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Review

Diagnosis of peanut allergy

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The diagnosis of food allergy requires both definitive symptom evoked episodes and the detection of the specific IgE. In addition, knowledge of the allergen components of each causative food is essential for the interpretation of specific IgE. In peanut allergy, Ara h 2, one of the 2S albumins, is the allergen component with the highest diagnostic accuracy. Though peanut allergens contain the similar types of protein families as other tree nuts allergens, the amino acid sequence homology between them are relatively low, so the clinical cross reactivity is not frequent. On the other hand, cross-reactive carbohydrate determinant which is a glycoprotein with high structural similarity seen in many plants sometimes cause the detection of clinically inactive IgE antibodies. This review gives an overview on the characteristics of peanut allergy and the interpretation of the allergen components.

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Diagnosis of the food allergy

Immediate type food allergy is IgE-mediated reaction which cause reactions within 2 h after the exposure to food allergens[1]. To diagnose it accurately needs both the confirmation of the definitive symptoms and the presence of the allergen specific IgE (s-IgE). When definitive symptom evoked episodes are not confirmed, an oral food challenge test is needed. The presence of s-IgE can be confirmed through specific serum IgE antibody test such as ImmunoCAP® assay, skin prick test, basophil histamine release test, or basophil activation test.

In addition to a crude allergen, measurement of allergen component-sIgE antibody confers higher diagnostic accuracy. To interpret each component-sIgE precisely, knowledges such as cross-reactivity with other antigens, stability against heat or digestion of each component is required.

It is important to note that s-IgE detection reflects sensitization but is not necessarily associated with symptoms. In other words, specific IgE test could be false positive because of the non-specific detection of IgE, detection of non-functional IgE, presence of some inhibitory factors, or the antigen is broken during digestion.

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Here, we give an overview of the antigenicity of the peanut (PN) allergens, which is one of the major food allergies.

Classification of the peanuts and tree nuts

In the biological classification, soy bean and PN belong to the family of Leguminosae, walnuts and pecans to the Juglandaceae, cashew and pistachio to the Anacardiaceae **(Table 1)**.

Clinically, patients allergic to PN or tree nut (TN) tend to eliminate all of them. However, more than 70% of patients with PN allergy do not have any other TN allergies[2, 3]. Thus, PN and each TN allergy must be diagnosed separately[4, 5].

On the other hand, cross-reactivity and cross-antigenicity between walnuts and pecans, cashew and pistachio which belong to the same families are reported [5, 6].

Table 1. Allergens of the legumes and tree nuts

		Prolamin		Cupin				
Common name	Family	LTP	2S albumin	Vicilin (7S globulin)	Legumin (11S globulin)	PR-10	Profilin	Oleosin
peanut	Leguminosae	Ara h 9 Ara h 16 Ara h 17	Ara h 2 Ara h 6 Ara h 7	Ara h 1	Ara h 3	Ara h 8	Ara h 5	Ara h 10 Ara h 11 Ara h 14 Ara h 15
soy bean		Gly m 1	Gly m 8	Gly m 5	Gly m 6	Gly m 4	Gly m 3	
pistachio	Anacardiaceae		Pis v 1	Pis v 3	Pis v 2 Pis v 5			
cashew			Ana o 3	Ana o 1	Ana o 2			
walnut	Juglandaceae	Jug r 3	Jug r 1	Jug r 2	Jug r 4	Jug r 5		
pecan			Car i 1	Car i 2	Car i 4			
hazel nut	Betulaceae	Cor a 8	Cor a 14	Cor a 11	Cor a 9	Cor a 1	Cor a 2	Cor a 12 Cor a 13
almond	Rosaceae	Pru du 3			Pru du 6		Pru du 4	
brazil nut	Lecythidaceae		Ber e 1		Ber e 2			
sesame	Pedaliaceae		Ses i 1 Ses i 2	Ses i 3	Ses i 6 Ses i 7			Ses i 4 Ses i 4

Allergen components of the legumes and tree nuts

Major PN and TN allergens are grouped into the common protein families **(Table 1)**. Among them, 2S albumin is reported to be the major allergen that related to the clinical symptoms. Pathogenesis-related protein (PR)-10, which is a cross-reactive homologue of the major birch pollen allergen Bet v 1, and profilin are involved in pollen associated allergy [4, 5]. Though PN and TN share some discrete sequences similar in physicochemical properties[2], overall amino acid sequence identities between PN and TN are quite low, supporting the low clinical cross-reactivity[7]. For example, the PN 2S albumin Ara h 2 is < 30% identical to the related walnut allergen, Jug r 1 **(Table 2)**[7]. On the other hand, amino acid sequence homology between walnuts and pecans, cashew and pistachio are quite high. For example, amino acid sequence of Jug r 1 is > 90% similar with the related pecan allergen,

Car i 1(**Table 2)**[7].

