components, and proliferation and cytokine production measured. Overall there were no significant differences between peanut-allergic and peanut-sensitized tolerant patients after stimulation with CPE. However, in short-time T-cell lines only peanut allergic children showed enhanced Th2 responses to Ara h 1, 3, and 6.

The body of evidence accumulated over recent years suggests that IgE response to Ara h 2 may be a useful marker in predicting peanut allergy in the USA [39••], Sweden [39••], the UK [42••,43•] and France [41••] but is unlikely an important predictor of peanut allergy in Spain. In this area, detection of Ara h 9 appears a more relevant marker of peanut allergy [39••,45,46].

Sensitization profiles in legume allergy

In a European multicenter study [32] investigating the characteristics of soybean allergy in 25 adults and 5 children, more than two-thirds of patients were sensitized to Gly m 4 (and 67% were peanut allergic). Gly m 4 may be under-represented in soy extracts, and recent review strongly recommended measurement of IgE response to Gly m 4 in birch pollen-sensitized patients with suspected soy allergy and undetectable IgE to soy extract [1].

Sensitization to Gly m 5 or Gly m 6 has recently been suggested as a potential marker of severe allergic reactions amongst patients with soy allergy [15••]. Sensitization to Gly m 5 or Gly m 6 was demonstrated in 6/7 (87%), 6/11 (55%) and 4/12 (33%) patients with severe, moderate and mild reactions to soy, respectively [15••], whereas IgE reactivity to Gly m 4 was found in 92% of those experiencing mild reactions (in patients with sensitization to Gly m 5 and/or Gly m 6 the odd for having severe vs. mild reaction was 12/1; in patients with IgE reactivity to Gly m 4 the odd for mild vs. severe reaction was 14/1). However, severe reactions after intake of soymilk during the peak of birch pollen have recently been reported in birch pollen-allergic children sensitized to Gly m 4, but not to Gly m 5 or Gly m 6 [47••].

Crossreactivity between peanut and other legume allergens

In a recent study in 12 peanut-allergic children from Italy, Ballabio *et al.* [48] investigated immunological crossreactivity with other legume (lupin, lentil, pea, kidney been, and soybean) and revealed a wide range of IgE-binding responses within and between patients. The most frequent polypeptides from other legumes recognised by peanut-allergic patients were the subunits of the 11S globulins. Results of in-vitro and in-vivo approaches were generally consistent, confirming marked crossreactivity between different leguminous seed proteins. However, few studies have explored clinical relevance of crossreactivity. In a recent study [49] of 39 unselected peanut-sensitized adults, 82, 55 and 87% of patients were also sensitized to lupine, pea, and soy, respectively. On the basis of DBPCFC, clinically relevant sensitization to lupine was demonstrated in 35% of study participants. On the basis of history clinically relevant sensitization to pea and soy was suggested in 29 and 33% of patients, respectively. Interestingly, a UK paediatric study [50] revealed lower incidence of lupine sensitization (~34%) and much lower clinical lupine allergy (~4%) in peanut allergic children, suggesting again marked differences in the clinical pattern of food allergy between different geographical areas.

Conclusion

Allergy to peanut and other legumes is becoming a significant health problem in many parts of the world, and accurate diagnosis is of critical importance. Currently used skin tests and specific IgE tests to the whole extracts have low specificity, particularly in pollen-sensitized patients. Recent data suggest that molecular diagnostic tests may reduce the need to perform OFCs. Quantification of Ara h 2 predicted peanut allergy with high accuracy in studies from the UK and France and may be a useful marker of peanut allergy in this geographical region. However, this allergenic molecule may not be a good prognostic marker in other areas where different allergenic components may be more relevant (e.g. LTP in patients from the Mediterranean area).

