Benefits and limitations of molecular diagnostics in peanut allergy

Part 14 of the Series Molecular Allergology

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Abstract

Allergic reactions to peanut (*Arachis hypogaea*, Ara h) are caused by immunoglobulin E (IgE)-mediated sensitizations to various proteins. The stability and relative proportion of these proteins in peanut determine the risk of hazardous reactions. Hazardous sensitization to seed storage proteins [S2 albumins (Ara h 2, 6 and 7) > other seed storage proteins (Ara 1 and 3) > oleosins (Ara h 10 and 11)] are distinct from sensitizations to lipid transfer protein (Ara h 9) with moderate risk or cross-sensitizations to Bet v 1-homologous PR-10 protein (Ara h 8) and to profilin (Ara h 5) with low risk. A specific IgE test, e.g. to Ara h 2 in the case of suspected systemic reaction, or where this should be ruled out, can facilitate easier risk assessment. Results, however, are only relevant in the presence of corresponding clinical symptoms. IgE sensitization to peanut extract without hazardous reactions is often caused in this part of the world by Bet v 1-related cross reactions (in birch pollen allergy sufferers), cross-reactive carbohydrate determinants (CCD) or profilin sensitizations. In the case of doubt, clinical relevance can only be established by means of oral challenge, particularly since not all peanut allergens (e.g., oleosins) are available as yet for diagnostic purposes.

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The relevance of peanut as an allergen

The peanut (*Arachis hypogaea*) belongs to the legume family (*Leguminosae*). As the most common trigger of food-induced anaphylaxis, which is responsible for the highest number of fatalities, it is considered the most important primary food allergen. Patients frequently reacted with respiratory symptoms following peanut challenge testing [1]. This hazard is probably linked to the high stability of the allergens and their high proportion of the total protein content. The peanut has a high protein content of 24 %–29 % [2], primarily seed storage proteins, such that even smallest amounts of allergens can provoke an allergic reaction; 5 % of peanut allergic sufferers react to as little as 1.6 mg of peanut protein [3].

Epidemiology

The prevalence of peanut allergy in the US and UK is between 1 % and 2 %, in Australia 3 %. Numbers are likely to be lower in Germany, even though 10.6 % of children and adolescents exhibit elevated peanut-specific IgE [5]. This high sensitization rate are explained by cross-reactivity due to:

- Bet v 1-homologous PR-10 proteins (Ara h 8)
- CCD-bearing glycoproteins
- Profilins (Ara h 5)

in patients sensitized to birch (PR-10 proteins) or grass pollen (CCD and profilins).

Peanut as a foodstuff

In Europe and the US peanuts are primarily consumed in roasted form, e.g. peanuts in the shell;

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Abbreviations

CCD	cross-reactive carbohydrate determinants
IgE	Immunglobulin E
nsLTP	non-specific lipid transfer protein

Table 1

Peanut allergens and their characteristics

	Name	Protein family	Stability	Proportion of total protein	Clinical relevance	Diagnostic availability
Storage proteins	Ara h 1	7S Globulins	+++	+++ (11–31%)	++	a, b, d*
	Ara h 2	2S Albumins	+++	++ (7–16%)	++++	a, b, c*, d*
	Ara h 3	11S Globulins	+++	+++ (38–76%)	++	a, b, d
Pollen-associated allergens	Ara h 5	Profilin	(+)	+	(+)	– ggfs. Phl p 12 a, b, c*
Storage proteins	Ara h 6	2S Albumins	+++	++ (4–14%)	+++	b, d*
	Ara h 7	2S Albumins	++?	++?	++?	d*
Pollen-associated allergens	Ara h 8	PR 10 (Bet v 1-homologue)	(+)	(+) (< 0,1%)	(+)	a, b, d*
Plant panallergens	Ara h 9	nsLTP	++	+	++ (primarily in Mediterranean countries)	a, b, d*
Structural protein	Ara h 10/11	Oleosins	++?	+?	?	–
Plant defensins	Ara h 12/13	Defensins	+?	+?	?	–

