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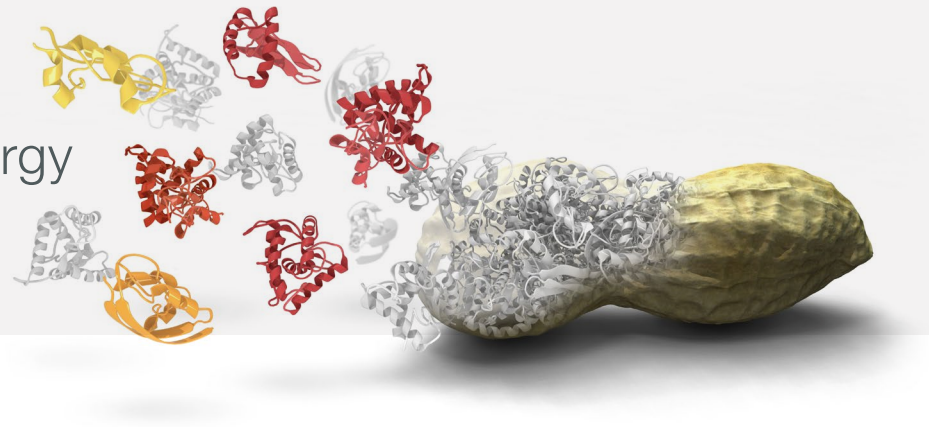
The Relevance of Ara h 6 in Peanut Allergy



Introduction

The diagnosis of peanut allergy is greatly improved by using component resolved diagnostics (CRD) in the clinical work up of patients[1]. By analyzing specific IgE antibodies (sIgE) to individual allergenic proteins in the peanut, the clinician obtains a better understanding of the underlying cause of the patient's symptoms. Sensitization(s) are revealed as being caused by primary peanut sensitization in the case of sIgE to Storage Proteins, or as a consequence of cross-reactivity in the cases of sIgE to Ara h 8 and Ara h 9. With this information, the clinician can judge the influence of other sensitizations and gains support for assessing the risk for severe reactions and improved management of the patient.

Three peanut specific storage proteins (Ara h 1, Ara h 2 and Ara h 3) and two cross-reactive proteins (Ara h 8 and Ara h 9, respectively) are widely used as diagnostic tools in clinical practice. The storage protein Ara h 2 appears to be dominating in terms of both sensitization frequency and in eliciting clinical symptoms in peanut allergic patients[2]. We now offer a test for a forth storage protein – Ara h 6 – which can contribute to an even higher certainty in the diagnosis of peanut allergy.

The nature of the Ara h 6 protein

Ara h 6 is a major peanut allergen showing similarity with Ara h 2 in many aspects. Both are storage proteins of the 2S albumin type that are heat stable and resistant to digestion in the gut, why they are associated with potentially systemic reactions[3, 4]. They are 58% similar on the amino acid level, and the IgE binding sites (epitopes) of Ara h 2 and Ara h 6 overlap to a large extent, although unique IgE binding epitopes of Ara h 6 have been demonstrated[3, 5].

Both proteins are highly immunogenic[6] and potent in functional assays such as histamine release and basophil activation tests[7, 8].

Ara h 6 is a major peanut allergen in children and adults

Peanut allergic patients show early and frequent sensitization to Ara h 6. In children with diagnosed peanut allergy more than two thirds (65-98%) have detectable sIgE to Ara h 6, as indicated by studies performed in France, Austria, Spain, Finland and Holland[9-14].

In a pan-European study of both children and adults, 85 % of subjects with early-onset peanut allergy (before 14 years of age) had elevated sIgE to any peanut storage protein, and of these 93% and 87% were positive to Ara h 2 and Ara h 6, respectively (calculated from[15]). In studies on only adults, the frequency of Ara h 6 sensitization among peanut allergic subjects is shown to between 50 % and 80 % of patients[9, 16, 17].

Differential Ara h 6 vs Ara h 2 sensitization

Sensitization to Ara h 6 without concomitant Ara h 2 sensitization has been detected in up to 4% of study subjects[11,13,16], indicating that although sensitization to Ara h 6 and Ara h 2 is mainly overlapping, selective Ara h 6 sensitization does occur. Indeed, Ara h 6 sensitization in the absence of Ara h 2 sIgE was reported in five Dutch children of which three reacted in peanut challenge[18], and in a Swedish boy negative (<0.35 kUA/L) for sIgE to Ara h 1 – 3, who reacted with anaphylaxis to an oral peanut challenge[19].