There is limited evidence to suggest that corecognition of several peanut allergens is associated with more severe allergy. In patients allergic to soybean, sensitization to Gly m 5 and Gly m 6 may be associated with more severe reactions, but sensitization to Gly m 4 appears to be a

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

marker of severity in those patients with concomitant birch pollen allergy. It is important to emphasize that further research in this field is essential before the widespread use of these novel tests in clinical practice.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 271).

1 Ballmer-Weber BK, Vieths S. Soy allergy in perspective. Curr Opin Allergy Clin Immunol 2008; 8:270–275.

2 • Custovic A, Nicolaou N. Peanut allergy: over-estimated in epidemiology or underdiagnosed in primary care? J Allergy Clin Immunol 2011; 127:631–632.
This editorial suggests a worrying possibility that a considerable proportion of children with peanut allergy are not diagnosed (and appropriately managed) by their primary care physicians.

3 • Sanz ML, de Las Marinas MD, Fernandez J, Gamboa PM. Lupin allergy: a hidden killer in the home. Clin Exp Allergy 2010; 40:1461–1466.
An excellent review article on lupin allergy.

4 Sicherer SH, Sampson HA. Peanut allergy: emerging concepts and approaches for an apparent epidemic. J Allergy Clin Immunol 2007; 120:491–503; quiz 504–505.

5 Bock SA, Munoz-Furlong A, Sampson HA. Further fatalities caused by anaphylactic reactions to food, 2001–2006. J Allergy Clin Immunol 2007; 119:1016–1018.

6 Avery NJ, King RM, Knight S, Hourihane JO. Assessment of quality of life in children with peanut allergy. Pediatr Allergy Immunol 2003; 14:378–382.

7 Bindslev-Jensen C, Ballmer-Weber BK, Bengtsson U, *et al.* Standardization of food challenges in patients with immediate reactions to foods: position paper from the European Academy of Allergology and Clinical Immunology. Allergy 2004; 59:690–697.

8 Asero R, Ballmer-Weber BK, Beyer K, *et al.* IgE-mediated food allergy diagnosis: current status and new perspectives. Mol Nutr Food Res 2007; 51:135–147.

9 Sampson HA. Utility of food-specific IgE concentrations in predicting symptomatic food allergy. J Allergy Clin Immunol 2001; 107:891–896.

10 Lidholm J, Ballmer-Weber BK, Mari A, Vieths S. Component-resolved diagnostics in food allergy. Curr Opin Allergy Clin Immunol 2006; 6:234–240.

11 • Guilloux L, Morisset M, Codreanu F, *et al.* Peanut allergy diagnosis in the context of grass pollen sensitization for 125 patients: roles of peanut and cross-reactive carbohydrate determinants specific IgE. Int Arch Allergy Immunol 2009; 149:91–97.
This study identified CCD sIgE in most of the false-positive peanut sIgE results.

12 Van der Veen MJ, van Ree R, Aalberse RC, *et al.* Poor biologic activity of cross-reactive IgE directed to carbohydrate determinants of glycoproteins. J Allergy Clin Immunol 1997; 100:327–334.

13 • Cabanos C, Tandang-Silvas MR, Odijk V, *et al.* Expression, purification, cross-reactivity and homology modeling of peanut profilin. Protein Expr Purif 2010; 73:36–45.
This study suggested that Ara h 5 could be used as a model and allergen of choice for profilin allergy diagnosis due to its close relation to other allergenic profilins.

14 • Guillamon E, Rodriguez J, Burbano C, *et al.* Characterization of lupin major allergens (*Lupinus albus* L.). Mol Nutr Food Res 2010; 54:1668–1676.
This in-vitro study has shown that the Lup-1 is a conglutin β (vicilin-like protein) while the Lup-2 corresponds to the conglutin α fraction (legumin-like protein). There was a high level of amino acid sequence homology of Lup-1 and Lup-2 with the major allergens of some legumes, which may explain both IgE and clinical crossreactivity of lupin and other legumes.

