

ORIGINAL ARTICLE

Clinical Allergy

Component-resolved diagnostics demonstrates that most peanut-allergic individuals could potentially introduce tree nuts to their diet

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Summary

Background: Nut allergy varies from pollen cross-allergy, to primary severe allergy with life-threatening symptoms. The screening of IgE antibodies to a wide spectrum of allergens, including species-specific and cross-reactive allergens, is made possible via microarray analysis.

Objective: We sought to study the association of variable IgE sensitization profiles to clinical response in peanut-challenged children and adolescents in a birch-endemic region. In addition, we studied the avoidance of tree nuts and species-specific sensitizations.

Methods: We studied 102 peanut-sensitized patients who underwent a double-blind placebo-controlled challenge to peanut. We analysed ISAC ImmunoCAP microarray to 112 allergens, singleplex ImmunoCAPs for hazelnut Cor a 14 and cashew Ana o 3, and performed skin prick tests to peanut, tree nuts and sesame seed. We surveyed avoidance diets with a questionnaire.

Results: Sensitization to PR-10 proteins was frequent (Bet v 1 90%), but equally high in the challenge negatives and positives. IgE to Ara h 2 and Ara h 6 discriminated peanut allergic (n = 69) and tolerant (n = 33) the best. Avoidance of tree nuts was common (52% to 96%), but only 6% to 44% presented species-specific sensitizations to tree nuts, so a great number could potentially introduce these species into their diet.

Conclusions and clinical relevance: PR-10-sensitizations were frequent and strong regardless of peanut allergy status. Component-resolved diagnostics can be employed to demonstrate to patients that sensitization to seed storage proteins of tree nuts is uncommon. Several tree nuts could potentially be reintroduced to the diet.

KEYWORDS

avoidance diet, cross-reactivity, immuno solid-phase allergen chip, peanut allergens, seed storage protein

1 | INTRODUCTION

Nut allergy is a heterogeneous disease including mild pollen-induced cross-allergy, as well as primary allergy with life-threatening anaphylaxis.

Double-blind placebo-controlled challenge is the reference standard in food allergy diagnostics, and studies on other diagnostic tests should be based on this.¹ By conducting a challenge, the threshold for reactivity and severity of symptoms can be determined. The threshold dose for 1% of patients to react is as low as 0.2 mg of peanut protein.²

Cross-reactivity between peanut and tree nuts is common when examined with whole extract-based tests,³ and avoidance of all species is commonly advised in case of suspicion of allergy to peanut or tree nut.⁴ The growing possibilities of examining species-specific stable allergen components increase knowledge of the diagnostic performance of the tests and of overall sensitization profiles of individuals.

As for Northern America and Europe,^{5,6} peanut allergy in the Finnish population can be accurately predicted with positivity to Ara h 2 and Ara h 6.⁷ These allergens are 2S albumins, which show good diagnostic performance also in hazelnut⁸ and cashew⁹ allergies. Other seed storage proteins in peanut are vicilin Ara h 1 (7S globulin) and legumin Ara h 3 (11S globulin).¹⁰ Simultaneous sensitization to several seed storage proteins indicates more severe allergy.¹¹ Sensitization to labile PR-10 protein Ara h 8 associates generally with asymptomatic sensitization or only mild symptoms, commonly oral allergy syndrome.¹² Ara h 9 sensitization is most prevalent in the Mediterranean¹³ and may associate with severe symptoms, but less frequently than Ara h 2 and Ara h 6.¹⁴ Sensitization rate to birch pollen increases with age until young adulthood and is as high as 25% in northern Europe.¹⁵ Bet v 1 drives sensitizations to other PR-10 proteins and is a major cause of cross-reactivity.¹⁶ This easily leads to suspicion of severe nut allergy and prescription of precautionary avoidance diets.

ImmunoCAP ISAC microarray of 112 allergens includes six peanut allergens: Ara h 1, 2, 3, 6, 8 and 9. Other 2S albumins, which can be cross-reactive with peanut Ara h 2 and Ara h 6,¹⁷ originate from Brazil nut (Ber e 1), walnut (Jug r 1), sesame seed (Ses i 1) and buckwheat (Fag e 2). Hazelnut and cashew 2S albumins Cor a 14 and Ana o 3 can be examined in singleplex tests. On ISAC, vicilins (Ara h 1) comprise walnut Jug r 2 and soya bean Gly m 5, and legumins (Ara h 3) comprise cashew Ana o 2, hazelnut Cor a 9 and soya bean Gly m 6. PR-10 proteins potentially cross-reactive with peanut Ara h 8 originate from birch, alder and hazel tree pollens, apple, celery, kiwi fruit, peach, hazelnut and soya bean. Ara h 9-cross-reactive lipid transfer proteins are derived from walnut, hazelnut, peach, apple, wheat and several pollens.

Our aim was to study the performance of ImmunoCAP ISAC 112 in peanut-allergic and peanut-sensitized, but tolerant, children and adolescents in an area with very high birch pollen exposure. In addition, we studied the avoidance of tree nuts and sesame seed, and

how the component-specific diagnostics prevent unnecessary avoidance diets.

2 | METHODS

We enrolled 102 children and adolescents in a study on peanut allergy in the Helsinki University Skin and Allergy Hospital. The clinical protocol for patient inclusion and double-blind peanut challenges are described in Kukkonen et al.⁷ Briefly, these children and adolescents were referred to a specialist care centre for suspicion of peanut allergy. Eligible patients had a strong sensitization to peanut (skin prick test ≥ 8 mm or peanut-specific IgE ≥ 15 kU/L) or a weaker sensitization, but lifelong avoidance of peanuts. Patients with poor asthma control (FEV1 < 80%) or any major chronic illness were excluded.

Patients underwent double-blind placebo-controlled challenges with ground peanut. As a matrix, we used a combination of crushed banana chips (Grefinn, Söderkulla, Finland) and blueberry powder (Kiantama, Suomussalmi, Finland), mixed with either Yosa oat yogurt (Bioferme, Piispanristi, Finland) or chocolate pudding (Arla Ingman, Söderkulla, Finland). The matrix itself served as a placebo. The challenge ended only when the patient experienced objective symptoms or when the highest dose of the challenge was achieved. Objective symptoms included diarrhoea, vomiting, conjunctivitis, urticaria, lip and throat swelling, bronchoconstriction, wheezing and angioedema. We assessed the challenge results as positive and negative, and based on severity. The severity was scored according to symptoms and the eliciting allergen dose. Score 1-5 indicated mild, 6-13 moderate and 14-23 severe reactions.^{7,18}

We examined avoidance diets with a questionnaire including peanut, hazelnut, almond, cashew, pistachio, walnut, pecan, Brazil nut, macadamia, coconut and sesame seed. Skin prick tests were conducted for the same species. For the skin tests, raw ingredients were ground and mixed with 0.9% saline. The skin of volar forearm was pricked with a single-headed lancet using a prick-prick method. Histamine hydrochloride (10 mg/mL) and saline were used as positive and negative controls. After 15 minutes, the weal sizes were measured and at least 3-mm weal sizes were documented as millimetres. We considered ≥ 3 mm weal positive.

We conducted ImmunoCAP ISAC microarray (Phadia AB; Uppsala, Sweden) analyses to 112 allergen components including Ara h 1, 2, 3, 6, 8 and 9 from peanut. In addition, we examined Cor a 14 and Ana o 3 with singleplex ImmunoCAPs. In species-specific sensitization analyses, we used Ana o 2 (ISAC) and 3 (CAP) for cashew and pistachio, Cor a 8, 9 (ISAC) and 14 (CAP) for hazelnut, Jug r 1, 2 and 3 (ISAC) for walnut and pecan, Ber e 1 (ISAC) for Brazil nut and Ses i 1 (ISAC) for sesame seed.

The study followed the principals of the Helsinki declaration. The ethics committee at the Helsinki University Hospital (ethics approval is 326/13/03/03/2010) approved the study protocol. One of the parents and the patient provided informed written consent. The study was registered at Clinicaltrials.gov (NCT01502878).

