

Ara h 1 and Ara h 6 Sensitization Causes Clinical Peanut Allergy in Ara h 2-Negative Individuals

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Abstract

Background: Of the major peanut allergens, sensitivity to Ara h 2 has the highest prediction for clinical allergy. In this study, we evaluated sensitization to peanut components in Iceland and related Ara h 2-negative sensitization to clinical allergy. **Methods:** Ara h 1, Ara h 2, Ara h 3, Ara h 8, and Bet v 1 IgEs were measured (ImmunoCAP) in 220 peanut IgE (Pn-IgE)-positive serum samples. Ara h 2 IgE-negative individuals were invited to an open peanut challenge and evaluated for Ara h 6 and 9 sensitization (ISAC microarray). **Results:** The Ara h 2 IgE-negative group (52.3%, 115/220) was older ($p = 0.04$) and more likely to have a history of pollen allergy than the Ara h 2-positive group ($p < 0.001$). Of the Ara h 2-negative participants, 24.3% were already consuming peanuts and 38.3% were unavailable. Of the 43 who underwent an open peanut challenge, 79% were negative, 14% were positive, and 7% were inconclusive. Those who reacted to peanuts had a higher Ara h 1 IgE than that of the tolerant participants,

and 3 were positive to Ara h 6 IgE, and 2 of those subjects were monosensitized. Ara h 8 may have caused a positive reaction, while Ara h 9 did not. **Conclusions:** Half of the peanut-sensitized individuals in Iceland were not sensitized to the major allergen Ara h 2. Ara h 1, Ara h 3, and Ara h 6 sensitizations resulted in a positive open peanut challenge and they are therefore clinically important for individuals with a peanut allergy in Iceland.

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Introduction

Peanut allergy is one of the most common food allergies, with a prevalence of approximately 1% in the general population, and recent studies have suggested that its prevalence is on the rise [1]. Peanut allergy is more persistent than other food allergies, and resolution is only seen in 20% of cases, unlike many other food allergies that are usually outgrown by the age of 16 years [2–5]. Peanut allergy tends to be more severe than other food allergies

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and is the main cause of food-induced fatalities from anaphylaxis [5–7]. Several risk factors, including the personal history and/or a family history of atopic diseases (asthma, atopic dermatitis, allergic rhinoconjunctivitis, and food allergy), are thought to increase the development of allergy or sensitization to peanuts [8–10].

Diagnosis is usually based on the clinical history along with positive specific serum IgE (sIgE) and/or a positive skin prick test (SPT). However, positive sIgE and SPT only diagnose peanut sensitization, and many subjects with peanut sensitization may not develop a clinical peanut allergy [11, 12]. Recent studies have shown that the prevalence of peanut sensitization is approximately 10%, while that of peanut allergy is approximately 1% [11, 13, 14]. The double-blind placebo-controlled food challenge is the gold standard for food allergy testing but it is time consuming and expensive and can lead to severe allergic reactions. Therefore, clinical cut-off points with a positive predictive value of $\geq 95\%$ have been proposed for sIgE and SPT as a surrogate for the double-blind placebo-controlled food challenge, but these cut-off points have relatively low sensitivity and low negative predictive values [15–18]. About half of those with a true peanut allergy have a peanut IgE (Pn-IgE) value below these cut-off points [17, 19]. Therefore, more accurate laboratory tests are greatly needed to distinguish a true peanut allergy from peanut sensitization alone.

Component-resolved diagnostics is a method to measure IgE antibodies to specific components of allergens, allowing us to measure peanut protein-specific IgE rather than measuring against whole peanut extracts with SPT or sIgE tests. The peanut is composed of many proteins (peanut components), and the immune response may differ from one protein to another [20, 21]; additionally, cross-reactivity between certain peanut proteins and their homologue allergen proteins from other species may explain a positive test [22].

Ara h 1, Ara h 2, Ara h 3, and Ara h 6 are seed storage proteins and they are considered the major peanut allergens due to their association with severe allergic reactions [23, 24]. Ara h 2 and Ara h 6 have higher predictive values for the diagnosis of clinical peanut allergy than the other Ara h proteins, but cosensitization to the 3 major peanut allergens Ara h 1, Ara h 2, and Ara h 3 correlates with more severe reactions [23, 25, 26]. Studies have shown that approximately 80% of Ara h 2-sensitized individuals (≥ 0.35 kUA/L) report allergic symptoms after peanut consumption, and of those who are sensitized to Ara h 2 plus Ara h 1 and/or Ara h 3, ninety-five percent report allergic symptoms after peanut consumption [27]. The