15 •• Holzhauser T, Wackermann O, Ballmer-Weber BK, *et al.* Soybean (Glycine max) allergy in Europe: Gly m 5 (beta-conglycinin) and Gly m 6 (glycinin) are potential diagnostic markers for severe allergic reactions to soy. J Allergy Clin Immunol 2009; 123:452–458.
Important study demonstrating that sensitization to Gly m 5 or Gly m 6 allergenic components may be a marker for identifying patients with soy allergy who are at high risk of experiencing more severe reactions.

16 Koppelman SJ, de Jong GA, Laaper-Ertmann M, *et al.* Purification and immunoglobulin E-binding properties of peanut allergen Ara h 6: evidence for cross-reactivity with Ara h 2. Clin Exp Allergy 2005; 35:490–497.

17 Marsh J, Rigby N, Wellner K, *et al.* Purification and characterisation of a panel of peanut allergens suitable for use in allergy diagnosis. Mol Nutr Food Res 2008; 52 (Suppl 2):S272–S285.

18 Restani P, Ballabio C, Corsini E, *et al.* Identification of the basic subunit of Ara h 3 as the major allergen in a group of children allergic to peanuts. Ann Allergy Asthma Immunol 2005; 94:262–266.

19 Stevenson SE, Chu Y, Ozias-Akins P, Thelen JJ. Validation of gel-free, label-free quantitative proteomics approaches: applications for seed allergen profiling. J Proteomics 2009; 72:555–566.

20 Vereda A, Andreae DA, Lin J, *et al.* Identification of IgE sequential epitopes of lentil (Len c 1) by means of peptide microarray immunoassay. J Allergy Clin Immunol 2010; 126:596–601; e1.

21 • Sastre J. Molecular diagnosis in allergy. Clin Exp Allergy 2010; 40:1442–1460.
An excellent review on the topic.

22 Ferrer M, Sanz ML, Sastre J, *et al.* Molecular diagnosis in allergology: application of the microarray technique. J Investig Allergol Clin Immunol 2009; 19 (Suppl 1):19–24.

23 International Union of Immunological Societies, Allergen Nomenclature Sub-Committee, List of allergens updated 2 December 2008.

24 Burks AW, Williams LW, Connaughton C, *et al.* Identification and characterization of a second major peanut allergen, Ara h II, with use of the sera of patients with atopic dermatitis and positive peanut challenge. J Allergy Clin Immunol 1992; 90:962–969.

25 Burks W, Sampson HA, Bannon GA. Peanut allergens. Allergy 1998; 53:725–730.

26 Flinterman AE, van Hoffen E, den Hartog Jager CF, *et al.* Children with peanut allergy recognize predominantly Ara h2 and Ara h6, which remains stable over time. Clin Exp Allergy 2007; 37:1221–1228.

27 Radauer C, Bublin M, Wagner S, *et al.*. Allergens are distributed into few protein families and possess a restricted number of biochemical functions. J Allergy Clin Immunol 2008; 121:847 e7–852 e7.

28 Mills EN, Jenkins JA, Alcocer MJ, Shewry PR. Structural, biological, and evolutionary relationships of plant food allergens sensitizing via the gastrointestinal tract. Crit Rev Food Sci Nutr 2004; 44:379–407.

29 Kleber-Janke T, Crameri R, Appenzeller U, *et al.* Selective cloning of peanut allergens, including profilin and 2S albumins, by phage display technology. Int Arch Allergy Immunol 1999; 119:265–274.

30 Mittag D, Akkerdaas 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; 114:1410–1417.

31 Beyer K, Morrow E, Li XM, *et al.* Effects of cooking methods on peanut allergenicity. J Allergy Clin Immunol 2001; 107:1077–1081.

32 Ballmer-Weber BK, Holzhauser T, Scibilia J, *et al.* Clinical characteristics of soybean allergy in Europe: a double-blind, placebo-controlled food challenge study. J Allergy Clin Immunol 2007; 119:1489–1496.