In conclusion, although Ara h 2 and Ara h 6 are similar and sensitization to these is overlapping, exclusive Ara h 6 sensitization is seen in an important minority of patients.

Ara h 2 and Ara h 6 for improved diagnostic accuracy

In studies on Ara h 6, different preparation of the protein and different assay methods have been used, making comparisons difficult. Nevertheless, taken together the collective data demonstrate that Ara h 6 is an important marker of

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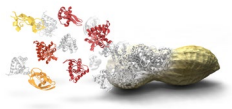

peanut allergy, with a diagnostic accuracy similar to that of Ara h 2. The sensitivity of Ara h 6 is reported to range from approximately 60 to 90%, while the specificity is reported to be less varied, and generally above 95%[9-11, 14]. Several studies indicate that when used together, Ara h 2 and Ara h 6 can provide the highest diagnostic accuracy[8, 11, 13, 14].

The importance of multiple storage protein sensitizations

Multiple sensitization to peanut storage proteins correlate not only with the probability of clinical symptoms per se, but is also associated with the symptom severity.

Although Ara h 2 is considered the most important peanut allergen many peanut allergic patients have additional sIgE directed against Ara h 1 and Ara h 3[2, 20, 21]. A number of studies using several diagnostic methods (skin prick testing, immunoblot, in vitro IgE), have shown that the severity score in food challenges or reported symptoms to peanut intake, correlate with the number of sensitizations to storage proteins[22-24], including Ara h 6[13, 17].

Thus, poly-sensitization to storage proteins appears to be indicative of more severe reactions. Multiple sensitizations to stable proteins may also be predictive of future symptoms in sensitized children. In longitudinal studies of Swedish children, the number of sensitizations at age four to storage proteins Ara h 1, 2, 3 and 6 and/or Ara h 9 (peanut LTP), correlated with allergic reactions to peanut intake at sixteen years of age[25]. Thus, it is conceivable that, by including all these peanut component tests in the diagnostic work up, the clinician can gain insight into the possible development of the patient's allergic status.

