

Letter to the Editor

Sensitization to cashew nut 2S albumin, Ana o 3, is highly predictive of cashew and pistachio allergy in Greek children

To the Editor:

Cashew allergy is relatively common and potentially severe, with immediate reactions to cashew being consistently associated with high risk of anaphylaxis, even to minute amounts of the nut.¹⁻⁴ Cashew nut is phylogenetically linked to pistachio, both species being members of the *Anacardiaceae* botanical family. A very high correlation between cashew- and pistachio-specific IgE levels (Spearman correlation coefficient $r_s = 0.95$) has been previously reported, suggesting significant serologic cross-reactivity.⁵ Conventional IgE assays have been shown to be suboptimally specific for either cashew nut or pistachio allergy, thus leaving a “gray area” with positive results of uncertain clinical significance.^{5,6} The aim of the present study was to investigate the utility of recombinant cashew 2S albumin Ana o 3 in the diagnosis of cashew nut allergy and to explore its relevance in the diagnosis of associated pistachio allergy.

For this purpose, a large cohort ($n = 100$) of children, characterized as allergic on the basis of either a positive oral food challenge (OFC) or a convincing history of a recent immediate-type, generalized skin or systemic reaction after the ingestion of cashew and/or pistachio, was assessed for IgE sensitization against rAna o 3, natural cashew, and pistachio extracts by using the ImmunoCAP fluoro-enzyme immunoassay (Thermo Fisher Scientific, Uppsala, Sweden). A control group ($n = 36$) of tolerant subjects was used for comparison, consisting of OFC-negative children having been referred for suspected cashew/pistachio reactivity or children with a clinical diagnosis of peanut, tree nut, or seed (sesame, legume) allergy, but with challenge-proven tolerance or reported regular consumption of both cashew and pistachio. To strengthen positive clinical diagnosis, only recent reactions, having occurred during the past 12-month period, were taken into account for inclusion.

A study group inclusion diagram, analyzing the selection of patients, is shown in Fig E1 in this article's Online Repository available at www.jacionline.org. Further data on study group designation, OFCs, *in vitro* testing, and statistical analysis are available in the **Methods** section in this article's Online Repository available at www.jacionline.org.

Of the 100 allergic children, 63 were documented to clinically react to cashew and 62 to pistachio. Importantly, in all 25 allergic children for whom definite clinical diagnosis regarding both nuts was reached, double allergy was the case, as we were unable to find a single case of selective allergy to any 1 of the 2 nuts. The clinical characteristics of allergic and control subjects are presented in **Tables E1-E3** in this article's Online Repository available at www.jacionline.org.

Ninety-six of the 100 allergic patients enrolled were found to have IgE levels of 0.35 kU_A/L or more for both cashew and pistachio, 1 patient was sensitized only to pistachio, and 3 patients tested negative to both. Among the 36 tolerant children, cashew- and/or pistachio-specific IgE was detected in 24 (67%). A strong relationship was noted between IgE levels to cashew and pistachio, with a correlation coefficient of 0.98 ($P < .001$)

(see Fig E2 in this article's Online Repository available at www.jacionline.org).

IgE sensitization to rAna o 3 (≥ 0.35 kU_A/L) was detected in 93 of the allergic children (93%). In contrast, only 2 (6%) of the tolerant patients were found positive to rAna o 3. Levels of IgE to cashew, pistachio, and rAna o 3 in allergic and tolerant groups are displayed in Fig E3 in this article's Online Repository available at www.jacionline.org.

In receiver-operating characteristic curve analysis of the ability of the tests to discriminate between allergic and tolerant patients, IgE to rAna o 3 performed superiorly to conventional extracts, yielding a near-optimal area under the curve of 97.7% (95% CI, 92.5-99.7) for cashew allergy (Fig 1, A), 97.4% (95% CI, 92.1-99.6) for pistachio allergy (Fig 1, B), and 97.9% (95% CI, 93.8-99.6) for cashew and/or pistachio allergy (see Table E4 in this article's Online Repository available at www.jacionline.org).

When attempting to assess performance characteristics for each assay at the 0.35 kU_A/L conventional cutoff point, IgE to rAna o 3 demonstrated marginally lower sensitivity (92% to 94%) than did IgE to cashew (95%) and pistachio (97%) extracts. In contrast, as presented in Table I, specificity was significantly higher for rAna o 3 (94%) than for cashew (58%) and pistachio extract assay (44%). As compared with either nut extract, the use of rAna o 3 alone correctly classified an additional 12 of 99 patients evaluated for cashew allergy and an additional 16 of 98 children evaluated for pistachio allergy. Moreover, in our population, 13 of 15 patients falsely labeled as allergic to cashew by detection of cashew-specific IgE would have been correctly classified by subsequent measurement of IgE levels to rAna o 3. Similarly, in the case of pistachio allergy, the additional detection of anti-rAna o 3 IgE in pistachio-sensitized patients was able to reduce the number of false-positive diagnoses from 21 down to a single patient. At the optimal cutoff point of 0.16 kU_A/L, rAna o 3 displayed a further increased sensitivity of 98% and a specificity of 94%, correctly classifying 132 (97%) of all 136 patients in this study.