33 Mittag D, Vieths S, Vogel L, *et al.* Soybean allergy in patients allergic to birch pollen: clinical investigation and molecular characterization of allergens. J Allergy Clin Immunol 2004; 113:148–154.

34 Zoccatelli G, Pokoj S, Foetisch K, *et al.* Identification and characterization of the major allergen of green bean (*Phaseolus vulgaris*) as a nonspecific lipid transfer protein (Pha v 3). Mol Immunol 2010; 47:1561–1568.

35 Beyer K, Ellman-Grunther L, Jarvinen KM, *et al.* Measurement of peptide-specific IgE as an additional tool in identifying patients with clinical reactivity to peanuts. J Allergy Clin Immunol 2003; 112:202–207.

36 Shreffler WG, Beyer K, Chu TH, *et al.* Microarray immunoassay: association of clinical history, in vitro IgE function, and heterogeneity of allergenic peanut epitopes. J Allergy Clin Immunol 2004; 113:776–782.

37 Astier C, Morisset M, Roitel O, *et al.* Predictive value of skin prick tests using recombinant allergens for diagnosis of peanut allergy. J Allergy Clin Immunol 2006; 118:250–256.

38 • Chiang WC, Pons L, Kidon MI, *et al.* Serological and clinical characteristics of children with peanut sensitization in an Asian community. Pediatr Allergy Immunol 2010; 21:e429–e438.
This is the first Asian study suggesting that Asian children with peanut sensitization have clinically similar presentations and respond to the same major peanut allergens (Ara h 1–3) as North American and European peanut-sensitized children.

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

39 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 2010. [Epub ahead of print]

This important study describes the clinical and immunological characteristics of patients with peanut allergy from the USA, Spain and Sweden using molecular diagnostics. Specific IgE responses to peanut, birch and grass allergenic components were measured using standardized ImmunoCAP method. The pattern and level of components recognition varied across patients from the three different geographical regions. American patients were commonly sensitized to Ara h 1–3, Spanish patients to the LTP Ara h 9, and Swedish patients to the Bet v 1 homologue Ara h 8. American patients developed peanut allergy at an earlier age, and along with Spanish patients tended to experience more severe symptoms. The results elegantly demonstrate the heterogeneity in the clinical and immunological phenotype of peanut allergy in distinct geographical areas which might reflect exposures to different environmental pollen and dietary traditions and emphasize the importance of molecular diagnostics in our understanding the puzzle of peanut allergy.

40 Asarnoj A, Moverare R, Ostblom E, *et al.* IgE to peanut allergen components:
•• relation to peanut symptoms and pollen sensitization in 8-year olds. Allergy 2010; 65:1189–1195.

This study investigated by microarray technique the IgE reactivity to peanut allergen components in Swedish school-age children in relation to pollen sensitization and reported peanut symptoms. The pattern of components recognition differed in the four groups of children with distinct peanut and birch pollen combination sensitizations. Peanut symptoms were more commonly reported and more severe in patients with reactivity to Ara h 1–3 than Ara h 8 and pollen sensitization. CRD diagnostics in peanut-sensitized patients with concomitant pollen sensitization may be a useful tool in distinguishing those with clinically relevant and potentially severe peanut allergy from those with clinically irrelevant peanut sensitization.

41 Codreanu F, Collignon O, Roitel O, *et al.* A novel immunoassay using
•• recombinant allergens simplifies peanut allergy diagnosis. Int Arch Allergy Immunol 2010; 154:216–226.