diagnostic value of Ara h 6 has been shown to be equivalent to that of Ara h 2, with a positive predictive value of 95% [26, 28]. Ara h 2 and Ara h 6 are both 2S albumins and have 59% amino acid homology, which causes Ara h 6 to cross-react extensively with Ara h 2 [29]. In rare cases, individuals can be sensitized to Ara h 6 without being sensitized to Ara h 2 [30, 31]. Ara h 8 is a homologue of the major birch pollen allergen Bet v 1 and it is rarely associated with severe allergic reactions. Studies have shown that only 20% of individuals with sensitization to Ara h 8 and without sensitization to Ara h 1, 2, and 3 report allergic symptoms after peanut consumption, and those symptoms are almost exclusively mild [32, 33]. Cross-reactivity between Ara h 8 and Bet v 1 may cause individuals with birch pollen allergy to be wrongly diagnosed with peanut allergy. Ara h 9 is a heat- and enzyme-stable non-specific lipid transfer protein and has cross-reactivity to other lipid transfer proteins, such as peach and hazelnut. Plant fruit allergy in the Mediterranean area is mainly caused by lipid transfer protein [34].

Component-resolved diagnostics results in peanut allergy have been shown to have geographical heterogeneity and are possibly country specific, which is probably related to differences in pollen counts and genetic backgrounds [35]. The purpose of this study is to investigate the characteristics of peanut-sensitized individuals in Iceland and to investigate whether Icelandic individuals who are sensitized to peanuts but negative to Ara h 2 can consume peanuts without risking their safety.

Study Participants and Materials and Methods

Study Population and Data Collection

Data were collected for individuals whose serum samples had tested positive by ImmunoCAP for Pn-IgE (≥ 0.35 kUA/L) during the period from November 2011 to December 2013 in the Department of Immunology of Landspítali University Hospital (LUH), Iceland, and who still had excess serum stored at the Department of Immunology. Information regarding the individual's age when the serum sample was drawn, gender, and personal and family history of atopy (asthma, allergic rhinitis, atopic eczema, and food allergy), and results from SPT were obtained from clinical records at LUH, Akureyri Hospital, and Egilsstaðir Hospital and from private allergy clinics. Those who were negative to Ara h 2 IgE were interviewed by telephone and, if not consuming peanuts, invited for an open peanut challenge at LUH.

All serum samples were tested by ImmunoCAP for Ara h 1, 2, and 3. Serum samples that tested negative for Ara h 2 IgE (<0.35 kUA/L) were further tested for Ara h 8 IgE and Bet v 1 IgE. Serum samples from all the participants who underwent the open peanut challenge were analyzed for sensitivity to Ara h 6 and Ara h 9.

Table 1. Demographic data, sensitivity profile, and allergic diseases for 220 individuals with positive peanut-IgE, grouped according to age

Variable	All (n = 220)	0–6 years (n = 85)	6–18 years (n = 92)	>18 years (n = 43)	p value
Males	66.8	76.5	67.4	46.5	0.003
Pn-IgE, kUA/L	17.25 (13.3–21.2)	14.0 (8.6–19.5)	23.9 (16.6–31.2)	9.4 (2.5–16.3)	0.014
Ara h 2-IgE, kUA/L	12.1 (8.6–15.6)	9.1 (4.2–14.0)	18.5 (12.0–25.0)	4.4 (0.03–8.9)	0.005
Ara h 1-IgE, kUA/L	6.78 (4.2–9.4)	3.9 (1.1–6.8)	11.7 (6.2–17.2)	1.94 (0.4–3.5)	0.006
Ara h 3-IgE, kUA/L	3.45 (1.8–5.0)	1.3 (0.3–2.2)	6.3 (2.7–10.0)	1.6 (0.03–3.3)	0.011
Ara h 2 IgE positivity	47.7	49.4	57.6	23.3	<0.001
Ara h 1 IgE positivity	35.0	34.1	40.2	25.5	0.246
Ara h 3 IgE positivity	28.2	24.7	34.8	20.9	0.165
Eczema	75.5	89.4	73.9	51.2	<0.001
Asthma	65.5	62.4	72.8	55.8	0.114
Rhinoconjunctivitis	65.5	38.8	82.6	83.7	<0.001
Other food allergy	85.5	90.6	79.3	88.4	0.088
Pollen allergy	52.3	20.0	70.7	76.7	<0.001
Pet allergy	56.8	36.5	71.7	65.1	<0.001

Values are presented as means (95% CI) or percents. Significant *p* values <0.05 were calculated using the *t* test and Fisher's exact test. *p* < 0.05 was considered statistically significant.