**No clinical diagnostic studies available to date.*

diagnostic availability: a) ImmunoCAP Singleplex (ThermoFisher; Freiburg); b) ImmunoCAP ISAC Multiplex (ThermoFisher); c) ALLERG-O-LIQ (Dr. Fooke Laboratorien); d) HYTEC (HYCOR).

husked and salted; or processed as peanut butter or peanut puffs. In its unrefined form, peanut oil can contain sufficient amounts of allergens to trigger allergic reactions. In Asia, raw peanuts are used more commonly as a cooking ingredient, whereby long cooking times reduce their allergenicity. Roasting at high temperatures likely promotes the formation of compact globular protein aggregates that can increase the allergenicity of Ara h 1 and 2 [6].

Individual peanut allergens

Clinical reactions are explained predominantly by the characteristics of the individual proteins (**Tab. 1**), particularly if sensitization involves only one allergen family. In this context, a distinction is made between primary and secondary allergens: in the case of the former, sensitization occurs to the allergen itself, whilst the latter involves cross-reactions to structurally similar epitopes following e.g. sensitization after inhalation.

Fig. 1 offers an overview about peanut allergens identified to date.

Primary major allergens: seed storage proteins

Ara h 1 is a vicilin-type 7S globulin and Ara h 3 an 11S globulin, both members of the cupin superfamily. Ara h 2, Ara h 6 and Ara h 7 are 2S albumins and belong to the prolamin superfamily [7]. Ara h 2 and Ara h 6 possess significant sequence homology, Ara h 7 less so. Although belonging to different protein families, Ara h 1, 2, and 3 exhibit high serological cross-reactivity, thereby hampering a selective diagnosis using individual seed storage proteins [8].

Seed storage proteins are the major peanut allergens in primary peanut allergy. Between 76 % and 96 % of peanut-allergic children and adolescents in the US and central and northern Europe show specific IgE to Ara h 2 and Ara h 6, compared with only 42 % in Spain. The sensitization rates for Ara h 1 are between 63 % and 80% and for Ara h 3 somehow lower, whilst the rate for Ara h 7 is only 43% [9, 10], thereby defining it as a minor allergen.

Primary minor allergens: oleosins

Oleosins are structural proteins found in plant cells and are potential allergens in legumes, oil seeds and tree nuts. Their three-dimensional hairpin structure with amphiphilic (both hydro- and lipophilic) ends and an extensive hydrophobic domain embedded in an oil matrix contributes to the formation and stability of oil bodies (oleosomes) and thus prevents the aggregation of individual lipid droplets. Several peanut oleosin isoforms with molecular weights of 14 (Ara h 11), 16 (Ara h 10) and 18 kDa have already been purified and produced as recombinants. They are apparently able to interact with one another and form larger complexes (oligomers) [11]. The prevalence of sensitization is not know and likely affects only a small number of peanut allergic sufferers. The fact that oleosins may be underrepresented or absent in aqueous peanut extracts repre-

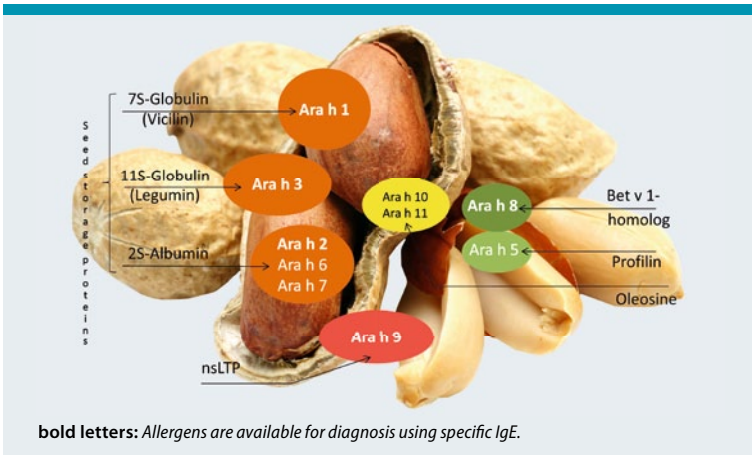

Fig. 1: Peanut allergens identified to date. The size of the ellipses approximately corresponds to their proportion in relation to the total protein content.

sents a diagnostic gap hampering the identification of affected patients [12].