2.1 | Statistical analysis

Random forest method was used to analyse the importance of allergens in predicting the challenge result. Mann-Whitney *U* test was used to compare individual allergens in ImmunoCAP ISAC and singleplex ImmunoCAPs. In multiple pairwise comparisons, we considered a *P* value of less than .0004 to be significant. This

TABLE 1 Baseline characteristics of peanut-challenged patients

	Negative n = 33	Positive n = 69	<i>P</i> value
Gender, female n (%)	16 (49)	27 (39)	.40
Age, y (median, range)	10 (6-18)	8 (6-18)	.03
Atopic eczema, previous or current, n (%)	18 (55)	50 (72)	.12
Asthma, n (%)	16 (48)	38 (55)	.53
Other food allergy than nuts, n (%)	26 (79)	29 (42)	.001
Sensitization to birch pollen, n (%) ^a	30 (91)	63 (91)	1
Total serum IgE, kU/L (median, range)	508 (70-5630)	583 (89-11 180)	.51
Blood eosinophil count, 10 ⁹ /L (median, range)	0.27 (0.09-1.07)	0.48 (0.02-1.85)	.014

^aSkin prick test ≥ 3 mm or specific IgE ≥ 0.35 kU/L.

Statistical significance is calculated with Fisher exact or Mann-Whitney *U* test. Significant *P* < .05 is in bold.

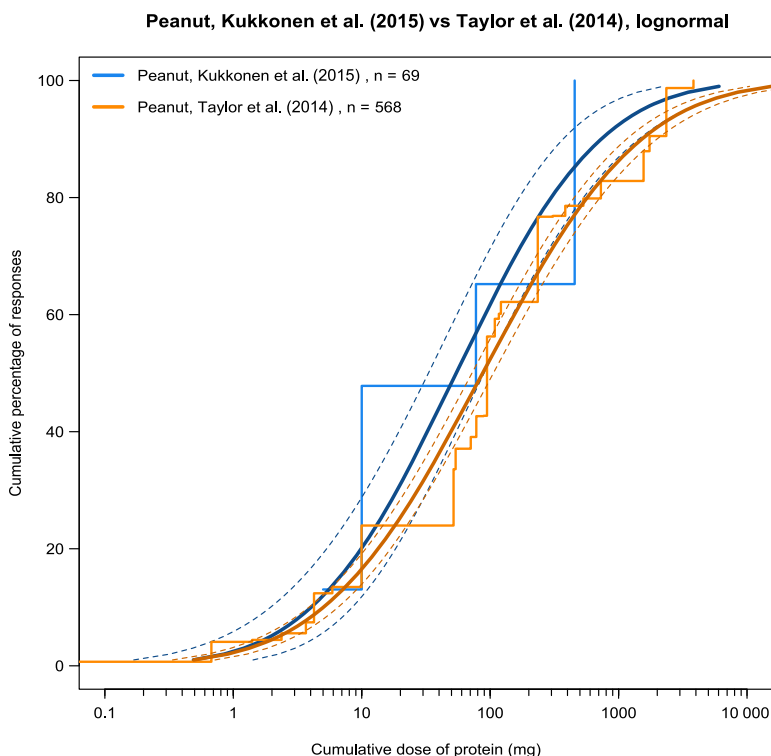
was based on Bonferroni method as the comparisons included a total of 114 allergens in ImmunoCAP and in ImmunoCAP ISAC. In other analyses, a *P* value below .05 was considered to be significant.

Spearman correlation was used to analyse the correlation of IgE level, challenge threshold dose and skin prick weal size. We considered correlation coefficients 0.20-0.39 weak, 0.40-0.59 moderate and 0.60-0.79 strong. Fisher's exact test was used to analyse dichotomous variables.

For threshold distribution curve, individual discrete and cumulative no observed adverse effect levels (NOAELs) and lowest observed adverse effect levels (LOAELs) were established in all patients for objective symptoms. The discrete and cumulative individual NOAELs and LOAELs were analysed with interval-censoring survival analysis (ICSA) and fitted to population threshold distribution curves (TDCs) from which eliciting doses (EDs) could be determined. The ED05, ED10 and ED50, or the eliciting doses respectively predicted to provoke reactions in 5%, 10% and 50% of the population, were estimated using the lognormal, log-logistic and Weibull parametric models.¹⁹

The detection limit for ImmunoCAP ISAC was 0.3 ISU-E and this was also defined as the sensitization cut-off. Detection limit for ImmunoCAP was 0.1 kU/L, and sensitization cut-off was defined as 0.35 kU/L.

The R software (<https://www.r-project.org/>) and Survival package (<https://cran.r-project.org/web/packages/survival/index.html>) and IBM SPSS 23 (IBM; Armonk, NY) were used.



Eliciting cumulative dose of peanut protein (mg)	Number of patients	Cumulative percent of all reactive patients
5	9	13
15	1	15
20	3	19
30	6	28
55	14	48
100	1	49
155	3	54
205	1	55
255	7	65
655	1	67
755	2	70
955	2	73
1255	19	100
Total	69	

FIGURE 1 Threshold distribution curves of the peanut-allergic Finnish children (blue lines) compared to the generic peanut-allergic population (between 0 and 18 y of age) studied in Taylor et al² (orange lines)

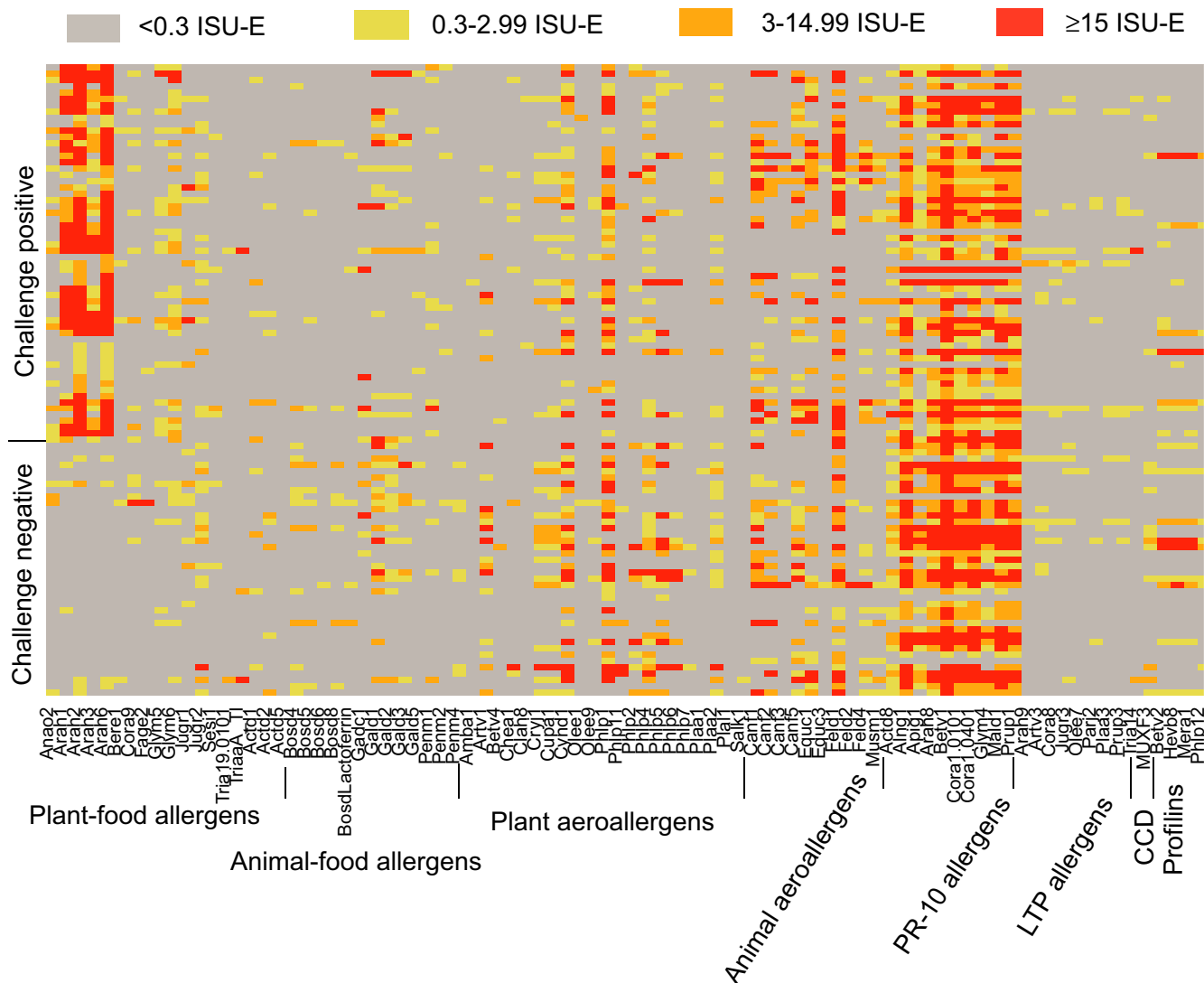


FIGURE 2 Sensitizations in IgE ISAC ImmunoCAP in challenge positives ($n = 67$) and negatives ($n = 33$). Dust mite, latex, mould, cockroach, insect venom and parasite allergens are not presented

3 | RESULTS

Of 102 patients, 69 were challenge positive and 33 negative. Of the positives, 25 (36%) were severe, 36 (52%) moderate and 8 (12%) mild. Baseline characteristics of the patients are presented in Table 1. Of the 69 challenge-positive patients, 9 (13%) reacted to the first dose of 5 mg (Figure 1). Calculated ED 50 values ranged from 55.3 to 75.0 mg of peanut protein (95% confidence interval of 33.4 to 124.5 mg peanut protein). IgE levels to Ara h 1, 2, 3 and 6 associated with the challenge threshold dose of peanut protein. With higher IgE levels, the threshold dose was lower. Ara h 8 and 9 showed no correlation with the threshold dose (Figure S1).