Serum-Specific IgE Testing

Serum-specific IgE antibodies to whole peanut, recombinant allergen components Ara h 1, 2, 3, and 8, and Bet v 1 were analyzed using the ImmunoCAP FEIA system (Thermo Fisher Scientific, Uppsala, Sweden) (results were expressed in kUA/L). The Ara h 6- and Ara h 9-specific IgE were measured by ImmunoCAP ISAC microarray (Thermo Fisher, Denmark), and the results were expressed in ISAC standardized units for specific IgE (ISU-E), where ISU-E ≥0.3 was considered positive.

Peanut Challenges

Ara h 2 IgE-negative individuals were invited to participate in an open peanut challenge at LUH during the summer of 2015. The iFAAM (Integrated Approaches to Food Allergen and Allergy Risk Management) protocol [36, 37] was used; it consisted of 7-dose step increases whereby 3, 10, 30, 100, 300, 1,000, and 3,000 mg of peanut proteins were given orally every 20 min. After the challenge, the individual was observed for 2 h. If any allergy symptoms, such as urticaria, angioedema, gastrointestinal symptoms, wheezing, stridor, rhinoconjunctivitis, or a drop in blood pressure, occurred during or 24 h after the test, the challenge was scored as positive and the appropriate medical treatment was administered.

Ethics

Ethics approval was obtained from the Landspítali Ethics Committee (5/2014), the National Bioethics Committee (VSN-14-061 and VSN-15-082), and the Data Protection Authority (reference No. 2013111365 hkg/-). Informed consent forms were signed by the participants or their parents prior to participation.

Statistical Analysis

Statistical analysis was performed using the R system. Comparison of means was done using an ANOVA, and comparison of

categorical data was done using Fisher's exact test. A receiver operating characteristic curve was used to estimate how the value of Pn-IgE predicts positive Ara h 1, 2, and 3 IgE. The Pn-IgE value representing the maximum area under the curve was chosen as the optimal predictive value. *p* < 0.05 was considered statistically significant.

Results

Study Population

The main demographic measures are shown in Table 1. Among the 220 enrolled individuals, the mean age was 12 years (median 8.15), and 66.8% were male. The mean Pn-IgE level was 17.25 kUA/L (95% CI 13.28–21.22) in the whole group. By dividing the population into 3 age groups (0–6 years, 6–18 years, and above 18 years of age), the age group of 6–18 years scored highest in peanut and Ara h 1–3 IgE measurements. They also scored highest for asthma and allergic rhinitis, while the youngest age group scored higher for eczema and other food allergies.

The outcome from the recombinant allergen components Ara h 1–3 measurements are outlined in Table 1 and Figure 1. In total, 105 individuals were positive for Ara h 2 IgE (47.7%), 77 were positive for Ara h 1 IgE (35.0%), and 61 were positive for Ara h 3 IgE (27.7%). A positive history of atopic dermatitis was recorded for 166 individuals (75.5%); 144 (65.5%) had a history of asthma

Table 2. Serum levels of peanut-IgE and Ara h 1, Ara h 2, and Ara h 3 IgEs in 43 participants with records on their history of anaphylaxis

Variable	History of anaphylaxis (n = 27)	No history of anaphylaxis (n = 16)	p value
Pn-IgE, kUA/L	46.38 (29.50–63.27)	6.68 (3.34–16.70)	0.0002
Ara h 2 IgE, kUA/L	34.43 (19.82–49.04)	4.02 (3.66–11.70)	0.0005
Ara h 1 IgE, kUA/L	20.72 (8.62–32.83)	0.28 (0.06–0.63)	0.0018
Ara h 3 IgE, kUA/L	13.40 (4.33–22.46)	0.11 (0.08–0.14)	0.0057

Values are presented as means (95% CI) Significant p values <0.05 were calculated using the *t* test.