Overall, performance of the recombinant cashew 2S albumin, rAna o 3, underlines the high diagnostic relevance of this allergen group, as has been previously demonstrated in the case of peanut, hazelnut, and soy.⁷⁻⁹ That this cashew-derived molecule may be used as a highly specific marker for pistachio allergy as well is not surprising, as we were able to show that cashew and pistachio appear immunologically nearly identical. Extensive serologic cross-reactivity is suggested by the near-perfect correlation of IgE levels to cashew and pistachio ($r_s = .98$, $P < .001$), while in subsequent reciprocal ImmunoCAP inhibition studies using a serum pool of 20 allergic patients, IgE binding to cashew was found to be strongly inhibited by pistachio and vice versa (84% to 86% compared to homologous inhibition), confirming a high level of cross-reactivity between the 2 nuts (see Table E5 in this article's Online Repository at www.jacionline.org).

To what extent serologic cross-reactivity may be reflected at the clinical level remains essentially unanswered. However, although the existence of a selective type of cashew or pistachio allergy cannot be excluded on the basis of the present study, the lack of even a single patient with proof of selective allergy or tolerance to only 1 of the 2 *Anacardiaceae* nuts corroborates the notion that

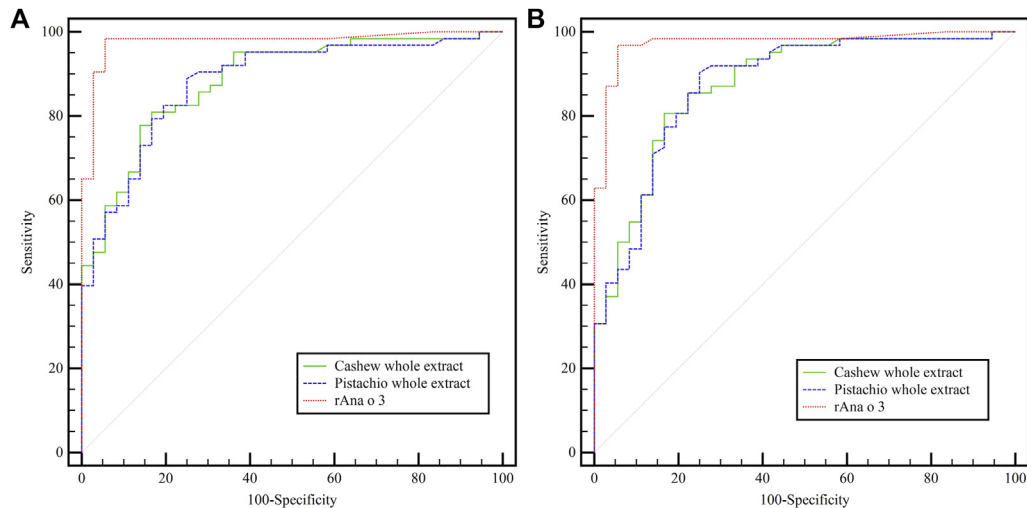


FIG 1. Receiver-operating characteristic curves for IgE to whole extracts and to individual components with regard to their ability to discriminate between allergy and tolerance to cashew (A) and pistachio (B).

TABLE I. Performance characteristics of specific-IgE level detection using whole extracts and rAna o 3 for the diagnosis of cashew and/or pistachio allergy

Diagnosis	Sample size (allergic/tolerant)	ImmunoCAP	Cutoff point (kU _A /L)	Sensitivity (%)	Specificity (%)	Correctly classified (%)
Cashew allergy	63/36	Cashew extract	0.35	95.2	58.3	81.8
		Pistachio extract	0.35	95.2	41.7	75.8
		rAna o 3	0.35	93.7	94.4	93.9
			0.16	98.4	94.4	97.0
Pistachio allergy	62/36	Cashew extract	0.35	93.6	58.3	81.6
		Pistachio extract	0.35	96.8	44.4	76.5
		rAna o 3	0.35	91.9	94.4	92.9
			0.16	96.8	94.4	95.9
Cashew and/or pistachio allergy	100/36	Cashew extract	0.35	95.0	58.3	86.0
		Pistachio extract	0.35	97.0	44.4	82.4
		rAna o 3	0.35	93.0	94.4	93.4
			0.16	98.0	94.4	97.1

The conventional cutoff point of 0.35 kU_A/L was used for all methods, whereas for rAna o 3, an optimal cutoff point (0.16 kU_A/L) was also calculated and used.

extensive cross-reactivity applies at the clinical level as well. It may therefore be especially prudent for physicians to advise strict avoidance of both *Anacardiaceae* members, if clinical reactivity to either nut is documented.

Bearing in mind the close phylogenetic relationship of the 2 allergenic sources and the present data, recognition of a *cashew/pistachio allergy syndrome* may be appropriate. Detection of IgE to Ana o 3 may serve as a highly accurate diagnostic marker for both arms of this apparently dual entity, significantly improving specificity over conventional, whole-extract serologic testing. To geographically and demographically explore the generalizability of our findings, further studies on different populations are required.