ISAC microarray results were available in 67 challenge-positive and 33-negative patients. In both the challenge negatives and positives, sensitizations to PR-10-proteins, especially Bet v 1, were high (90% for Bet v 1) (Figure 2). All PR-10 proteins showed higher IgE

levels in the challenge negatives, although the differences were not statistically significant (Table 3).

For the whole patient population, skin prick weal sizes were highest to peanut, hazelnut and almond. Cashew, peanut, walnut, sesame seed, pecan and pistachio weal sizes correlated moderately to strongly with IgE levels to the respective 2S albumins, whereas hazelnut correlated only weakly (Table 2).

Sensitization profiles of the peanut allergic and tolerant were similar except for peanut seed storage proteins Ara h 1, 2, 3, 6 and soya bean Gly m 6. Specific IgE to Ara h 1, 2, 3 and 6 was higher in the challenge positives ($P < .0001$). IgE to Ara h 8 and 9 showed no difference between the groups (Table 3). Monosensitization to Ara h 8 was present in 25 patients, of which four had mild reaction (1-3 points) and other 21 had no reaction in the peanut challenge. Monosensitization to Ara h 9 was not present in our study population. IgE to Gly m 6, an 11S globulin from soya bean, showed a higher level in the challenge positives, and overall 41 patients (41%)

TABLE 2 Sensitization in skin prick tests, IgE levels to 2S albumins and correlations between weal sizes and IgE levels

Allergen	Skin prick tests			2S albumins				Positive		Correlation (rho) of weal size with 2S albumin	P value
	Median (mm)	Minimum	Maximum	Positive (≥3 mm) n (%)	Corresponding 2S albumin	Median (ISU-E or kU/L)	Minimum	Maximum	Positive (≥0.35 kU/L) n (%)		
Peanut	8	0	28	99 (97)	Ara h 2	2.5	0	175	60 (60)	0.578	<.001
					Ara h 6	2.8	0	156	62 (62)	0.639	<.001
Hazelnut	6	0	15	94 (92)	Cor a 14	0.06	0	273	25 (25)	0.240	.02
Sesame seed ^a	3	0	23	69 (70)	Ses i 1	0	0	3.1	6 (6)	0.528	<.001
Cashew	3	0	18	55 (54)	Ana o 3 ^a	0.06	0.01	317	26 (27)	0.621	<.001
Brazil nut	0	0	11	38 (37)	Ber e 1	0	0	1.0	6 (6)	0.239	.02
Pecan	0	0	12	41 (40)	Jug r 1	0	0	85.9	15 (15)	0.502	<.001
Pistachio	4	0	15	77 (76)	Ana o 3 ^a	0.06	0.01	317	26 (27)	0.539	<.001
Walnut	3	0	13	54 (53)	Jug r 1	0	0	85.9	15 (15)	0.527	<.001
Almond	5	0	15	92 (90)	NA					NA	
Coconut	0	0	8	24 (24)	NA					NA	
Macadamia ^b	4	0	10	67 (66)	NA					NA	

^an = 98.
^bn = 101.

TABLE 3 Specific IgE to plant food allergens and two additional PR-10 aeroallergens in the challenge negatives and positives

Allergen	Challenge negative (n = 33)			Challenge positive (n = 67)			P value	No of patients with positive IgE result	
	Median	25 Percentile	75 Percentile	Median	25 Percentile	75 Percentile		Challenge negative	Challenge positive
Actd8	0.66	0	2.14	0.19	0	1.68	.17	22	30
Anao2	0	0	0.09	0.10	0	0.55	.01	3	20
Apig1	1.23	0	7.31	0.22	0	1.27	.03	20	30
Arah1	0	0	0	6.84	0.15	29.1	1.8 E – 10	1	43
Arah2	0	0	0	16.0	1.95	58.7	7.6 E – 14	3	57
Arah3	0	0	0	0.54	0	6.57	6.8 E – 8	0	36
Arah6	0	0	0	17.7	2.84	70.9	7.5 E – 18	2	60
Arah8	7.17	1.91	22.5	4.47	0.45	13.7	.43	26	53
Arah9	0	0	0	0	0	0	.15	1	5
Cora1.0101	8.07	3.01	22.8	3.84	0.56	15.6	.14	28	53
Cora1.0401	18.5	7.57	37.1	9.87	1.52	27.2	.10	30	60
Glym4	2.68	0	15.0	0.92	0	9.66	.50	23	40
Glym5	0	0	0	0	0	0.50	.03	3	20
Glym6	0	0	0.12	0.30	0	2.90	.00019	6	35
Jugr2	0.18	0	0.86	0.13	0	0.42	.33	12	22
Mald1	15.5	2.87	44.6	14.2	1.18	40.7	.88	29	57
Prup1	9.37	3.89	26.1	7.77	1.30	28.8	.70	28	55
Cora14 (ImmunoCAP)	0.03	0.01	0.22	0.08	0.03	0.53	.02	8	17
Anao3 (ImmunoCAP) ^a	0.05	0.02	0.16	0.06	0.03	0.62	.17	6	20
Alng1	18	5.9	54	11	1.4	31	.20	29	57
Betv1	58	17	107	51	12	117	.86	30	60

Statistical significance tested with Mann-Whitney *U*, *P* < .0004 in bold. Cut-off for positive is 0.3 ISU-E in ISAC microarray and 0.35 kU/L in ImmunoCAP. Allergens with medians and 75 percentile <0.3 ISU-E are presented in the Table S1 except peanut and soya bean allergens.

^an = 98.

were sensitized to Gly m 6. However, only seven patients (17%) reported allergy to soya. Gly m 5 sensitization was observed in 4 of 7 and Gly m 6 sensitization in 7 of 7 patients. Peanut challenge was positive in 6/7 of the soya allergic patients. In patients with soya allergy, both Gly m 5 and Gly m 6 were higher (*P* < .001) 0 vs 0.6 ISU-E and 0.1 vs 3.6 ISU-E, respectively. Levels of all allergens are represented in Tables 3 and S1.

All patients with moderate-to-severe reaction were sensitized to Ara h 2 and/or Ara h 6. One patient showed sensitization only in ImmunoCAP to Ara h 2 (1.28 kU/L) and was negative to Ara h 2 and Ara h 6 in ImmunoCAP ISAC. Overall, in the moderate-to-severe reactors, the levels of Ara h 2 and Ara h 6 were equally high, measuring 25 ISU-E (*P* = .38: 4.3–62 IQR vs 4.6–72 respectively).

Sensitizations to Ara h 1 (10%) and Ara h 3 (2%) were rare in the negative-to-mild group. In the moderate-to-severe group, IgE levels were significantly higher for both Ara h 1 and Ara h 3 (*P* < .0001 for both allergens) than in the negative-to-mild group. Overall, in the moderate-to-severe group, Ara h 1 showed higher levels than Ara h 3 8.9 ISU-E (0.2–36) median (IQR) vs 0.9 (0–7.9), *P* < .001

respectively. Ara h 8 sensitization was frequent in the whole population (78%), and the levels did not differ between the negative-to-mild group 7.9 (2.1–27) median (IQR) compared to moderate-to-severe group 3.8 (0.4–13) *P* = .15. Ara h 9 sensitization was generally rare, 6 (6%) (Figure S2).

For discriminating challenge positives and negatives, Ara h 2 and Ara h 6 were the most important allergens in the ImmunoCAP ISAC (Figure S3).