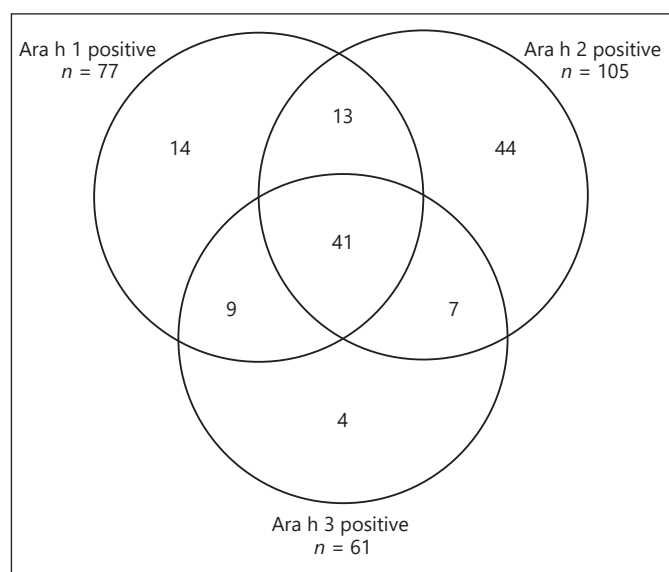


Fig. 1. Venn diagram showing Ara h 1, Ara h 2, and Ara h 3 IgE positivity in 220 serum samples that were positive for peanut IgE by ImmunoCAP.

and 144 (65.5%) had allergic rhinitis. The values of Pn-IgE and Ara h 1, 2, and 3 IgE did not differ between the individuals with a history of atopic dermatitis, asthma, or allergic rhinitis and those without.

Of the 43 individuals who had information recorded on whether they had anaphylaxis or not after consuming peanuts, 27 (62.8%) had a history of anaphylaxis, whereas 16 (37.2%) did not. As shown in Table 2, those who had a history of anaphylaxis to peanuts had significantly higher Pn-IgE values as well as Ara h 1, 2, and 3 IgE values. Of the 27 individuals with a history of anaphylaxis, 5 individuals (18.5%) were Ara h 2 negative.

Using the receiver operating characteristic graphical plot, the optimal value of Pn-IgE was estimated, predict-

ing positive values of Ara h 1, 2, and 3 IgE, which showed that 79.3% of Ara h 2 IgE-positive individuals had a Pn-IgE value ≥ 4.2 kUA/L, 85.5% of Ara h 1 IgE-positive individuals had a Pn-IgE value ≥ 12.2 kUA/L, and 89.1% of Ara h 3 IgE-positive individuals had a Pn-IgE value ≥ 8.5 kUA/L. The levels of Ara h 8 and Bet v 1 showed no significant correlation with Pn-IgE values.

All 3 major peanut components (Ara h 1, 2, and 3) correlated well with Pn-IgE ($p_s = 0.83$ – 0.92 and $p < 0.01$).

Ara h 2 IgE-Positive versus Ara h 2 IgE-Negative Participants

Of the 220 individuals who were positive for Pn-IgE, 115 (52.3%) were negative for Ara h 2 IgE. Table 3 shows the main characteristics of the individuals grouped based on whether they were positive for Ara h 2 IgE or not. Those who were negative for Ara h 2 IgE were older, they were more likely to have allergic rhinoconjunctivitis, a pollen allergy, a family history of atopy, and a much lower Pn-IgE level, and they were more likely to have a negative peanut SPT than those who tested positive for Ara h 2 IgE. In addition, those who tested positive for Ara h 2 IgE were more likely to be positive for Ara h 1 and 3 IgE and have a history of asthma compared to Ara h 2-negative individuals.

Open Peanut Challenges

All of the 115 Ara h 2 IgE-negative individuals were invited to participate in an open peanut challenge, as seen in Figure 2. Forty-four individuals rejected or were not available to participate in the open peanut challenge or had a medical condition. Twenty-eight individuals were already consuming peanuts. Nine had previously undergone an open peanut challenge; 6 of them were negative and 3 were positive. In total, 34 individuals underwent an open 7-step peanut challenge, with 28 negative challenges and 3 positive challenges, and 3 remained inconclusive

Table 3. Demographic data, sensitivity profile, and allergic diseases for 220 individuals with positive peanut IgE, grouped according to whether they had positive Ara h 2 IgE or not

Variable	Ara h 2 IgE positive (n = 105)	Ara h 2 IgE negative (n = 115)	p value
Age, years			0.0395
Mean	9.3	14.6	
Median (range)	7.5 (0.3–69.4)	10.3 (0.5–76.2)	
Male	66 (62.9)	81 (70.4)	0.2537
History of eczema	84 (80.0)	82 (71.3)	0.1589
History of asthma	76 (72.4)	68 (59.1)	0.0470
History of allergic rhinoconjunctivitis	62 (59.0)	83 (72.2)	0.0467
Self-reported history of other food allergies	88 (83.8)	100 (87.0)	0.5682
History of pollen allergy	42 (40.0)	73 (63.5)	0.0007
History of pet allergy	60 (57.1)	65 (56.5)	1
Family history of atopy ^a	69 (65.7)	88 (85.7)	0.0009
Positive peanut SPT ^b	82 (95.3)	73 (78.5)	0.0027
Pn-IgE, kUA/L			<0.0001
Mean	32.1	3.7	
Median	11.5	1.55	
Ara h 1 IgE positive	54 (51.4)	23 (20.0)	<0.0001
Ara h 3 IgE positive	48 (45.7)	13 (11.3)	<0.0001