Both seed storage proteins and oleosins have a high degree of thermal and digestive stability and are therefore important as primary food allergens (**Tab. 1**).

Secondary allergens: non-specific lipid transfer proteins and cross-reactive aeroallergens

Ara h 9, a non-specific lipid transfer protein (nsLTP), is considered a secondary food allergen particularly in Mediterranean countries. This secondary sensitization/cross-reaction is probably due to other nsLTP (e. g., Pru p 3 in peach). Since nsLTP possess thermal and digestive stability, affected patients can develop systemic symptoms [13].

Sensitizations to the Bet v 1-homologous PR-10 protein Ara h 8, the profilin Ara h 5 and glycoproteins (CCD) are usually caused by cross-reactions to pollen allergens. Sensitization rates vary depending on regional pollen exposure. Birch trees are responsible for a considerable north-south gradient in Europe in terms of cross-reactions to Ara h 8; in regions of higher grass pollen exposure, increased cross-reactive IgE to Ara h 5 and CCD-containing peanut extracts can be expected. The relevant proteins are largely labile to heat and digestion. Since peanuts are generally not consumed raw in this part of the world, only mild and predominantly oropharyngeal symptoms develop.

Clinical data on molecular diagnostic methods

No other food allergen has been the subject of more clinical studies on the relevance of molecular allergy diagnosis than the peanut.

Sensitization to the three seed storage proteins Ara h 1–3 is more commonly associated with systemic and severe clinical symptoms compared with sensitization to Ara h 8 [14]. Children and adolescents with only Ara h 8 sensitization and no IgE to seed storage proteins (Ara h 1–3, 6) do not generally demonstrate systemic reactions [15]. Determining IgE antibodies to Ara h 2 yields the best predictive value [16, 17, 18, 19, 20]. However, several case reports describe patients with systemic reactions following peanut exposure due to sensitization to Ara h 6 without IgE to Ara h 1–3 [21]. This means in turn that, in the absence of IgE to seed storage proteins Ara h 1–3 and 6, a clinically relevant allergy is highly unlikely in central Europeans.

In southern Europe, specific IgE to the lipid transfer protein Ara h 9 is also predictive of a systemic allergic reaction [22]. The majority of these patients is not sensitized to Ara h 2, but rather to Ara h 9 [9].

Ultimately, it is not possible to reliably predict the risk of anaphylaxis by determining IgE to Ara h 2 alone. Thus there are sensitized but peanut-tolerant patients particularly in the area of lower IgE titers (under 2 kU/l) [18], and even in case of high-titer Ara h 2 sensitizations individual patients can demonstrate clinical tolerance [23].

Diagnostic approaches using peanut allergens

Available individual allergens

It is possible to determine specific IgE antibodies to whole peanut extract, the seed storage proteins Ara h 1, h 2, h 3 and h 6, to nsLTP Ara h 9 and to the PR-10 protein Ara h 8 (**Tab. 1**).

Approach to peanut allergy diagnosis

Depending on patient history and previous findings, a number of diagnostic questions arise (**Figs. 2 and 3**):

- Requirement to exclude a peanut allergy (e.g., in patients with atopic dermatitis or other food allergies) prior to the consumption of peanut-containing products (**Fig. 2**).
- Incidental finding of sensitization (e.g., elevated IgE to peanut in panel or screening tests) (**Fig. 2**).
- Allergic reaction following exposure to/consumption of peanut (**Fig. 3**).