The avoidance of tree nuts was common (52% coconut to 96% pecan) in patients with suspected peanut allergy. The most common species that patients had never eaten were pecan (96%), macadamia (94%) and Brazil nut (94%). In contrast, almond and coconut had been eaten by nearly half of the patients. Only 26% reported no known ingestion of sesame seed. Skin prick tests were commonly positive also in patients who had always avoided a specific species, and this was observed especially for hazelnut. Although most patients had never knowingly eaten a specific species and had sensitization in skin prick tests, sensitization to the respective seed storage proteins was uncommon (sesame seed 2% to pecan 44%), Pecan

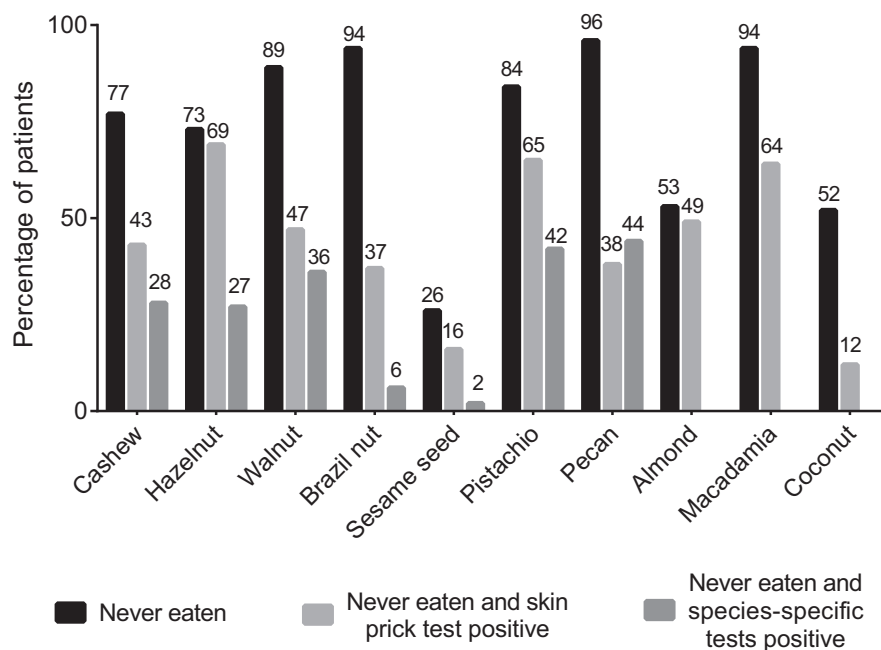


FIGURE 3 Avoidance of tree nuts and sesame seed, and the outcome of skin prick tests and species-specific and/or stable allergen components. Available species-specific tests: cashew and pistachio Ana o 2 (ISAC), 3 (CAP), hazelnut Cor a 8, 9 (ISAC), 14 (CAP), walnut and pecan Jug r 1, 2, 3 (ISAC), Brazil nut Ber e 1 (ISAC) and sesame seed Ses i 1 (ISAC). No available species-specific tests for almond, macadamia and coconut. Skin weal size ≥ 3 mm, ISAC ImmunoCAP microarray ≥ 0.3 ISU-E and singleplex ImmunoCAP ≥ 0.35 kU/L were considered positive.

was assessed with the cross-reactive walnut Jug r-allergen components. In patients who had never eaten a specific tree nut, almond and hazelnut skin prick tests were positive in nearly all patients whereas Brazil nut, pecan and coconut showed skin prick positivity in under half of the patients (Figure 3).

When considering skin prick positive patients, some had eaten the specific species. Especially sesame seed and almond were eaten by several sensitized patients without symptoms (Table 4).

4 | DISCUSSION

We have shown that peanut-allergic and tolerant patients in a birch-endemic area have similarly high prevalence of PR-10 sensitization. Peanut-allergic patients had higher IgE levels to peanut seed storage proteins, especially Ara h 2 and Ara h 6. In these highly birch-sensitized patients, skin prick tests were positive to several tree nuts, and patients avoided many of these species. However, sensitization to species-specific proteins in tree nuts was uncommon.

When comparing the threshold distribution curves based on our data to a larger, previously published multinational allergen threshold database, no significant population differences were observed.² This indicates that our study population is representative of the generic allergic population (from UK, USA, the Netherlands, France, Germany and Australia). However, it must be noted that the 5 mg first dose results in 15%-20% allergic response and it is not valid to extrapolate and calculate the ED₀₅ and ED₁₀ values as we have no data points in that range. The calculated ED₅₀ values are comparable to the values established by Taylor et al.² For clinical

purposes, a starting dose of 5 mg peanut protein seem to have little impact on symptoms presented during the challenges and clinical diagnosis. As expected from Taylor et al.² however, nearly 20% of Finnish individuals reacted to the first challenge dose of 5 mg peanut protein. The level of sensitization to peanut seed storage proteins associated with the allergen threshold dose. With high IgE levels to the four seed storage proteins, patients experienced symptoms at lower peanut protein doses. Previously Eller et al.²⁰ showed weak correlation of Ara h 1, 2 and 3 with the peanut threshold dose.

Ara h 8 sensitization was very common in our patients, and it occurred equally in peanut tolerant and allergic patients highlighting the fact that it is pollen cross-reactivity and unassociated with true challenge-confirmed peanut allergy. In somewhat contrast to Ackerbauer et al, we do not recommend testing Ara h 8 as it seems not to add any value in differentiating severely allergic from tolerant.²¹ In line with IgE to Ara h 8, neither of the other PR-10 protein sensitizations differed between the negative and positive challenge groups. IgE levels to PR-10 proteins were slightly higher in the challenge negatives, though not statistically significant. We hypothesize that strongly PR-10-sensitized patients were more probably suspected of having true peanut allergy and therefore have been more probably selected to our study population. In our previous study with a large registry of skin prick tests,³ mean skin prick weal size to birch pollen was 2.3 mm in 6- to 18-year-olds, whereas in this population it was 4.9 mm. In addition, seasonal mild symptoms due to PR-10 sensitization may have occurred in the history of these patients, which has further enhanced the suspicion of true peanut allergy. Thus, in these patients, the food challenge is particularly

TABLE 4 (a) Patients that reported never having eaten a specific tree nut or sesame. Proportions of patients that had SPT and/or species-specific tests negative and could potentially introduce the species into the diet. (b) Patients that had SPT-positive and reported having tried the specific species. Proportions of patients with no symptoms and positive birch SPT presented. Examined species-specific tests: cashew Ana o 2, 3; hazelnut Cor a 8, 9, 14; walnut Jug r 1, 2, 3; Brazil nut Ber e 1; sesame seed Ses i 1; pistachio Ana o 2, 3; pecan Jug r 1, 2, 3. Almond, macadamia and coconut tested only in skin prick tests, no available species-specific tests. Cut-offs for positivity ≥ 3 mm in SPT, ≥ 0.3 ISU-E in ISAC microarray and ≥ 0.35 kU/L in singleplex ImmunoCAP. SPT; skin prick test

(a)	Never eaten, n		Never eaten & SPT-negative, n		Never eaten & species-specific tests negative, n		Never eaten & SPT & species-specific tests negative, n	
Cashew n = 96	74	77%	33	34%	46	48%	28	29%
Hazelnut n = 98	72	73%	4	4%	46	47%	4	4%
Walnut n = 96	89	93%	42	44%	50	52%	34	35%
Brazil nut n = 96	93	97%	55	57%	87	91%	54	56%
Sesame n = 91	26	29%	11	12%	25	27%	10	11%
Pistachio n = 94	81	86%	18	19%	49	52%	16	17%
Pecan n = 97	95	98%	56	58%	53	55%	39	40%
Almond n = 101	54	53%	4	4%				
Macadamia n = 98	95	97%	30	31%				
Coconut n = 98	53	54%	41	42%				
(b)	SPT-positive, n		Has eaten, n		No symptoms, n		Birch SPT-positive, n	
Cashew n = 96	53	55%	12	23%	5	42%	5	100%
Hazelnut n = 98	90	92%	22	24%	14	64%	13	93%
Walnut n = 96	51	53%	4	8%	2	50%	2	100%
Brazil nut n = 96	38	40%	0					
Sesame n = 91 ^a	64	70%	49	77%	41	84%	39	95%
Pistachio n = 94	73	78%	10	14%	6	60%	6	100%
Pecan n = 97	39	40%	0					
Almond n = 101	91	90%	41	45%	25	61%	24	96%
Macadamia n = 98	67	68%	2	3%	1	50%	1	100%
Coconut n = 98	23	23%	11	48%	9	82%	9	100%

^aOne patient did not report whether or not had symptoms from sesame.

useful in ending unnecessary avoidance of peanut. We conducted the study challenges out of pollen season in all patients that had pollen sensitization.