Values are presented as numbers (%) unless otherwise stated. Significant *p* values <0.05 were calculated using the *t* test and Fisher's exact test. ^a The number of individuals with available information on their family history of atopy was 77 in the Ara h 2 IgE-positive group and 105 in the Ara h 2 IgE-negative group. ^b The number of individuals with available information on the peanut SPT outcome was 86 in the Ara h 2 IgE-positive group and 83 in the Ara h 2 IgE-negative group.

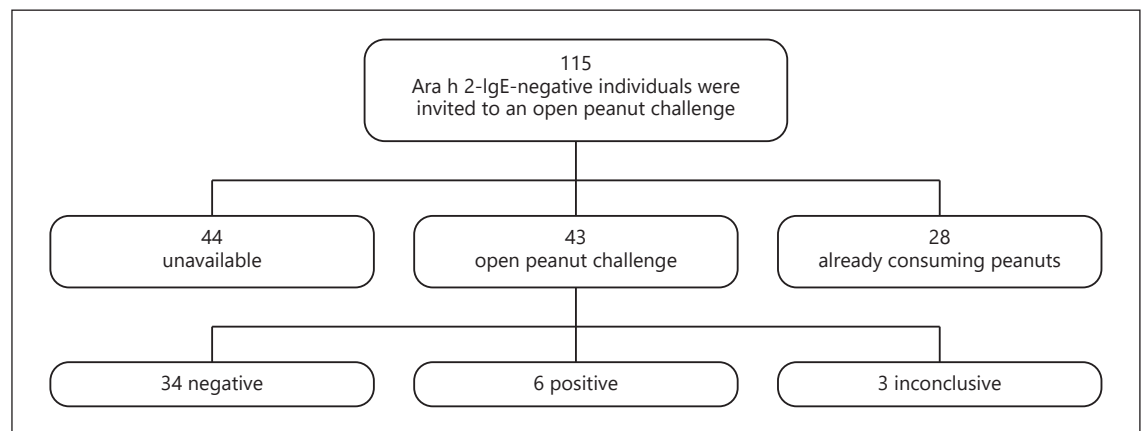


Fig. 2. Algorithm outlining results from the open peanut challenge study for 115 individuals with negative Ara h 2 on ImmnoCAP.

where the child refused to finish the peanuts required. Thus, of the 71 individuals who participated in the challenge study, 28 were already consuming peanuts and not challenged and 34 had a negative challenge, which means that 87.3% were able to consume peanuts (62/71); their characteristics are shown in Table 4. Sixty-eight individu-

als underwent an open peanut challenge and had a conclusive response, and 6 of them had a positive challenge (8.8%).

The prevalence of a clinical peanut allergy in Icelandic Ara h 2-negative individuals is 8.8% (6/68; 43 challenges plus 25 consuming).

Table 4. Demographic data, history of allergic diseases, and sensitization profile of 68 Ara h 2 IgE-negative individuals, grouped according to whether they had a positive challenge or a negative challenge or were already eating peanuts

Variables	Positive challenge (n = 6)	Negative challenge or eating peanuts (n = 62)	p value
Age, years			0.097
Mean	4.9	45	
Median	3.5	11.5	
Male	4 (66.7)	25 (72.6)	1
History of eczema	6 (100)	44 (71.0)	0.183
History of asthma	4 (66.7)	35 (56.5)	1
History of allergic rhinoconjunctivitis	4 (66.7)	49 (75.4)	0.607
History of other food allergies	4 (66.7)	52 (83.9)	0.285
History of pollen allergy	2 (33.3)	46 (74.2)	0.057
History of pet allergy	2 (33.3)	37 (59.7)	0.390
Family history of atopy ^a	6 (100)	48 (78.7)	0.624
PS-IgE, kUA/L			0.056
Mean	8.9	3.0	
Median	7.2	1.4	
Ara h 1 IgE, kUA/L			<0.001
Mean	4.4	0.26	
Median	3.5	0.1	
Ara h 3 IgE, kUA/L			0.126
Mean	0.91	0.30	
Median	0.5	0.1	
Ara h 8 IgE, kUA/L			0.631
Mean	0.35	1.2	
Median	0.1	0.1	
Bet v1 IgE, kUA/L			0.892
Mean	3.2	3.9	
Median	0.1	0.1	

Values are presented as numbers (%) unless otherwise stated. Significant *p* values <0.05 were calculated using the *t* test and Fisher's exact test. ^a The number of individuals with available information on their family history of atopy was 61 in the negative challenge/peanut-eating group.