1. Exclusion of peanut allergy: IgE to peanut extract is well suited as a screening parameter (particularly for the exclusion of) peanut allergy: the absence of peanut IgE has a remarkably high negative predictive value (rare exceptions: relevant sensitization to the oleosins Ara h 10/11). A positive IgE result is only clinically relevant in the presence of corresponding symptoms (low diagnostic specificity). In the case of negative specific IgE, an additional prick-to-prick test with native peanut serves to detect or

exclude sensitization. If positive, an oral peanut challenge test should be considered.

2. Incidental finding of sensitization: Incidental unexpected findings of positive IgE to peanut are also seen in clinical practice. A stepwise approach (**Fig. 2**) takes the potential consequences, advantages and costs of diagnostic tests into consideration. The most important initial question relates to the frequency (e. g., more than once a month) and recency (e. g., within the previous 6 weeks) of consumption of relevant quantities of peanuts.

3. Allergic reaction following exposure to peanut: Determining IgE to Ara h 2 plays an essential role in case of suspected primary peanut allergy. Significantly elevated specific IgE and a clearly positive patient history are highly suggestive of a clinically relevant allergy. Due to heterogenous study data and varying threshold (cut-off values) in the investigated collectives, it is currently not possible to define a reliable threshold dose for specific IgE to Ara h 2 for the prediction of an allergic reaction. Although IgE to Ara h 6 represents a parameter of similar predictive value, only scant data have been available to date compared with data for Ara h 2.

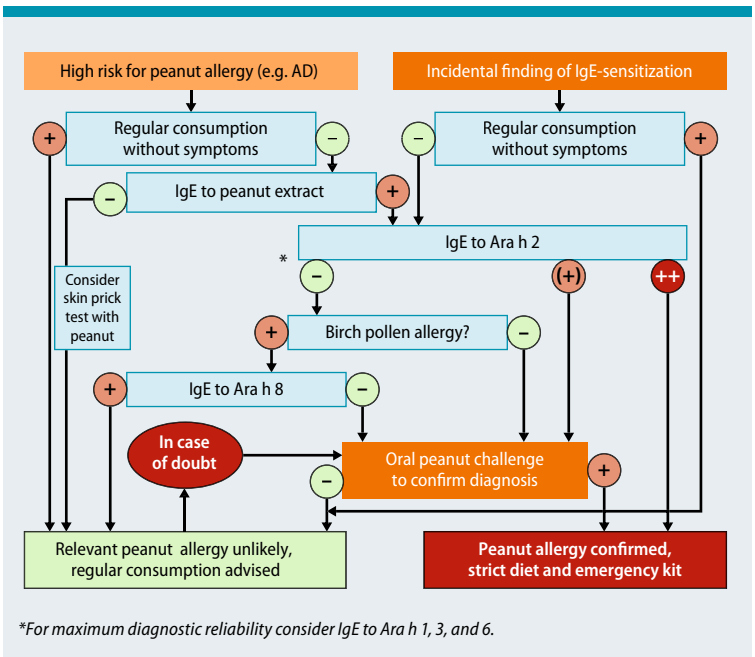

Fig. 2: A model of a diagnostic algorithm for the detection or exclusion of peanut allergy (see text for more details).