	Ara h 2	Gly m 8	Ses i 1	Jur r 1	Ana o 3	Cor a 14	Pis v 1	Car i 1
Ara h 2		34	25	23	25	26	24	20
Gly m 8	55		19	18	17	19	20	18
Ses i 1	40	40		38	35	43	32	38
Jur r 1	41	37	57		38	65	37	87
Ana o 3	39	42	57	63		42	64	39
Cor a 14	42	42	59	78	63		37	61
Pis v 1	42	38	54	62	79	59		34
Car i 1	34	36	57	92	63	76	63	

Modified from Maruyama N: JJACI 2015; 29: 303-11

Table 2. Amino acid sequence identity and homology of the 2S alubmins
Light-gray columns shows the amino acid sequence identity, and dark-gray columns shows the homology.

2S albumins

Ara h 2, Ara h 6, and Ara h 7 are 2S albumin seed storage proteins which are the members of the prolamin superfamily. 2S albumin is transcribed from a single precursor gene that is cleaved to form a small subunit and a large subunit and linked by S-S bonds [8]. Ara h 2 has a bundle of five alpha-helices held together by four disulfide bonds [8]. Due to these structural features, Ara h 2 is resistant to digestive enzymes. Ara h 2 and Ara h 6 have similar molecular weights (17 kDa and 15k Da, respectively), share approximately 60% sequence identity, and are expressed at similar levels. Though both are confirmed as a major PN allergen and most patients are co-sensitized, levels of Ara h 6-sIgE were lower compared to Ara h 2, and the degree of IgE inhibition was higher with Ara h 2. Moreover, Ara h 2 induced significantly greater maximum reactivity by the mast cell activation test[9].

Ara h 1

Ara h 1 is one of the major peanut allergens belongs to the cupin superfamily, named vicilin. Ara h 1 has trypsin inhibitory activity, that might play a role in plant defense against insects[4]. N-terminus are cleaved off to yield mature Ara h 1. It contains 6 cysteine residues that help to form bi-cupin, then assemble to form a highly stable homotrimetric complex. According to this trimetric structure, Ara h 1 possesses resistance to heat and protease digestion, and increases the number of IgE-binding epitopes in one molecule, that might increase the antigenicity[10]. Epitopic regions of Ara h 1 are exposed on the surface of the homotrimers, and contain identical or structurally homologous amino acids with Cor

a 11 and Jug r 2. These are considered as one of the causes of the cross-antigenicity between PN and TN[10].

Cross-reactive Carbohydrate Determinant

Cross-reactive carbohydrate determinant (CCD) is a glycoprotein with high structural similarity seen in many plants (fruits, vegetables, and pollens). Bromelain from pineapple and horseradish peroxidase (HRP) are known as the representative CCD binding to the asparagine residue**(Fig. 1)**[1, 11]. Regarding the peanut, Ara h 1 contains one glycosylation site that bears mainly xylosylated N-glycans of the composition $Man_3XylGlcNAc_2$[4].

IgE antibodies recognizing the CCD confer cross-antigenicity to a wide variety of legumes and TN, but are less likely to cause allergic symptoms due to their low ability of degranulating mast cells[4]. Thus, the presence of IgE antibody to CCD is considered to the clinical false positive detection of IgE antibodies. We have actually confirmed that both anti-bromelain and anti-HRP IgE antibodies were detected in the clinical false positive sera, but not in the sera from patients with peanut allergy[11]. Moreover, while peanut IgE antibodies from peanut allergy patients were not inhibited by HRP, most of the false positive peanut IgE antibodies were significantly inhibited by HRP **(Fig. 2)**[11].