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Methods

Study population

Children (0-18y) referred for suspected cashew and/or pistachio allergy to a tertiary Pediatric Allergy Center (University of Athens, Greece) between 2010 and 2012 were evaluated for inclusion in the study. Children with a convincing history of recent immediate-type systemic reaction after ingestion of cashew or pistachio were considered allergic and were included as case subjects. In these patients, confirmatory oral food challenges (OFC) were largely avoided on ethical grounds, given the clear-cut clinical history, the propensity of the studied nuts to induce systemic reactivity even at low doses, the fact that the majority of allergic subjects were young children and parents' unwillingness to consent to a possibly hazardous and unnecessary challenge. However, in order to strengthen positive clinical diagnosis, only recent reactions, having occurred during the past 12-month period, were taken into account for inclusion. Furthermore, reported systemic reactivity upon isolated exposure to cashew or pistachio was a prerequisite for inclusion by clinical history, with the sole exception of immediate generalized urticaria and/or angioedema, which was considered marginally systemic in nature and thus objectively indicative of clinical allergy. In any other condition, as with history of local or subjective symptoms, isolated vomiting or skin rash not entirely compatible with an IgE-mediated reaction, as well as with any reaction reported to have taken place prior to the last 12 months, an open OFC was required for demonstration of current clinical reactivity.

Children with negative oral challenge to cashew nut were always offered an OFC to pistachio and vice versa. Patients with a negative OFC were enrolled as control subjects for the nut being challenged. The control group additionally included children followed at our Allergy Center with a clinical diagnosis of allergy to a seed-storage protein-containing plant food (peanut, tree nut, seed or legume), whose clinical tolerance to cashew and pistachio was established by negative OFCs or reported regular asymptomatic consumption of both nuts at home.

A study group inclusion diagram, analyzing selection of patients, is shown in Fig E1.

The study was approved by the local Research Ethics Committee and written informed consent was obtained from the parents of all participating children.

Oral food challenges

Open oral challenges with cashew or pistachio were performed in 5 dose escalation steps, with an interval of 20 minutes between doses. Amount of ingested nut was approximately tripled at each step, ranging

from 0.2 g to a maximum of 14g per dose (cumulative dose 21.2g). A challenge was only considered positive if objective symptoms of an IgE-mediated reaction occurred during or within two hours of the procedure.

IgE-binding inhibition experiments

Specific IgE-binding inhibition experiments were performed as previously described,^{E1} using serum from four individual patients (no. 2, 23, 42 and 81) and a pool of 20 allergic patients' sera. Prior to use, each sample was diluted to approximately 5 kU_A/L. Cashew and pistachio extracts were used both as solid phase reagents and as liquid phase inhibitors. Briefly, serially diluted inhibitor (highest concentration 5.8 mg/ml for cashew and pistachio) and equal volume of patient serum were mixed and incubated for two hours at room temperature prior to IgE measurement. All assays were run in duplicate. For each inhibitor/solid phase combination, a dose-response curve was established from which the highest level of inhibition was recorded. Extracts of *Dermatophagoides pteronyssinus* and *Aspergillus fumigatus* were used as controls, and did not cause any inhibition of IgE-binding to the nut allergen preparations studied.

Data analysis

Total and specific IgE levels were measured in all patients and correlations among IgE levels were calculated using Spearman's rank order correlation coefficient. To demonstrate each assay's ability to discriminate between patients allergic or tolerant to either nut, receiver-operating characteristic (ROC) curve analysis was performed and the area under the curve (AUC) was used as a measure of diagnostic accuracy.^{E2} Performance characteristics for each assay were calculated both at the generally applied cut-off point of 0.35 kU_A/L and the optimal cut-off point for each method, the latter defined as the IgE concentration providing the most efficient discrimination between allergic and tolerant subjects.

The p-value for inter-group comparisons was obtained using the Mann-Whitney U test for total and specific IgE levels and with the Fisher's exact test for age, gender and asthma prevalence. A p-value of <.05 was considered significant. All statistical analyses were performed with Medcalc 12.5.0 (Medcalc Software, Ostend, Belgium).

Results

Participants' characteristics

In line with other studies, a male predilection among allergic patients was noted, with 2 out of 3 allergic children being male.^{E3} Allergic and tolerant children differed in age at the time of inclusion (median 6.5y, IQR 4.1-9.1 for the allergic group vs. median 9.8y, IQR 5.8-13.3 for the tolerant group, $p<.001$), whereas there were no significant differences with respect to gender, asthma prevalence, aero-allergen sensitization frequency and total IgE levels (Table E1).

Discussion

There are some limitations relevant to the study design, which, to our opinion, should not substantially affect the results.

Firstly, population of the allergic group was not entirely based on the outcome of OFCs, but to a large extent relied on the accuracy of reported clinical history. However, the requirement for immediate, objective, generalized symptoms, and the fact that patients who reported local, subjective or atypical symptoms were always challenged before inclusion, served to reduce the risk of incorrect labeling of patients as allergic. Furthermore, the fact that only recent reactions (up to 12 months) were taken into account for inclusion diminished recall bias and ensured current clinical reactivity at the time of serum collection.

Secondly, the allergic and control groups were shown to differ in age at time of inclusion. While it could be theorized that this age gap might account for the contrasting rAna o 3 results between the two study groups, the magnitude of discrepancy in anti-rAna o 3 IgE levels between allergic and tolerant subjects, along with the fact that tree nut allergy is generally regarded as a lifelong disorder,^{E4} highly unlikely to have resolved within a few years in a whole population, discredits the above hypothesis. Additionally, by carrying out an age-stratified analysis, we were able to demonstrate that the rAna o 3 assay performed consistently well (AUC>94%) across all 4 age quartiles (data not shown).