Ara h 9 sensitization was rare in our study population and was unassociated with the threshold dose. This is in line with other reports from northern European populations.¹³ No differences were present in either of the patient groups in other lipid transfer proteins.

Vicilin Gly m 6 from soya bean showed higher sensitization rates in peanut-allergic patients. This is similar to Hong et al²² who found Gly m 5 and 6 sensitizations more prevalent in peanut-allergic patients. The difference of Gly m 5 was not statistically significant in our population. Gly m 5 and 6 are associated with severe reactions in soya allergic patients,²³ but the soya bean 2S albumin (Gly m 2S albumin) associates best with severe reactions.^{24,25} Soya allergy was reported only by 17% of the Gly m 6-sensitized subjects, and IgE levels to both Gly m 5 and Gly m 6 were higher in patients who reported soya allergy. The soya bean 2S albumin is needed to assess species-specific sensitization in soya allergy. Our results on reported soya allergy and ISAC microarray indicate that soya allergy needs to

be examined with further component-based measurements and food challenges.

Avoidance of tree nuts was common in our patients. Of all species, coconut, almond and sesame seed showed lowest rates of avoidance, which might be due to higher consumption of these species in our diet, and the fact that sesame is a seed, and almond and coconut are sometimes considered unclassified to the group of culinary nuts. Coconut has been shown to have minor cross-reactivity with other tree nuts and peanut and to be botanically distant.^{26,27} As sesame seeds are included in many convenience food hamburgers as well as Middle-Eastern and Oriental foods, their consumption may also have increased in Finland during the past years. The very high rate of avoidance of Brazil nut and macadamia is probably affected by an overall lower consumption of these species and their strangeness to the families. By studying IgE to the available seed storage proteins, we were able to show that sensitization to these proteins was infrequent, even though sensitization to the whole tree nut species in skin prick tests was common. The high rate of sensitization in skin prick tests is caused by birch cross-reactivity. The low

correlation of hazelnut skin prick test and 2S albumin may be due to the effect of birch pollen, which induces strong hazelnut cross-sensitization,³ but does not affect 2S albumin sensitization. Almond and hazelnut are strongly birch cross-reactive tree nuts,³ which can be seen in our results of the prevalence of skin prick test positivity and the asymptomatic sensitization findings in the patients that have tried these species despite of their skin prick test positivity. We consider the available species-specific tests, when negative, sufficient to rule out hazelnut,⁸ cashew and pistachio,⁹ walnut,²⁸ Brazil nut,²⁹ and sesame seed³⁰ allergies in most cases. Species-specific pecan sensitizations were assessed by analysing the cross-reactive walnut allergens.³¹ The ISAC platform includes several important peanut, tree nut and seed allergens, although it would benefit from including hazelnut and cashew 2S albumins Cor a 14 and Ana o 3, which currently are available only in singleplex tests.

We hypothesize that the families' vision of cross-contamination risk is an important factor leading to avoidance of all species that may be considered nuts or seeds in a culinary sense.^{32,33} Avoidance of species, of which specific allergens the child is not sensitized to, may be feasible considering the risk of cross-contamination.³³ However, the unnecessary avoidance may prevent the development of oral tolerance to these species.³⁴

One strength of our study is that all patients were double-blind placebo-control challenged. We are limited by the fact that lower doses in the challenge protocol are needed if one would like to conduct a more detailed analysis of the Finnish ED05 and ED10. However, the challenge doses were optimally selected to diagnose clinical allergy to peanut, and the study population was representative of the generic allergic population. By conducting a comprehensive questionnaire, we were able to analyse the avoidance of wide spectrum of tree nuts in addition to peanut. We are limited to assessing sensitization to these species as challenges were performed only to peanut. We want to emphasize that despite negative species-specific tests, previously avoided tree nuts should be introduced with caution because some allergens may still remain unknown and could induce severe symptoms.³⁵

In conclusion, ImmunoCAP ISAC shows high sensitization to PR-10 proteins in peanut-allergic and sensitized, but tolerant patients, and we do not recommend Ara h 8 for diagnosing peanut allergy. IgE to peanut seed storage proteins are high in truly peanut-allergic patients, and the 2S albumins Ara h 2 and Ara h 6 have the best diagnostic accuracy. Avoidance of several species is common in patients with suspicion of allergy to peanut, and skin prick tests are positive in the majority of patients. However, species-specific sensitization to tree nuts is uncommon. Thus, based on species-specific allergen tests, most patients could potentially introduce several previously avoided tree nuts into their diet.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Each named author has (1) substantially contributed to conception and design or acquisition of data, or analysis and interpretation of data; (2) drafting the article or critically revising it for important intellectual content; and (3) finally approved the version to be published.