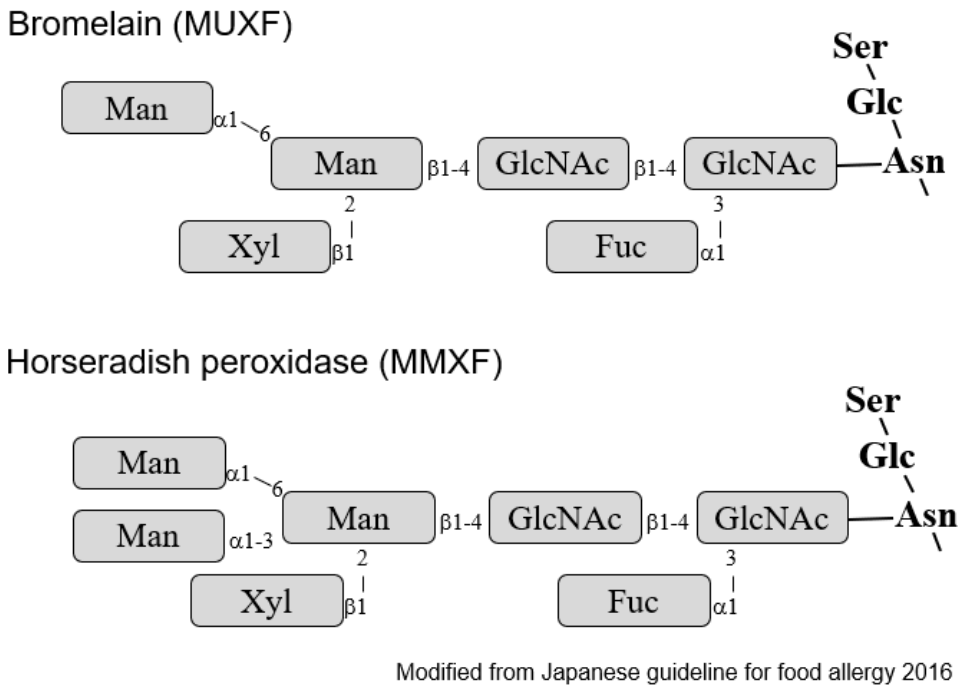

Fig. 1. Structures of the Cross-reactive carbohydrate determinant

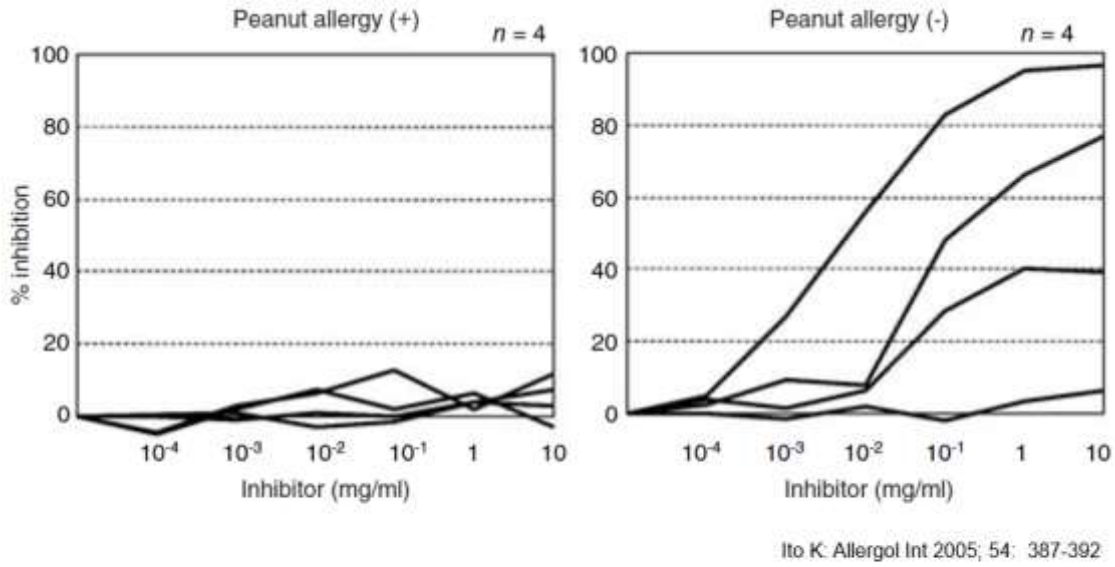

Fig. 2 Peanut IgE inhibition tests with HRP, a representative CCD antigen

Indivisual sera from 4 patients with and without peanut allergy were pre-incubated with the indicated concentrations of HRP, and peanut IgE antibodies were detected by UniCAP.
Percent inhibition of peanut IgE titers are shown.