Thirdly, rAna o 3 appears to be far superior than whole cashew or pistachio extracts in terms of specificity, partly as a result of the high false-positive rate of whole-extract testing. Indeed, 24 (67%) of 36 tolerant patients were found sensitized to cashew or pistachio extract. As demonstrated by subsequent micro-array testing, 16 of 24 false-positive patients were sensitized to at least one widely cross-reactive panallergen (lipid transfer protein, profilin or cross-reactive carbohydrate determinant), which may account for the asymptomatic sensitization to cashew and/or pistachio (data not shown). We recognize that the specificity of extract-based testing would be expected to be somewhat higher in populations with lower frequency of multi-sensitization. Nonetheless, the high rate of multi-sensitization in our control group illustrates the fundamental utility of component-resolved testing; that is to generate test results “purified” from irrelevant or less informative IgE-sensitizations.

It may concludingly be noted that this study was carried out in a Greek pediatric population, so generalizability of the results is open to question. Whether rAna o 3 is equally diagnostic in adults or in patients from a different geographic latitude remains to be evaluated in subsequent studies. In the single relevant published study, examining the frequency of Ana o 3 sensitization among US patients with self-reported cashew allergy, 6 of 7 children (86%) and 15 of 19 adults (79%) were found positive by

95 immunoblotting.^{E5} The numbers are slightly lower in comparison to our findings (we found 93 of 100
96 allergic children positive to rAna o 3); however, given that there was no requirement for recent clinical
97 reactivity, and that historical reactions were taken into account even if decades had since passed, it is
98 possible that allergy may have had resolved in at least some cases at the time of serum collection,
99 especially in adults, potentially reducing the observed frequency of Ana o 3 sensitization. Additionally, it
100 is likely that the immunoblot assay used in that study had a lower analytical sensitivity than the assay
101 technology used in the study reported here.

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Fig E1. Schematic diagram of patient assignment to study groups. Numbers across lines denote specific number of patients.

^a Twenty-one children with suspected allergy were excluded from the study (20 refused to participate, 2 due to concomitant eosinophilic esophagitis). The baseline characteristics of these children did not differ from those of the included allergic children (data not shown).

^b Thirteen of 94 patients reported generalized urticaria (+/- angioedema), which was considered marginally systemic, and were included as case subjects.

^c Children without a recent convincing history of systemic reaction, who declined an OFC, were not included in the study.

^d Challenge to pistachio following negative challenge to cashew and vice versa.

Fig E2. Cashew- and pistachio-specific IgE levels in 100 children allergic to cashew and/or pistachio.

Fig E3. Levels of IgE-sensitization to cashew extract and rAna o 3 in patients evaluated for cashew allergy (n=99) (A), and to pistachio extract and rAna o 3 in patients evaluated for pistachio allergy (n=98) (B). CA indicates cashew-allergic (n=63); PA, pistachio-allergic (n=62); CPT, cashew- and pistachio-tolerant (n=36); dotted lines indicate 0.1 kU_A/L and 0.35 kU_A/L levels; bold lines indicate median values.