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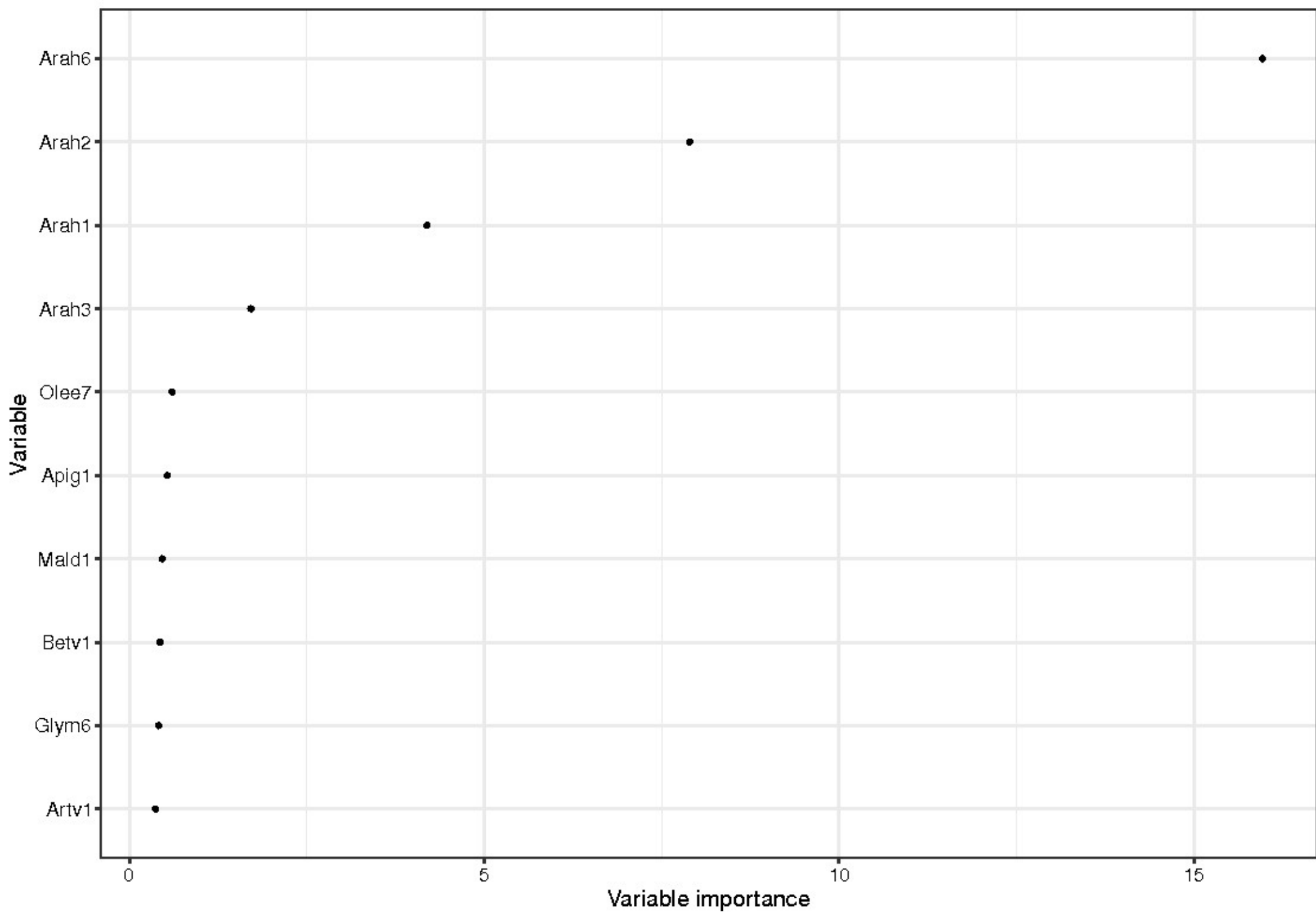
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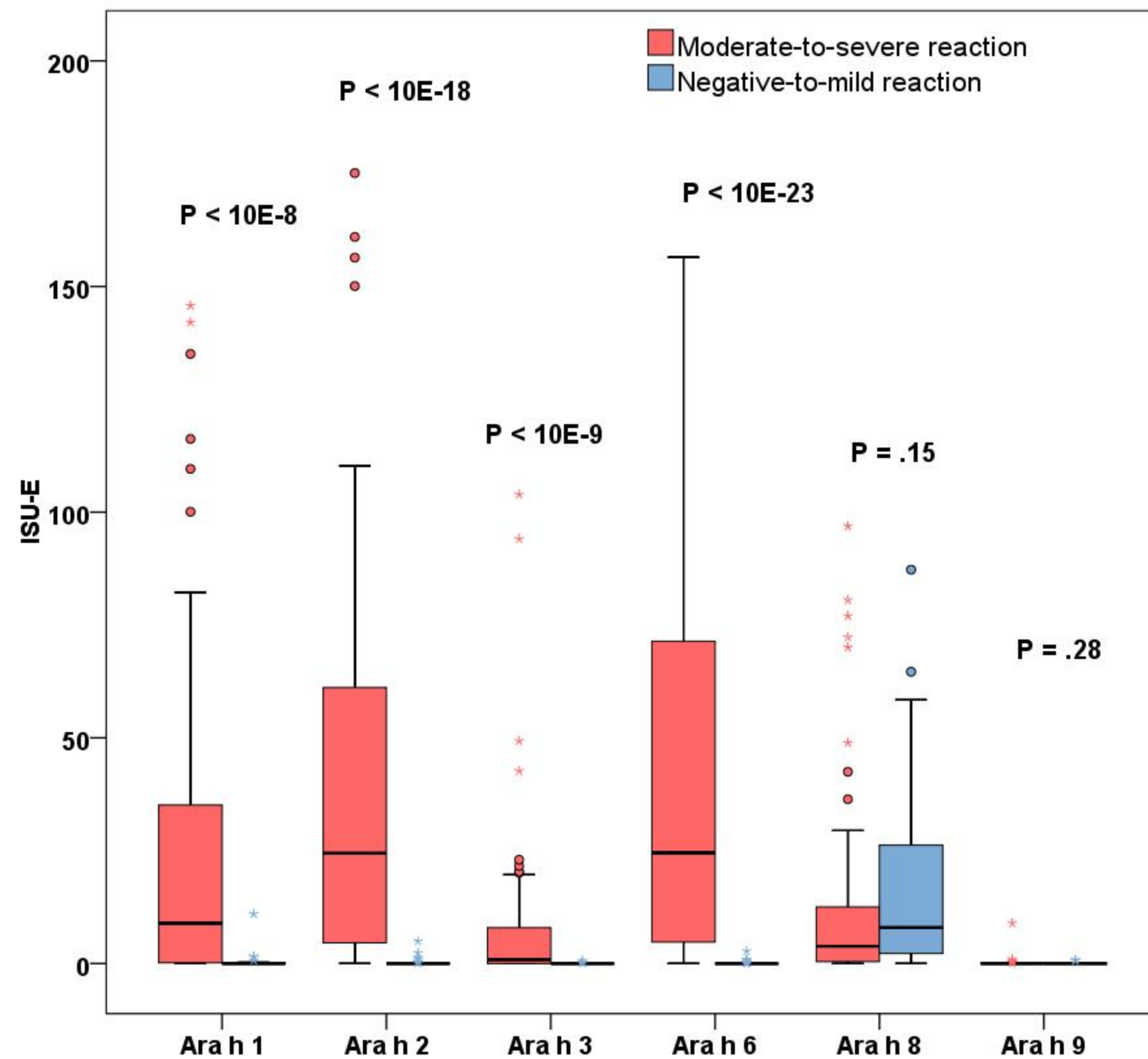
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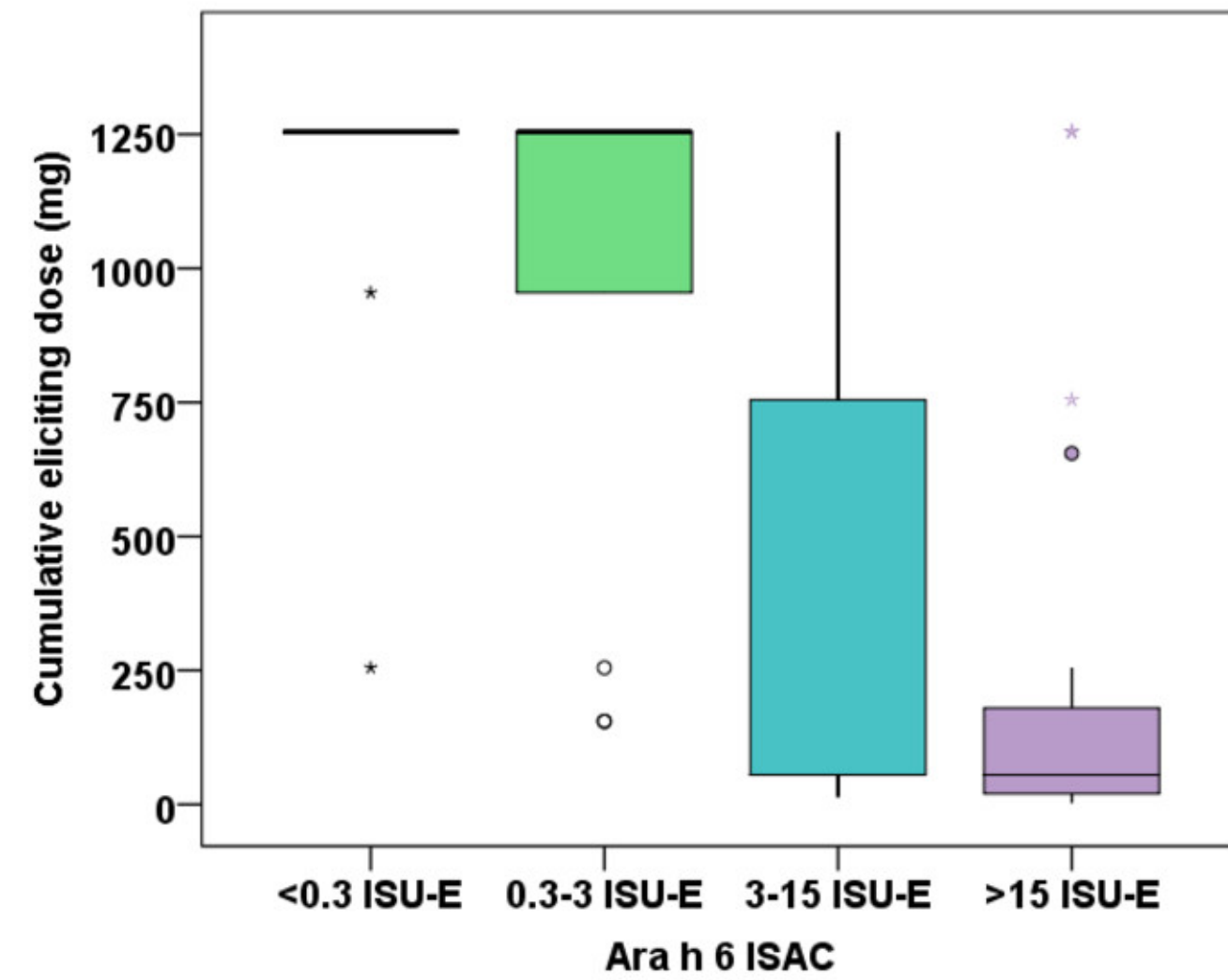
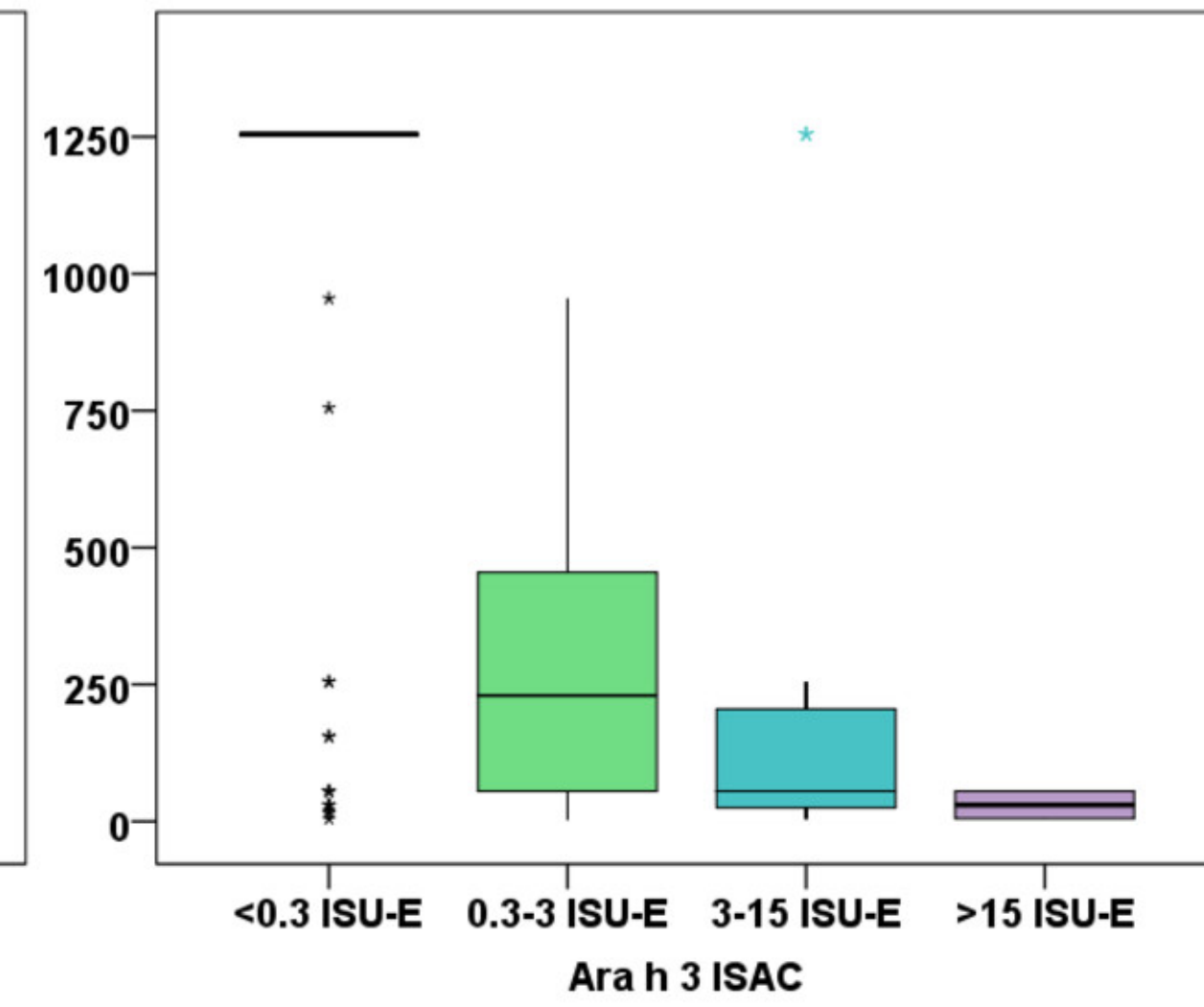
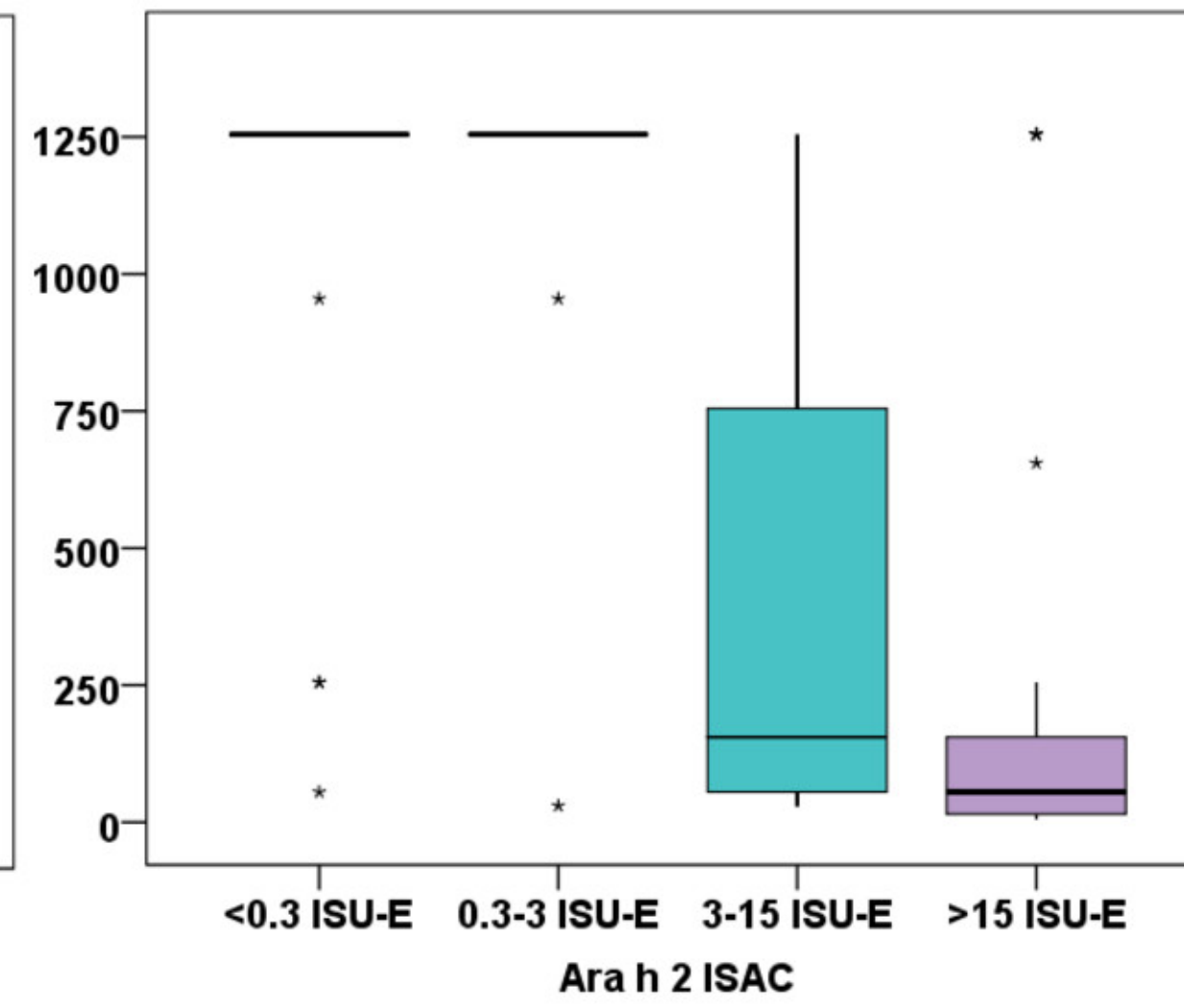