References

1. Matricardi PM, Kleine-Tebbe J, Hoffmann HJ, Valenta R, Hilger C, Hofmaier S, et al. *EAACI Molecular Allergology User's Guide.* Pediatr Allergy Immunol. 2016;27 Suppl 23:1-250.
2. Klemans RJ, van Os-Medendorp H, Blankestijn M, Bruijnzeel-Koomen CA, Knol EF, Knulst AC. *Diagnostic accuracy of specific IgE to components in diagnosing peanut allergy: a systematic review.* Clin Exp Allergy. 2015 ;45(4):720-30
3. Lehmann K, Schweimer K, Reese G, Randow S, Suhr M, Becker WM, et al. *Structure and stability of 2S albumin type peanut allergens: implications for the severity of peanut allergic reactions.* Biochem J. 2006;395:463-72.
4. Suhr M, Wicklein D, Lepp U, Becker WM. *Isolation and characterization of natural Ara h 6: evidence for a further peanut allergen with putative clinical relevance based on resistance to pepsin digestion and heat.* Mol Nutr Food Res. 2004;48(5):390-9.
5. Koid AE, Chapman MD, Hamilton RG, Van Ree R, Versteeg SA, Dreskin SC, et al. *Ara h 6 Complements Ara h 2 as an Important Marker for IgE Reactivity to Peanut.* J Agric Food Chem. 2014;62(1):206-13
6. Bernard H, Mondoulet L, Drumare MF, Paty E, Scheinmann P, Thai R, et al. *Identification of a new natural Ara h 6 isoform and of its proteolytic product as major allergens in peanut.* J Agric Food Chem. 2007;55(23):9663-9.
7. Blanc F, Adel-Patient K, Drumare MF, Paty E, Wal JM, Bernard H. *Capacity of purified peanut allergens to induce degranulation in a functional in vitro assay: Ara h 2 and Ara h 6 are the most efficient elicitors.* Clin Exp Allergy. 2009;39(8):1277-85.
8. van Erp FC, Knol EF, Pontoppidan B, Meijer Y, van der Ent CK, Knulst AC. *The IgE and basophil responses to Ara h 2 and Ara h 6 are good predictors of peanut allergy in children.* J Allergy Clin Immunol. 2017;139(1):358-60 e8.
9. Ackerbauer D, Bublin M, Radauer C, Varga EM, Hafner C, Ebner C, et al. *Component-Resolved IgE Profiles in Austrian Patients with a Convincing History of Peanut Allergy.* Int Arch Allergy Immunol. 2015;166(1):13-24.
10. Agabriel C, Ghazouani O, Birnbaum J, Liabeuf V, Porri F, Gouitaa M, et al. *Ara h 2 and Ara h 6 sensitization predicts peanut allergy in Mediterranean pediatric patients.* Pediatr Allergy Immunol. 2015;25(7):662-7.
11. Codreanu F, Collignon O, Roitel O, Thouvenot B, Sauvage C, Vilain AC, et al. *A Novel Immunoassay Using Recombinant Allergens Simplifies Peanut Allergy Diagnosis.* Int Arch Allergy Immunol. 2011;154(3):216-26.
12. Flinterman AE, van Hoffen E, den Hartog Jager CF, Koppelman S, Pasmans SG, Hoekstra MO, 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(8):1221-8.
13. Kukkonen AK, Pelkonen AS, Makinen-Kiljunen S, Voutilainen H, Makela MJ. *Ara h 2 and Ara 6 are the best predictors of severe peanut allergy: a double-blind placebo-controlled study.* Allergy. 2015;70(10):1239-45.
14. Pedrosa M, Boyano-Martinez T, Garcia-Ara C, Caballero T, Quirce S. *Utility of specific IgE to Ara h 6 in peanut allergy diagnosis.* Ann Allergy Asthma Immunol. 2015;115(2):108-12.
15. Ballmer-Weber BK, Lidholm J, Fernandez-Rivas M, Seneviratne S, Hanschmann KM, Vogel L, et al. *IgE recognition patterns in peanut allergy are age dependent: perspectives of the EuroPrevall study.* Allergy. 2015;70(4):391-407.
16. Klemans RJ, Knol EF, Bruijnzeel-Koomen CA, Knulst AC. *The diagnostic accuracy of specific IgE to Ara h 6 in adults is as good as Ara h 2.* Allergy. 2014; 69(8):1112-4
17. Peeters KA, Koppelman SJ, van Hoffen E, van der Tas CW, den Hartog Jager CF, Penninks AH, et al. *Does skin prick test reactivity to purified allergens correlate with clinical severity of peanut allergy?* Clin Exp Allergy. 2007;37(1):108-15.
18. van der Valk JP, Schreurs MW, El Bouch R, Arends NJ, de Jong NW. *Mono-sensitisation to peanut component Ara h 6: a case series of five children and literature review.* Eur J Pediatr. 2016;175(9):1227-34.
19. Asarnoj A, Glaumann S, Elfstrom L, Lilja G, Lidholm J, Nilsson C, et al. *Anaphylaxis to Peanut in a Patient Predominantly Sensitized to Ara h 6.* Int Arch Allergy Immunol. 2012;159(2):209-12.
20. 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(3):684-5.
21. Valcour A, Jones JE, Lidholm J, Borres MP, Hamilton RG. *Sensitization profiles to peanut allergens across the United States.* Ann Allergy Asthma Immunol. 2017;119(3):262-6 e1.
22. Asarnoj A, Moverare R, Ostblom E, Poorafshar M, Lilja G, Hedlin G, et al. *IgE to peanut allergen components: relation to peanut symptoms and pollen sensitization in 8-year-olds.* Allergy. 2010;65(9):1189-95.
23. Astier C, Morisset M, Roitel O, Codreanu F, Jacquenet S, Franck P, et al. *Predictive value of skin prick tests using recombinant allergens for diagnosis of peanut allergy.* J Allergy Clin Immunol. 2006;118(1):250-6.
24. Lewis SA, Grimshaw KE, Warner JO, Hourihane JO. *The promiscuity of immunoglobulin E binding to peanut allergens, as determined by Western blotting, correlates with the severity of clinical symptoms.* Clin Exp Allergy. 2005;35(6):767-73.
25. Asarnoj A, Hamsten C, Lupinek C, Melen E, Andersson N, Anto JM, et al. *Prediction of peanut allergy in adolescence by early childhood storage protein-specific IgE signatures: The BAMSE population-based birth cohort.* J Allergy Clin Immunol. 2017;140(2):587-90 e7.