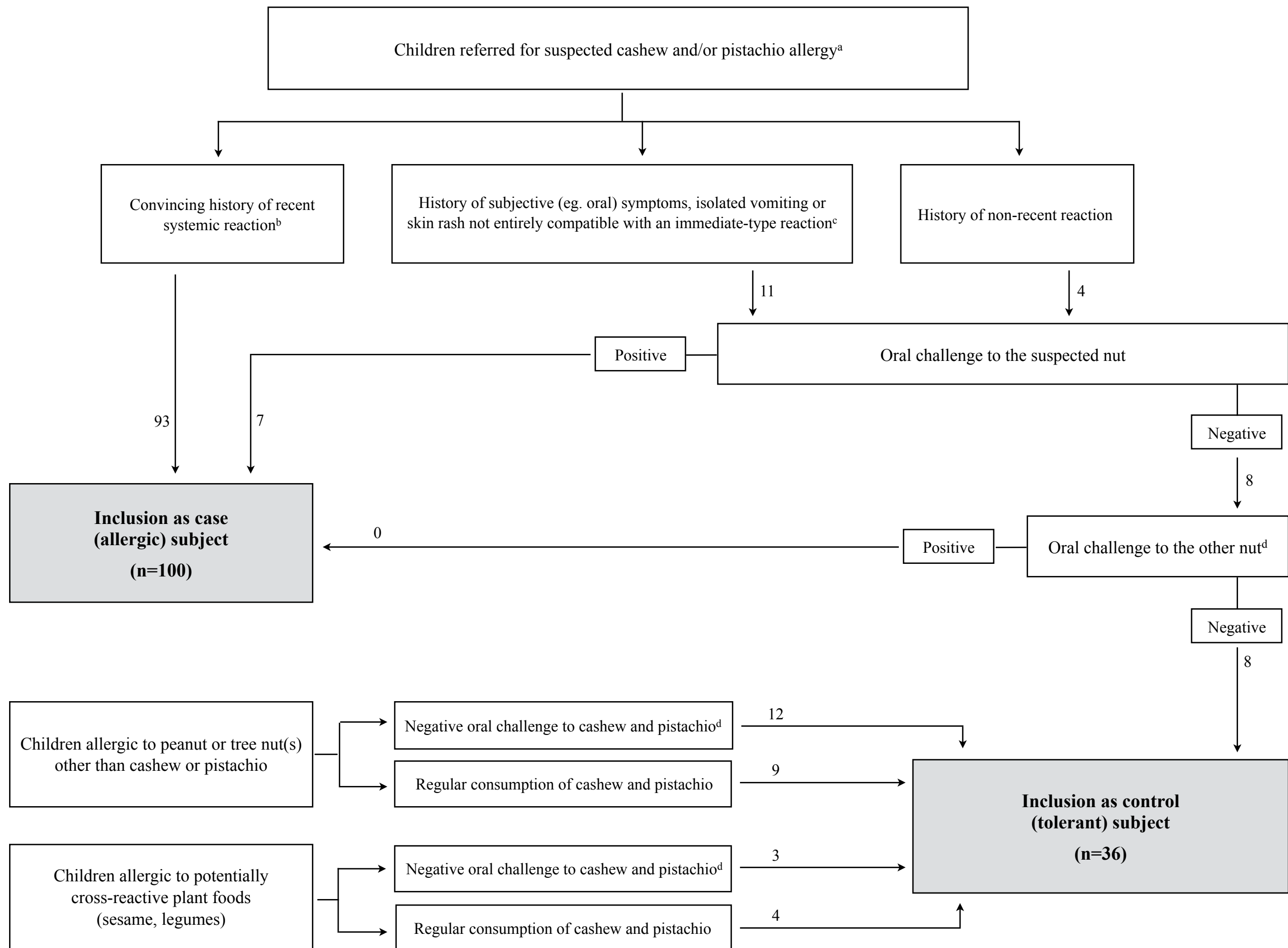


Table E1. Allergic and tolerant patient group characteristics

Group characteristics	Allergic				Tolerant (n=36)	P value ^b
	Confirmed allergy to cashew (n=63)	Confirmed allergy to pistachio (n=62)	Confirmed allergy to both cashew and pistachio (n=25)	Total (n=100)		
Male (%)	43 (68.3)	42 (67.7)	18 (72)	67 (67)	29 (80.1)	.14
Female (%)	20 (31.7)	20 (32.3)	7 (28)	33 (33)	7 (19.9)	
Age, years ^a	5.8 (3.5-8.5)	7.5 (5.2-9.9)	7.4 (5.2-10)	6.5 (4.1-9.1)	9.8 (5.8-13.3)	<.001
Current asthma (%)	27 (42.9)	27 (43.5)	13 (52)	41 (41)	13 (36.1)	.69
Aero-allergen sensitization (%)	52 (82.6)	54 (87.1)	24 (96)	82 (82)	34 (94.4)	.10
Total IgE ^a (kU _A /L)	497 (166-913)	418 (150-1008)	596 (213-1021)	427 (151-960)	442 (245-1063)	.45
Cashew-specific IgE levels ^a (kU _A /L)	5.5 (1.7-16.2)	3.9 (1.3-8.7)	6.3 (2.5-16.8)	4.2 (1.3-11.6)	0.3 (0.1-0.9)	<.001
Pistachio-specific IgE levels ^a (kU _A /L)	7.7 (2.5-23.9)	5.6 (2.3-16.4)	8 (3.5-23)	5.9 (2.3-17.3)	0.5 (0.2-1.3)	<.001

^a Median value and interquartile range (IQR)

^b Comparison between allergic (n=100) and tolerant (n=36) patient groups for gender, asthma and aero-allergen sensitization frequency using Fisher's exact test, and for age and IgE levels using the Mann-Whitney *U* test (two-tailed probability).