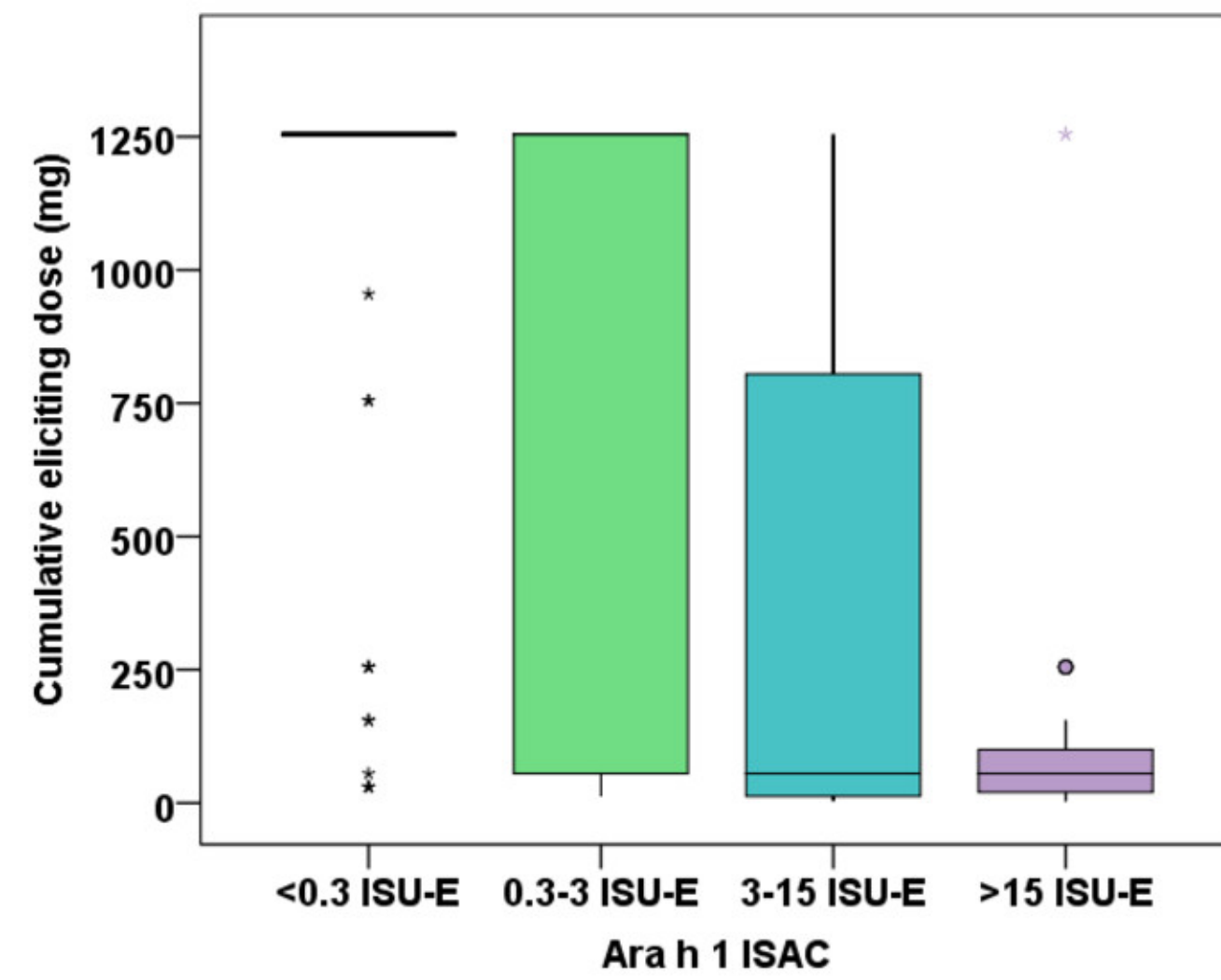
SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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ISAC allergen	Correlation with the cumulative threshold dose, rho (n=67)	P value
Ara h 1	-.491	<. 001
Ara h 2	-.510	<. 001
Ara h 3	-.541	< .001
Ara h 6	-.546	< .001
Ara h 8	.212	.09
Ara h 9	-.023	.85