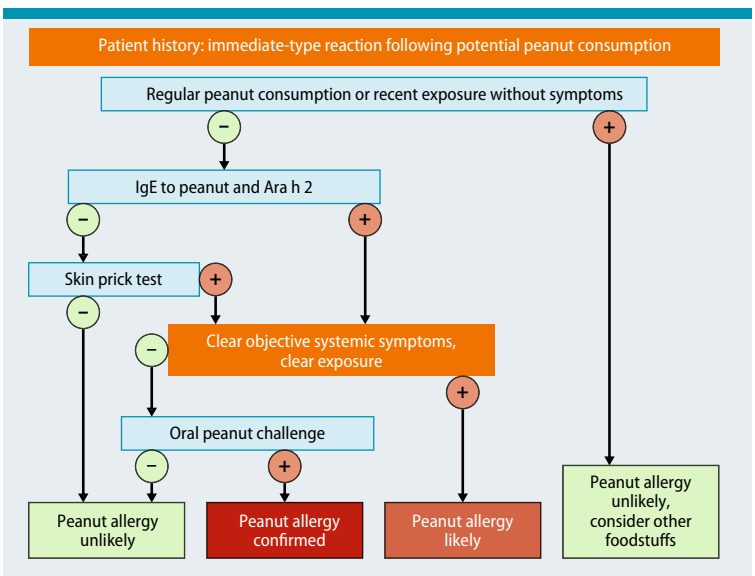

Fig. 3: Diagnostic algorithm for immediate-type reaction following potential peanut consumption

Common peanut cross-reactivities in birch pollen sensitization

Determining IgE to Ara h 8 and Ara h 2 is recommended in the case of suspected birch pollen-related sensitization. Negative Ara h 2 and unequivocally positive Ara h 8 indicate a Bet v 1-related cross-reaction of low clinical relevance. Other causes of positive IgE findings of limited clinical relevance would be cross-reactions due to CCD or profilins.

Rare constellations in peanut allergy

The detection of sensitization to Ara h 1 and 3 is often not necessary, since there is high cross-reactivity between these seed storage proteins [8] and monosensitizations to Ara h 1 and/or 3 are rare. When in doubt, performing a food challenge can clarify cases of negative or low IgE to Ara h 2. If none of the seed storage proteins test positive, a clinically relevant peanut allergy is highly unlikely, although it cannot be ruled out completely in the presence of sufficient clinical suspicion (diagnostic gap due to, e. g., oleosins Ara 10/11). IgE to nsLTP Ara h 9 should be additionally determined in patients from the Mediterranean region.

Cross-reactive allergens

Clinically relevant cross-reactions are mediated primarily by seed storage proteins. Reactions to legumes such as lupins and lentils, as well as tree nuts such as hazelnuts and walnuts or oil seeds such as sesame seeds are possible. Serological cross-reactions need to be viewed with critical scrutiny in terms of their clinical relevance; thus, the presence of antibodies to soy in peanut-allergic sufferers is usually of no relevance.

Open questions

The following questions related to molecular diagnosis in peanut-allergic sufferers remain unanswered:

- Are all relevant peanut allergens already available or are further components other than the oleosins still lacking for the purpose of comprehensive diagnosis?
- Which value does the monitoring of IgE to seed storage proteins (e. g., Ara h 2) have in the prediction of tolerance development?
- How relevant is the additional analysis of IgE to Ara h 6 for the prediction of peanut allergy?

Conclusion

Molecular allergy diagnosis plays an essential role in peanut allergy:

- Many cases of sensitization to peanut extracts in this part of the world are caused by pollen-associated cross-reactions, which can be differentiated by determining IgE to available marker allergens (e. g., Bet v 1-homologous Ara h 8, CCD MUXF 3, profilin Phl p 12)
- The associated clinical reactions are often mild and generally restricted locally to the mouth and throat
- In the case of peanut-allergic sufferers from the Mediterranean region, Ara h 9 as an nsLTP forms part of IgE diagnosis and may be associated with systemic reactions
- Significantly elevated specific IgE values to stable seed storage proteins such as Ara h 2 (and probably Ara h 6) are frequently associated with systemic reactions and a clinically relevant peanut allergy
- Oral food challenge can be omitted with in a number of these patients (those with a clear and reliable patient history of systemic reactions caused by peanut and with proven sensitization)
- In cases of doubt, the clinical diagnosis of peanut allergy should be established by an oral challenge, since some patients with Ara h 2-specific IgE may be tolerant to peanut, whilst others show a systemic reaction to peanut despite the absence of Ara h 2-spefic IgE to peanut. Another reason is, that not all peanut allergens relevant to diagnosis are available as yet. In addition, detectable concentrations of specific IgE are consistent with a sensitization (predisposition to allergies) being clinically relevant only in the presence of corresponding symptoms.

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Conflict of interest
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