Table E2. Clinical characteristics of allergic patients

Patient No.	Gender	Age (y)	Documented clinical reactivity	Reactivity based on history (H) or challenge (C)	Systems involvement ^a	Nut quantity ^b	Known allergy to other tree nuts or peanut ^c	Known allergy to other plant foods ^c
1	M	3.2	Cashew	H	G-R	1		
2	F	6.3	Cashew	C	G	3		
3	F	4.7	Cashew	H	S-G-R	Tr		
4	F	11.2	Cashew	H	S-R-C	1		
5	M	4.0	Cashew	H	S-R	Tr		
6	M	3.6	Cashew	H	S-G-C	Tr		Pc, Se, Le, Pe, Mu
7	M	5.3	Cashew	H	S-G-R	1		
8	M	6.1	Cashew	H	S-G-R-C	Tr		
9	F	4.9	Cashew	H	S-R	Tr		
10	M	6.9	Cashew	H	S-G-R	1	H	Se, Le, Pe, Wh
11	M	11.5	Cashew	H	S-R	Tr		Pc, To
12	F	7.9	Cashew	H	S-R	1	W	
13	M	3.1	Cashew	H	S-G-R-C	Tr		
14	M	8.5	Cashew	H	S-R	Tr		Pc, Le, To
15	M	1.8	Cashew	H	S	4		
16	F	3.4	Cashew	H	S-R	Tr		
17	M	11.3	Cashew	C	G-R	Tr		
18	M	6.6	Cashew	H	S-G-R	1		
19	M	2.3	Cashew	H	S	3	W	
20	M	4.2	Cashew	H	S-G	2		
21	F	2.8	Cashew	H	S-R	Tr	W	
22	M	6.4	Cashew	H	S-G	1	P	
23	F	5.3	Cashew	H	S-R	1	W, H	
24	M	8.4	Cashew	H	S-G-R	1	H	Se
25	F	2.4	Cashew	H	S	1		
26	F	6.5	Cashew	H	S-G	Tr	W, H	
27	M	5.5	Cashew	H	S-R	1	H	Gr
28	M	4.3	Cashew	H	S-G-R	1		
29	M	2.4	Cashew	H	S-R	Tr		Wh
30	M	2.5	Cashew	C	S	Tr		
31	M	5.7	Cashew	H	S-G-R	1		
32	F	2.6	Cashew	H	S-G-R	2		
33	M	14.5	Cashew	H	S-R	1		Se, Ca, Sp
34	M	11.4	Cashew	H	S-R	Tr	H	
35	F	8.3	Cashew	H	S-G-R-C	2		
36	F	4.1	Cashew	H	S	Tr	H	
37	M	2.4	Cashew	H	S-R	2		
38	M	4.8	Cashew	H	S-G-R	Tr		
39	F	6.6	Pistachio	H	G-R	1		Wh
40	M	4.9	Pistachio	H	S-R	Tr		Se
41	M	6.4	Pistachio	H	S-R	Tr		
42	M	6.1	Pistachio	H	S-G	Tr	H	Wh, Ct
43	M	3.8	Pistachio	C	G	1		Le
44	M	7.8	Pistachio	H	S	1		
45	F	7.3	Pistachio	H	S-G-R	2		Ap, Ba
46	M	11.5	Pistachio	H	S-G-R	1		
47	M	8.6	Pistachio	H	S	Tr		
48	F	13.7	Pistachio	H	S-G-R	1		Pc, Me
49	F	10.7	Pistachio	C	G	Tr	W	Se, Ba, Ki, Gr
50	M	7.5	Pistachio	H	S-R	1	W	Ba
51	F	10.9	Pistachio	H	S-C	3	H, P	To
52	F	3.3	Pistachio	H	S-R-C	1		
53	M	6.8	Pistachio	H	S	1		
54	M	15.2	Pistachio	H	S	3	H	
55	M	3.0	Pistachio	H	S-R	Tr		
56	M	6.7	Pistachio	H	S-G-R	1	H	Wh
57	M	9.1	Pistachio	H	S-G-R	Tr	H	Pc, Le, Pe, Ba, Gr, Ca
58	M	5.2	Pistachio	H	S	5	W, H	Wh, Ba
59	M	9.0	Pistachio	H	S-R	Tr	P	Pc, Le, Pe, Me
60	M	8.7	Pistachio	H	G-R	Tr		Le
61	M	3.0	Pistachio	H	S-G-R	Tr		

62	M	5.6	Pistachio	H	S-G	Tr		
63	M	12.1	Pistachio	H	S-G-R	Tr	P	
64	M	2.8	Pistachio	C	S-G	1		Wh, Po, Ba
65	F	9.9	Pistachio	C	G-R	Tr	W	
66	F	7.0	Pistachio	H	S-R	Tr		
67	M	2.5	Pistachio	H	S	7		
68	F	11.9	Pistachio	H	S-G	Tr		Se
69	F	4.8	Pistachio	H	S-R	Tr	W, H	Pc, Se
70	M	9.9	Pistachio	H	S-G	Tr		
71	F	8.9	Pistachio	H	S-G-R	1		Pc, Le, Pe
72	M	7.5	Pistachio	H	S-G-R	1		
73	M	3.9	Pistachio	H	S-G-R	1	H	Wh, Se, Le, Pe
74	F	10.0	Pistachio	H	S-R	1	H, P	Me
75	F	7.7	Pistachio	H	S-R	1	W, H	
76	F	18.2	Cashew, pistachio	H	S-R	1	W, A	Se
77	M	9.8	Cashew, pistachio	H	S-G	2		
78	M	8.7	Cashew, pistachio	H	S-G	Tr		
79	M	7.4	Cashew, pistachio	H	S-G-R	1		
80	M	1.9	Cashew, pistachio	H	S-R	Tr		
81	F	5.4	Cashew, pistachio	H	S-G	Tr		
82	F	8.3	Cashew, pistachio	H	S-G-R	Tr	W, P	Wh, Le, Pe, Ch, Ap
83	M	12.5	Cashew, pistachio	H	S-G-R	Tr		
84	M	3.4	Cashew, pistachio	H	S	1	H	Le, Pe, Ch
85	F	4.4	Cashew, pistachio	H	S-G	1		
86	F	3.0	Cashew, pistachio	H	S-G-R	Tr		
87	M	5.8	Cashew, pistachio	H	S-R	Tr		
88	M	5.9	Cashew, pistachio	H	S-R	2		
89	M	5.7	Cashew, pistachio	H	S-G-R-C	Tr	W, H	Pc, Gr, Sp
90	M	9.4	Cashew, pistachio	H	G-R-C	3	W, H	
91	M	15.3	Cashew, pistachio	H	S-R	Tr	H	Pc, Se, Le, Ch, Pu
92	M	3.2	Cashew, pistachio	H	S-R	Tr	W, H	Le, Pe
93	M	9.5	Cashew, pistachio	H	S-G-R-C	1	H, P	Ba
94	F	8.3	Cashew, pistachio	H	G-R	1		
95	M	6.5	Cashew, pistachio	H	S-G-R	2	W, H	Le, Pe
96	F	12.8	Cashew, pistachio	H	S-G-C	1		Pc, Ki, Gi
97	M	15.0	Cashew, pistachio	H	S-G	Tr	W, H, P	Le, Pe
98	M	6.5	Cashew, pistachio	H	S-G-R-C	1	W, H	Pc, Le, Pe
99	M	2.0	Cashew, pistachio	H	S	Tr		
100	M	10.4	Cashew, pistachio	H	S	Tr		Pc, Le, Pe, Ch, Sp