Clinical Significance of Recombinant Allergen Components Findings

Symptomatic Peanut-Sensitive, Ara h 2-Negative Individuals

Ara h 1 IgE was significantly higher in those with a challenge-proven peanut allergy compared to the tolerant participants. In our population of Ara h 2 IgE-negative individuals, 4 had a Pn-IgE value ≥ 15 kUA/L, which has been suggested as the cut-off value for symptomatic peanut allergy [17], and 2 of them did not show any clinical reactivity.

The 6 peanut challenge-positive individuals showed variable combinations of sensitization (Table 5). Mono-sensitivity was seen for Ara h 6 in 2 positive individuals. One was a 5.8-year-old child with an Ara h 6 IgE level of 17 ISU-E who, upon challenge, developed gastrointestinal

symptoms, asthma, and increased eczema. The other child was a 10-year-old with an Ara h 6 IgE level of 1.3 ISU-E who developed itching and urticaria. The sensitivity patterns for positive and inconclusive challenges are shown in Table 5. One individual who developed urticaria during the peanut challenge was positive to Ara h 1 and 3 on ImmunoCAP (12.1 and 1.12 kUA/L, respectively) but showed no sensitivity to any of the Ara h proteins on ISAC.

Tolerant Peanut-Sensitive, Ara h 2-Negative Individuals

Four of the 62 individuals who were able to consume peanuts had both positive Ara h 1 and 3 IgE (6.5%), 6 had only Ara h 1 IgE (9.7%), and 2 had only positive Ara h 3 IgE (3.2%). Of the 34 who had a negative challenge, 32 had enough serum sample for ISAC analyses of their sen-

Table 5. Peanut-sensitive and Ara h 2-negative individuals who underwent an open peanut challenge

Variable	Individuals with a positive open peanut challenge						Individuals with an inconclusive challenge			Negative challenge ^c (total: <i>n</i> = 32)
	1	2	3	4	5	6	1	2	3	
Age at sample drawing, years	10.7	4.2	2.5	8.7	0.7	2.7	1.7	2.5	14.8	15.6
Age at challenge, years	10.7	5.8	3.0	11.5	3.4	5.5	4.0	5.0	16.0	17.2
Pn-IgE, kU _A /L	0.38	5.71	8.67	3.31	18.10	17.00	1.95	1.95	2.88	3.6
Ara h 1 IgE, kU _A /L			6.69	0.30	12.10	7.19	1.36			6 (10)
Ara h 2 IgE, kU _A /L				0.33						1 (3)
Ara h 3 IgE, kU _A /L			0.72	0.27	1.12	3.13				2 (6)
Ara h 6 IgE, ISU-E	1.30	17.00	2.60				1.70	7.40		3 (9)
Ara h 8 IgE, kU _A /L				18.70					14.1	4 (13)
Ara h 9, ISU-E										2 (6)
Bet v 1 IgE, kU _A /L				1.58					35.9	8 (25)
Bet v 2, ISU-E				5.69						10 (31)
Bet v 4, ISU-E										1 (3)
Allergic reaction	I/U	GI/Ez/A	? ^a	I/U	U	I/U/GI	^b	^c	^d	
Other sensitizations		Oa/L/D/CS/		Om/Oa/aL/ bL/C/W/B/ P/G/M/C/A	ISAC NEG		W	Om	G/B/ D/C	

ImmunoCAP measurements of IgE ≥ 0.35 kU_A/L and ISAC IgE values ≥ 0.3 ISU-E were considered positive. Empty fields indicate negative values. I, itchy mouth; U, urticaria; GI, gastrointestinal symptoms; Ez, appearance/worsening of eczema; A, appearance/worsening of asthma; Oa, ovalbumin; Om, ovomucoid; aL, α -lactalbumin; bL, β -lactoglobulin; W, walnut; S, shrimp; D, dog; C, cat; M, mugwort; G, grass; B, birch (Bet v 1); P, plane tree; A, alternaria. ^a There was no recorded data on this individual's peanut challenge, which was performed in Denmark after blood had been obtained in Iceland. ^b Spit out 1–2 doses in multiple attempts. ^c Denial after 3 doses. ^d Denial after 2 doses. ^e Only the number (%) of positive components is shown.

sitivity to Ara h 6 and Ara h 9. Three had Ara h 6 IgE, and 2 of them were monosensitized and 2 had Ara h 9. Thirteen of the 32 (40.6%) subjects with a negative challenge did not show any sensitivity on the ISAC microarray. Ten (29.4%) of the 34 individuals with a negative challenge were Bet v 1 IgE and/or Ara h 8 IgE positive, and 3 of them reported a little itch in the mouth that disappeared during or after the challenge.