This is one of the two recently published important studies which have identified quantification of Ara h 2 as the best predictor of clinical reactivity to peanut. The researches measured the IgE reactivity to peanut extract and recombinant peanut components (Ara h 1, 2, 3, 6, 7 and 8) in 166 peanut-allergic patients (challenge-confirmed), 61 birch and/or grass pollen-sensitized peanut-tolerant patients, and 10 healthy controls (without atopic disease) from two different centres in France. Despite being tolerant to peanut 79% of pollen-sensitized patients were sensitized to peanut extract confirming the low specificity of this test in diagnosis of peanut allergy. Receiver Operating Characteristic (ROC) curve analysis revealed that at the cut-off level of 0.1 kU/l both Ara h 2 and Ara h 6 gave high sensitivity and specificity in both centres (center 1, 99%, 91%, and center 2, 92%, 78%) suggesting that Ara h 2 and/or Ara h 6 could be useful in differentiating peanut allergy from tolerance. Ara h 2 sIgE was the most important predictor of peanut allergy with cut-off level of 0.23 kU/l conferring 93% sensitivity and 96% specificity. However, only on the basis of these markers some patients would still require DBPCFC to confirm the diagnosis.

42 Nicolaou N, Poorafshar M, Murray C, *et al.* Allergy or tolerance in children
•• sensitised to peanut: prevalence and differentiation using component-resolved diagnostics. J Allergy Clin Immunol 2010; 125:191–197 e1–e13.

This is the first published study suggesting that measurement of IgE response to major peanut allergen Ara h 2 is more useful in predicting clinical peanut allergy than currently used skin or blood tests based on whole extract. Amongst peanut-sensitised children (SPT and/or sIgE to whole extract) within a population-based birth cohort, the researchers determined the presence or absence of clinical peanut allergy using oral challenge tests. Then they proceeded to investigate the utility of a novel component-resolved diagnostic test using microarray technology (major peanut and potentially crossreactive components, including grass allergens) to correctly identify those children with clinical peanut allergy. Marked differences in the component sensitization profile between peanut-allergic ($n = 29$) and peanut-tolerant patients ($n = 52$) were demonstrated. Measurement of specific IgE to Ara h 2 was the most important predictor of peanut allergy.

43 Nicolaou N, Murray C, Belgrave D, *et al.* Quantification of specific IgE to whole
• peanut extract and peanut components in prediction of peanut allergy. J Allergy Clin Immunol 2011; 127:684–685.

This study demonstrated the value of sIgE Ara h 2 quantification using routinely available laboratory test (ImmunoCAP) in predicting clinical reactivity to peanut among peanut-sensitized school-age children in the UK. A cut-off of 0.35 kU_A/l of Ara h 2 IgE conferred 100% sensitivity and 96.1% specificity. Using this cut-off point, 97.5% of patients in the study were correctly classified, with all children with peanut allergy given the correct classification.

44 Flinterman AE, Pasmans SG, den Hartog Jager CF, *et al.* T cell responses to
• major peanut allergens in children with and without peanut allergy. Clin Exp Allergy 2010; 40:590–597.

Interesting study addressing the question of whether T-cell component-resolved diagnostics could be useful in differentiating peanut allergy from tolerance demonstrating a considerable overlap between peanut-allergic and peanut sensitized-tolerant patients in component-specific T-cell responses.

45 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 e5–778 e5.

46 Lauer I, Dueringer N, Pokoj S, *et al.* The nonspecific lipid transfer protein, Ara h 9, is an important allergen in peanut. Clin Exp Allergy 2009; 39:1427–1437.

47 Kosma P, Sjolander S, Landgren E, *et al.* Severe reactions after the intake of
•• soy drink in birch pollen-allergic children sensitized to Gly m 4. Acta Paediatr 2010; 100:305–306.

This is the first report of a soybean-dependant pollen-food crossreaction of children experiencing reactions during the birch pollen season.

48 Ballabio C, Magni C, Restani P, *et al.* IgE-mediated cross-reactivity among leguminous seed proteins in peanut allergic children. Plant Foods Hum Nutr 2010; 65:396–402.

49 Peeters KA, Koppelman SJ, Penninks AH, *et al.* Clinical relevance of sensitization to lupine in peanut-sensitized adults. Allergy 2009; 64:549–555.

50 Shaw J, Roberts G, Grimshaw K, *et al.* Lupin allergy in peanut-allergic children and teenagers. Allergy 2008; 63:370–373.

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.