^a Systems involved in reported recent reaction or positive food challenge to cashew or pistachio. For patients reactive to both nuts, features of the most severe reaction are taken into account. *S* indicates generalized skin findings (urticaria, angioedema); *G*, gastrointestinal (emesis, abdominal cramping); *R*, respiratory (rhinorrhea, nasal congestion, repetitive sneezing, repetitive cough, wheezing, dyspnea); *C*, circulatory or neurological (hypotension, cyanosis, severe bradycardia, lightheadedness, loss of consciousness).

^b Approximate quantity of the nut that provoked the respective reaction. *Tr* indicates trace amount up to less than one nut, whereas numerical figures indicate number of single nuts.

^c Clinical history of immediate type reaction or positive oral challenge. *W* indicates walnut; *H*, hazelnut; *A*, almond; *P*, peanut; *Pc*, peach; *Se*, sesame; *Le*, lentil; *Pe*, pea; *Mu*, mustard; *Wh*, wheat; *To*, tomato; *Gr*, grapes; *Ca*, cabbage; *Sp*, spices; *Ct*, chestnut; *Ap*, apple; *Ba*, banana; *Me*, melon; *Ki*, kiwi; *Po*, potato; *Ch*, chickpea; *Pu*, pumpkin seed; *Gi*, ginger.

Table E3. Clinical characteristics of control (tolerant) patients

Patient No.	Gender	Age (y)	Documented clinical tolerance	Tolerance based on history (H) or challenge (C)	Type of control subject	Known allergy to other tree nuts or peanut ^a	Known allergy to other plant foods ^a
101	M	7.9	Cashew, pistachio	C	Suspected pistachio allergy	W	
102	M	3.8	Cashew, pistachio	C	Suspected cashew allergy		
103	M	5.9	Cashew, pistachio	C	Suspected cashew allergy		
104	F	1.7	Cashew, pistachio	C	Suspected cashew allergy		
105	F	5.5	Cashew, pistachio	C	Suspected pistachio allergy		
106	M	12.8	Cashew, pistachio	C	Suspected pistachio allergy		
107	F	5.7	Cashew, pistachio	C	Suspected cashew allergy		
108	M	17.7	Cashew, pistachio	C	Suspected cashew allergy		
109	M	2.7	Cashew, pistachio	C	Nut-allergic control	W	
110	F	16.5	Cashew, pistachio	C	Nut-allergic control	W	
111	M	3.8	Cashew, pistachio	C	Nut-allergic control	W	
112	M	15.4	Cashew, pistachio	H	Nut-allergic control	A	
113	M	13.8	Cashew, pistachio	H	Nut-allergic control	W	
114	M	8.3	Cashew, pistachio	C	Nut-allergic control	H	
115	F	3.8	Cashew, pistachio	C	Nut-allergic control	H	
116	M	12.9	Cashew, pistachio	C	Nut-allergic control	W, H, A	
117	M	12.5	Cashew, pistachio	H	Nut-allergic control	W, H	
118	M	8.1	Cashew, pistachio	H	Nut-allergic control	H, P	
119	M	9.7	Cashew, pistachio	H	Nut-allergic control	W	
120	M	17.7	Cashew, pistachio	H	Nut-allergic control	W, P	
121	M	13.6	Cashew, pistachio	H	Nut-allergic control	P	
122	M	10.6	Cashew, pistachio	C	Nut-allergic control	W, P	Le, Pe, Ba, Me, Ct
123	M	9.1	Cashew, pistachio	C	Nut-allergic control	W, H	Le, Pe, Ch
124	M	10.8	Cashew, pistachio	C	Nut-allergic control	W	Pc, Ap, Gr, Cr, Su
125	M	13.9	Cashew, pistachio	H	Nut-allergic control	W	Pc, Le, Gr, Cr
126	M	15.5	Cashew, pistachio	C	Nut-allergic control	A	Pc
127	M	8.9	Cashew, pistachio	C	Nut-allergic control	H	Se, Le, Pe, Ch
128	F	9.7	Cashew, pistachio	C	Nut-allergic control	H	Ba, Gr
129	F	4.4	Cashew, pistachio	H	Nut-allergic control	H	Se, Le
130	M	9.8	Cashew, pistachio	H	Plant food-allergic control		Le, Pe
131	M	10.7	Cashew, pistachio	H	Plant food-allergic control		Se
132	M	11.1	Cashew, pistachio	C	Plant food-allergic control		Pc, Pe, To
133	M	12.4	Cashew, pistachio	C	Plant food-allergic control		Pe, Ca, Co
134	M	3.5	Cashew, pistachio	C	Plant food-allergic control		Le, Pe, Ch
135	M	14.6	Cashew, pistachio	H	Plant food-allergic control		Le, Ba, Ca
136	M	9.6	Cashew, pistachio	H	Plant food-allergic control		Le

^a Clinical history of immediate type reaction or positive oral challenge. *W* indicates walnut; *H*, hazelnut; *A*, almond; *P*, peanut; *Le*, lentil; *Pe*, pea; *Ba*, banana; *Me*, melon; *Ct*, chestnut; *Ch*, chickpea; *Pc*, peach; *Ap*, apple; *Gr*, grapes; *Cr*, cherry; *Su*, sunflower seed; *Se*, sesame; *To*, tomato; *Ca*, cabbage; *Co*, corn.