Clinical utility of the component specific IgE

The pattern of sensitization to PN allergens varies among populations in different geographical regions[4, 12]. Ara h 1, 2, and 3 are the main elicitors of allergic reactions in the USA. Spanish patients recognized these allergens less frequently and were more often sensitized to the LIPID TRANSFER PROTEIN (LTP). Swedish patients recognized Ara h 1 and 3 more frequently than Spanish patients, but had the highest sensitization rate to Ara h 8 (PR-10). In a study involving PN allergic subjects from 11 European countries sensitized to Ara h 1, 2 and 3 since childhood, Ara h 2 was identified as the sole major allergen[13]. We also confirmed that Ara h 2 had the highest diagnostic accuracy compared to Ara h 1, 3, 5, 8 AND 9 in Japanese children[14].

It was difficult to differentiate PN allergy and non-PN allergy by the crude peanut-sIgE **(Fig. 3)**[3]. Peanut-sIgE was not statistically different between PN allergy and non-PN allergy subjects. Moreover, there were non-PN allergy subjects despite the titer of greater than 50 UA/ml. Ara h 2 could well differentiate PN allergy when the cut-off value of 4.0 UA/ml was adopted. However, some were not sensitized to Ara h 2 reflecting sensitization to some other components **(Fig. 3)**.

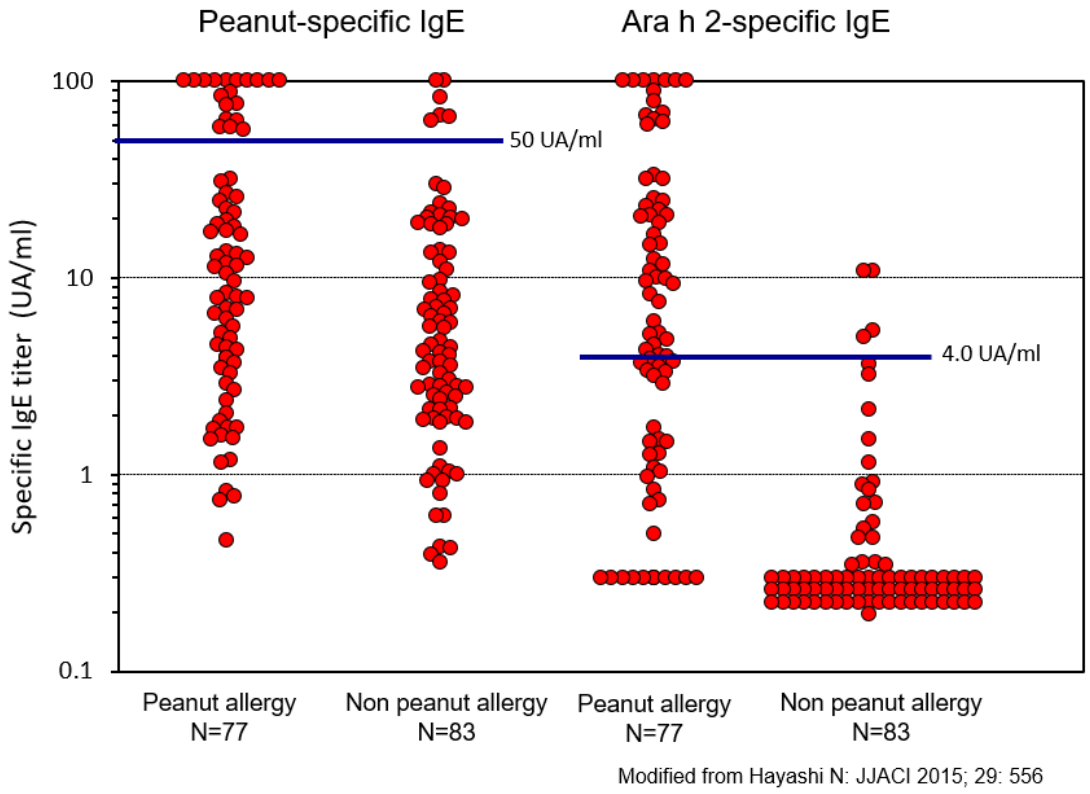

Fig. 3. The specific IgE levels of the peanut allergy and non allergy subjects.