Bet v 1 Sensitivity within Ara h 2-Negative Individuals

Of the 115 individuals who tested positive for Pn-IgE and negative for Ara h 2 IgE, 26 (22.6%) were positive for Bet v 1 IgE; of those, 17 (65.4%) were also positive for Ara h 8 IgE. Those who tested positive for Bet v 1 IgE were older and more likely to be females and have a history of pollen and pet allergy than those who tested negative. There was no difference between the 2 groups regarding positive Ara h 1 IgE and Ara h 3 IgE. Those who were positive for Bet v 1 IgE were more likely to be positive for Ara h 8 IgE.

Of the 115 individuals who were positive for Pn-IgE and negative for Ara h 2 IgE, 18 (15.7%) were positive for Ara h 8 IgE and all but 1 were positive for Bet v 1 IgE (94.4%).

Discussion

In this study of 220 individuals with IgE sensitivity to peanuts, 47.7% were positive for Ara h 2, the most potent of the peanut allergens. This is similar to the results of another Scandinavian study where 47% of those individuals who were sensitized to peanuts were sensitized to Ara h 2 IgE. [38]. Individuals who tested positive for Ara h 2 IgE were also more likely to be positive for Ara h 1 and/or Ara h 3; this cosensitization has been associated with reports of peanut allergy in more than 95% of cases [27]. All 3 major peanut components (Ara h 1, 2 and 3) correlated significantly with Pn-IgE. Those who tested negative for Ara h 2 IgE were older and more likely to have a history of pollen allergy, had a lower Pn-IgE level, and

were more likely to have a negative peanut SPT than those who tested positive for Ara h 2 IgE, which may indicate a harmless sensitization to peanuts due to cross-reactivity.

Peanut allergy has been reported to be more common in males and tends to develop at an early age [39]. In this study, 66.8% were males; however, based on the Bet v 1 sensitivity data, the negative participants were more likely to be males, whereas those positive for Bet v 1 were equally likely to be male or female. A quarter of the participants who tested negative for Ara h 2 IgE were positive for Bet v 1 IgE. The Bet v 1 IgE-positive participants were older, which could be explained by the fact that birch pollen allergy and pollen food syndrome develop later in life than peanut allergy [40, 41]. Those who tested positive for Bet v 1 IgE were also significantly more likely to be allergic to airborne allergens and they were more likely to be sensitized to Ara h 8 IgE, which further supports the possibility of a birch pollen allergy rather than a peanut allergy. A history of allergic rhinitis did not predict higher Ara h 2 IgE levels, but individuals with allergic rhinitis had a significantly higher Pn-IgE, which could indicate cross-reactivity when whole peanut extracts are measured instead of the major peanut allergens.

Our study population with peanut sensitivity was highly atopic. According to the ISAAC study of 773 Icelandic 10- to 11-year-old children in 2008, the 12-month prevalence was 27% for atopic dermatitis, 8.9% for asthma, and 11.5% for allergic rhinoconjunctivitis [42]. These numbers are much lower than those in our study population, where 75.5% had a history of atopic dermatitis, 65.5% had a history of asthma, and 65.5% had allergic rhinitis, which underscores the atopic bias in this patient population. However, in this study, the subjects were not questioned on asthma, which may partly explain this difference.

Forty-three individuals had information recorded on whether they had anaphylaxis or not after consuming peanuts; of those subjects, 27 had a positive history of anaphylaxis to peanuts and 5 (18.5%) were Ara h 2 negative. Those who had a history of anaphylaxis due to peanuts had much higher Pn-IgE and Ara h 2 IgE levels, which is in line with other studies showing that increasing IgE concentrations are associated with more severe allergic reactions [27, 43].