Table E4. Comparison of area under the ROC curve (AUC) for the three different serological methods and pairwise comparison of AUC for rAna o 3 versus natural cashew or pistachio extract.

	Comparison of ROC curves			Pairwise comparison of ROC curves	
	AUC % (95% CI)			Δ AUC % (p value)	
	Cashew extract	Pistachio extract	rAna o 3	rAna o 3 vs. cashew extract	rAna o 3 vs. pistachio extract
Cashew allergy diagnosis	88.6 (80.6-94.1)	88.2 (80.1-93.8)	97.7 (92.5-99.7)	9.2 (<.01)	9.6 (<.01)
Pistachio allergy diagnosis	87.6 (79.4-93.4)	87.5 (79.3-93.3)	97.4 (92.1-99.6)	9.9 (<.01)	9.9 (<.01)
Cashew and/or pistachio allergy diagnosis	87.9 (81.2-92.8)	87.7 (81-92.7)	97.9 (93.8-99.6)	10.0 (<.01)	10.1 (<.01)

Table E5. Percentage of IgE-binding inhibition between cashew and pistachio extracts, using 4 individual sera and a pool of sera from 20 allergic patients.

		Patient 2		Patient 23		Patient 42		Patient 81		Serum pool (n=20)	
		Solid phase									
		Cashew	Pistachio	Cashew	Pistachio	Cashew	Pistachio	Cashew	Pistachio	Cashew	Pistachio
Inhibitor	Cashew	98	74 (76%)	98	94 (96%)	91	86 (92%)	98	94 (96%)	96	81 (84%)
	Pistachio	89 (91%)	98	94 (96%)	98	86 (95%)	93	95 (97%)	98	83 (86%)	96

Numbers in parentheses denote the ratio of heterologous to homologous IgE-binding inhibition.

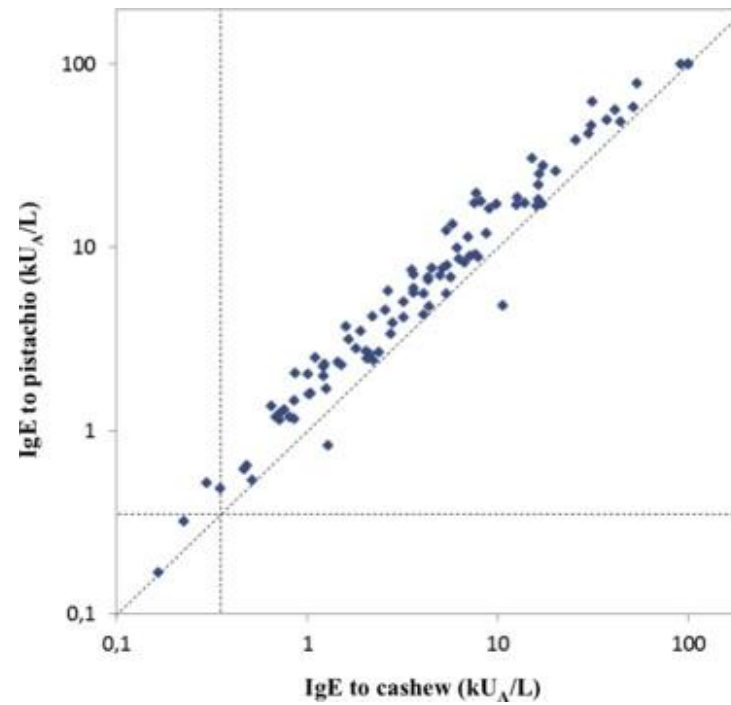


Fig E2

Savvas Savvatanos , Anastasios P. Konstantinopoulos , Åse Borgå , George Stavroulakis , Jonas Lidholm , Magnus P. ...

Sensitization to cashew nut 2S albumin, Ana o 3, is highly predictive of cashew and pistachio allergy in Greek children

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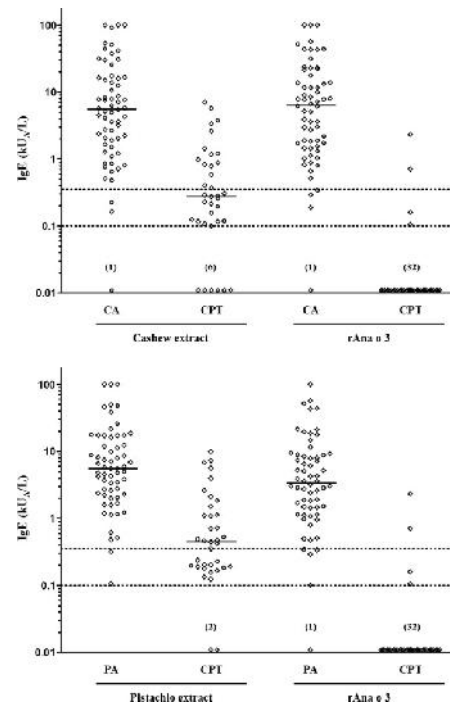


Fig E3

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