The severity of PN allergy is difficult to predict from neither the PN-sIgE nor the Ara h 2-sIgE. The severity of allergy is determined by both the threshold dose that elicits symptoms and the severity of evoked symptoms. We have developed a scoring system to evaluate the symptom severity, named Anaphylaxis Scoring Aichi (ASCA)[15]. Then, the overall severity is expressed by dividing the total score (TS) of ASCA by the cumulative total protein dose (Pro) at the oral food challenge test (TS/Pro)[3]. Not only peanut-sIgE but also Ara h-2-sIgE could not strongly correlate with the TS/pro **(Fig. 4)**[3].

Overall, Ara h 2 could well predict the presence of PN allergy, but is difficult to predict its severity.

Conclusions

In PN allergy, Ara h 2 is the allergen component with the highest diagnostic accuracy. Though PN allergens contain the same types of protein families as other tree nut allergens, the amino acid sequence homology between them are relatively low, so the clinical cross reactivity is not frequent. On the other hand, CCD cause the false positive detection of sIgE.

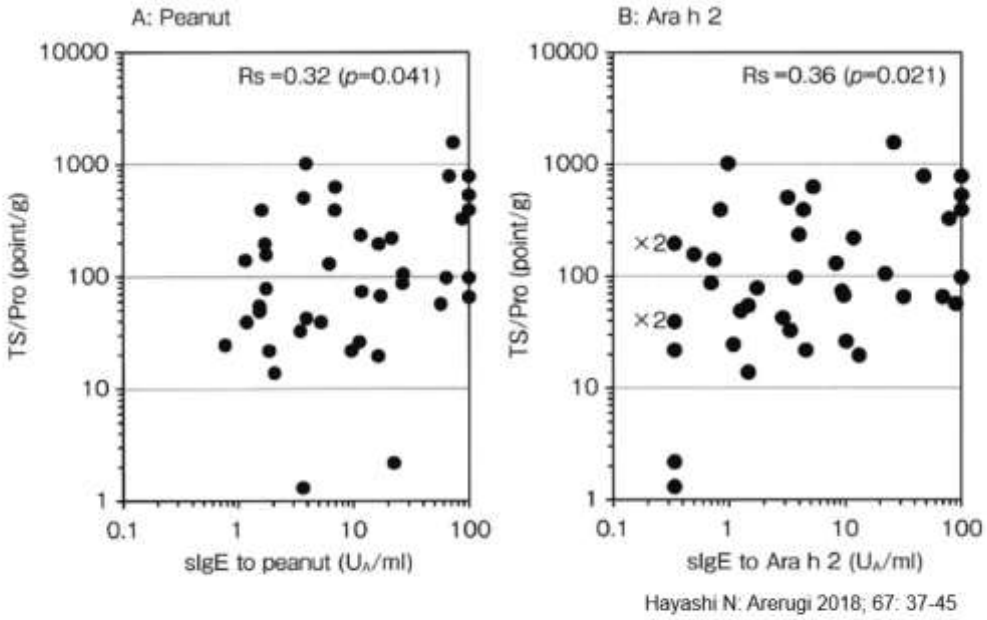

Fig. 4. The specific IgE levels and the results of the oral food challenge.

The severity of the symptoms provoked in the OFC was evaluated based on the total score (TS) as assessed by the ASCA system. The concurrent severity od the provoked symptom and the threshold dose Was expressed as TS/Pro (point/g) by dividing the TS by the cumulative protein dose. The scatterplots show The correlation between the TS/Pro and the sIgE to peanut (A) or Ara h 2 values (B). The correlation coefficient was calculated using Spearman's Rank sum test. "×2" indicates the presence of 2 overlapping Patients at the dot.

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