Supplemental table 1. Allergens of the ISAC microarray stratified by the challenge result. Uncorrected P values are presented. Plant-food allergens with ≥ 0.3 ISU-E medians and PR-10 proteins are presented in separate tables.

	Challenge negative			Challenge positive					Challenge negative			Challenge positive			
	Median	Percentile 25	Percentile 75	Median	Percentile 25	Percentile 75	Uncorrected P value		Median	Percentile 25	Percentile 75	Median	Percentile 25	Percentile 75	Uncorrected P value
Alta1	0.10	0	0.17	0	0	0.17	0.58	Feld1	3.82	0	13.65	7.03	0	28.56	0.56
Alta6	0	0	0	0	0	0	0.87	Feld2	0	0	0	0	0	0	0.27
Amba1	0	0	0	0	0	0	0.30	Feld4	0	0	0.57	0	0	0.45	0.99
Anis1	0	0	0	0	0	0	1	Gadc1	0	0	0	0	0	0	0.53
Anis3	0	0	0	0	0	0	0.87	Gald1	0	0	3.39	0	0	0.48	0.33
Apim1	0	0	0	0	0	0	0.01	Gald2	0	0	0.31	0	0	0.23	0.46
Apim4	0	0	0	0	0	0.49	0.004	Gald3	0	0	0	0	0	0	0.73
Artv1	0	0	2.34	0	0	0	0.004	Gald5	0	0	0	0	0	0.08	0.45
Artv3	0	0	0.16	0	0	0	0.09	Hevb1	0	0	0	0	0	0	0.55
Aspf1	0	0	0	0	0	0	0.01	Hevb3	0	0	0	0	0	0	1
Aspf3	0	0	0	0	0	0	1	Hevb5	0	0	0	0	0	0	1
Aspf6	0	0	0	0	0	0	0.55	Hevb6.01	0	0	0	0	0	0	1
Betv2	0	0	0	0	0	0.23	0.58	Hevb8	0	0	0	0	0	0	0.48
Betv4	0	0	0	0	0	0	0.66	Lepd2	0	0	0	0	0	0	0.33
Blag1	0	0	0	0	0	0	0.60	Mera1	0	0	0	0	0	0	0.47
Blag2	0	0	0	0	0	0	0.69	Musm1	0	0	0	0	0	0	0.98
Blag5	0	0	0	0	0	0	0.55	MUXF3	0	0	0.31	0	0	0	0.02
Blag7	0	0	0	0	0	0	0.23	Olee1	0	0	0	0	0	0	0.39
Blot5	0	0	0	0	0	0	0.25	Olee7	0	0	0	0	0	0	0.13
Bosd4	0	0	0.47	0	0	0	0.02	Olee9	0	0	0	0	0	0.05	0.99
Bosd5	0	0	0	0	0	0	0.16	Parj2	0	0	0	0	0	0	0.70
Bosd6	0	0	0	0	0	0	0.87	Penm1	0	0	0	0	0	0	0.32
Bosd8	0	0	0	0	0	0	0.28	Penm2	0	0	0	0	0	0.10	0.14
BosdLactoferrin	0	0	0	0	0	0	0.12	Penm4	0	0	0.13	0	0	0.11	0.53
Canf1	0	0	11.43	0	0	8.40	0.82	Phlp1	10.59	0	39.74	4.37	0	20.28	0.43
Canf2	0	0	3.30	0	0	0.45	0.76	Phlp11	0	0	0	0	0	0	0.10
Canf3	0	0	0	0	0	0	0.93	Phlp12	0	0	0	0	0	0	0.27
Canf5	0.32	0	2.66	0	0	3.35	0.45	Phlp2	0	0	0	0	0	0	0.09

Chea1	0	0	0	0	0	0	0.52	Phlp4	0.48	0.12	3.23	0.21	0	1.50	0.17
Clah8	0	0	0	0	0	0	0.55	Phlp5	0	0	9.45	0	0	0	0.06
Cryj1	0	0	0.71	0	0	0.18	0.25	Phlp6	0	0	0.13	0	0	0	0.04
Cupa1	0.11	0	0.80	0	0	0.21	0.22	Phlp7	0	0	0	0	0	0	0.19
Cynd1	1.33	0	10.13	0.32	0	3.52	0.35	Plaa1	0	0	0	0	0	0	0.50
Derf1	0	0	0	0	0	0	0.72	Plaa2	0.20	0	0.95	0.14	0	0.40	0.29
Derf2	0	0	0	0	0	0	0.80	Plaa3	0	0	0	0	0	0	0.48
Derp1	0	0	0	0	0	0	1	Plal1	0	0	0	0	0	0	0.55
Derp10	0	0	0	0	0	0.09	0.12	Pold5	0	0	0	0	0	0	0.79
Derp2	0	0	0	0	0	0	0.80	Salk1	0	0	0	0	0	0	0.03
Equc1	0	0	1.39	0	0	3.56	0.98	Vesv5	0	0	0.19	0	0	0.13	0.74
Equc3	0	0	0	0	0	0	0.94	Plant food allergens with <0.3 ISU-E medians							
								Actd1	0	0	0	0	0	0	0.61
								Actd2	0	0	0	0	0	0	0.29
								Actd5	0	0	0	0	0	0	1
								Bere1	0	0	0	0	0	0.13	0.08
								Cora8	0	0	0	0	0	0	0.36
								Cora9	0	0	0	0	0	0.20	0.20
								Fage2	0	0	0	0	0	0	0.29
								Jugr1	0	0	0	0	0	0	0.54
								Jugr3	0	0	0	0	0	0	0.15
								Prup3	0	0	0	0	0	0	0.42
								Sesi1	0	0	0	0	0	0	0.40
								Tria14	0	0	0	0	0	0	0.36
								Tria19.0101	0	0	0	0	0	0	0.97
								TriaaA_TI	0	0	0	0	0	0	0.36

Figure legends for supplemental figures:

Figure 1. IgE levels to peanut allergens and the threshold dose of peanut protein.

Figure 2. Levels of IgE to peanut allergens in negative-to-mild and moderate-to-severe reactions.

Figure 3. Peanut seed storage proteins (Ara h 1, 2, 3, and 6), and especially 2S albumins Ara h 2 and 6, were the best allergens to discriminate positive and negative challenges. Result of a random forest analysis.