Only 12 individuals of the 220 had previously already undergone a peanut challenge, and 9 of them were Ara h 2 negative. The peanut challenge is recommended for all individuals who have a negative Ara h 2 IgE and are not consuming peanuts [35]. In this study, there were 105

(91.3%) Ara h 2 IgE-negative individuals who had not undergone a peanut challenge. Since many peanut-positive and Ara h 2-negative individuals may tolerate eating peanuts, this finding indicates that food challenges are underused in our community, which may possibly lead to the overdiagnosis of peanut allergy. According to Finnish research, Ara h 6 is the best marker for moderate to severe peanut allergy, with 95% sensitivity and specificity and a cut-off level of 0.8 ISU [26]. With the addition of Ara h 2 measurements, Ara h 6 reaches 100% prediction accuracy. The current study focuses on the Ara h 2-negative population, revealing the clinical characteristics and component-resolved diagnostics results characterizing clinical peanut allergy in that group. The prevalence of clinical peanut allergy in Icelandic Ara h 2-negative individuals is 8.8% (6/68; 43 challenges plus 25 consuming). Of the Ara h 2-negative individuals undergoing the peanut challenge, the ones most likely to react to peanuts tend to be younger and have not yet developed a pollen allergy (median age: 3.5 vs. 11.5 years, $p = 0.097$); they also tend to have higher Pn-IgE (median: 7.2 vs. 1.4 kUA/L, $p = 0.056$) and Ara h 1 (median: 3.6 vs. 0.1 kUA/L, $p < 0.001$) levels. When evaluating peanut-sensitive individuals for a peanut challenge, this result should be kept in mind. This study shows that peanut allergy in Iceland can be caused by monosensitization to Ara h 6 and 8, which has been demonstrated before, as well as to Ara h 1 and 3 [44]. A cut-off level of Pn-IgE of more than 15 kUA/L has been proposed to predict clinical reactivity in 95% of allergic patients [17]. If this cut-off level is applied to our study population of Ara h 2 IgE-negative individuals, 4 individuals would have been diagnosed with peanut allergy; however, 2 out of those children did not show any clinical reactivity in the peanut challenge. This suggests that the Icelandic population differs somewhat from other populations when considering cut-off values for Pn-IgE, and the evaluation of Ara h 1, 2, 3 and 6 sensitivity may help in selecting patients for peanut challenges.

Considering the individuals with a positive peanut challenge, they all had sensitization to one of the peanut components other than Ara h 2. Two individuals were sensitized to Ara h 1 and 3 IgE, one individual was sensitized to Ara h 1, 3, and 6 IgE, 2 individuals were monosensitized to Ara h 6, and 1 individual was monosensitized to Ara h 8 IgE. All of these sensitizations have been shown to cause allergic reactions [32, 43, 45]. Ara h 6 monosensitization has been found on rare occasions in other studies, but in our study 2 individuals out of 6 peanut allergic and Ara h 2-negative individuals were mono-

sensitized to Ara h 6, which could indicate that Ara h 6 monosensitization is common in the Icelandic peanut allergic population [30]. In contrast to what has been shown in Spain, sensitization to Ara h 9 was not shown to be clinically important in Iceland, although the cases may be too few to generalize [46].

One weakness of this study is that 1.5–3.5 years elapsed between the blood drawing and performance of the peanut challenge. Some of the children were 2–3 years old at the time of the blood drawing, and it is possible that the sensitivity profile may have changed. Since there was not an approval for a new blood sample at the time of the challenge, it is uncertain if they had become Ara h 2 sensitized. Two of the 3 children who had a positive peanut challenge were under 3 years of age at the time of the blood drawing, showing a negative Ara h 2 IgE (0.7 and 2.7 years of age).

A cut-off level for Ara h 2 cannot be proposed since Ara h 2-positive individuals were not included in this study. However, in that group, there may be individuals who can tolerate peanuts.

Conclusion

In our Icelandic population of peanut-sensitized individuals, half were negative to Ara h 2 IgE, which has been considered the best predictor for peanut allergy. This study has shown that various combinations of Ara h 1, 3, 6, and 8 IgE and monosensitization to Ara h 6 and, possibly, Ara h 8 IgE, may result in an allergic reaction upon peanut challenge, which stresses the importance of the other components. Measurement of Ara h 1, 3, and 6 in addition to Ara h 2 is helpful in evaluating peanut sensitivity but, in the end, a food challenge may be the only way to discriminate peanut sensitivity versus peanut allergy.

Disclosure Statement

The authors of this paper declare no conflict of interests aside from support from Thermo Fisher in the way of providing antigens for the ImmunoCAP and microarray measurements for component-resolved diagnostics. This study was completely researcher initiated, and Thermo Fisher did not influence the study design, research question, or results.

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