

Ara h 2 and Ara h 6 sensitization predicts peanut allergy in Mediterranean pediatric patients

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Abstract

Background: Peanut allergy (PA) management was improved by the introduction of molecular allergology, but guidelines for Mediterranean patients are lacking. We aimed at evaluating peanut component-resolved diagnosis as a diagnostic and prognostic tool in children from Southern France.

Methods: In 181 pediatric patients, PA diagnosis was founded on medical history, skin prick testing, serum-specific IgE to *Arachis hypogaea* extract and components, Pru p 4, and plant carbohydrates, and oral food challenge. Allergen microarray was also performed in 68 of these patients.

Results: In peanut-allergic children ($n = 117$), IgE to Ara h 6 were most prevalent (64%), followed by Ara h 2 (63%), Ara h 1 (60%), and Ara h 9 (52%). Ara h 6 was the best predictor of PA. The second best predictor was the ratio of Ara h 2 IgE to peanut IgE (cutoff 0.113). Persistent childhood PA was associated with complex molecular profiles. Comparison of singleplex and microarray results showed poor concordance for Ara h 2 and Ara h 9.

Conclusion: Ara h 6 and Ara h 2 are the best predictors of PA at diagnosis in Mediterranean pediatric patients. Ara h 1, Ara h 8, and molecular complexity are associated with PA persistence.

Peanut allergy (PA) is one of the most prevalent, persistent, and severe food allergies in children (1). Prevalence of PA is still on the rise, and children who outgrow it tend to do so at elder ages (1, 2). Because of safety, cost, logistic, and reliability reasons, oral peanut challenge (OPC) is not consensual as a first-line test (3–7). The search for safe and reliable *in vitro* tests has led to constant progress since the Sampson team estab-

lished threshold peanut IgE levels for positive oral food challenge (OFC) (3). In recent years, PA work-up has been reshaped by the introduction of component-resolved diagnosis (CRD), with serum-specific IgE (sIgE) to the major allergen Ara h 2 now widely accepted as the best laboratory predictor for PA (6, 8–14). On the other hand, frequent sensitization to nonspecific lipid transfer proteins (LTP) including Ara h 9 has been reported in Mediterranean patients from Italy and Spain (15, 16). Food LTP sensitization is inconsistently associated with severe clinical symptoms, thus adding to the difficulty of proper clinical interpretation of CRD. Moreover, a recent report from Spain shows that Ara h 2 sensitization is the best predictor for pediatric PA, despite lower prevalence (13). The question of the prevalence and clinical relevance of 2S

Abbreviations

CRD, component-resolved diagnosis; IgE, immunoglobulin E; LTP, nonspecific lipid transfer protein; OFC, oral food challenge; OPC, oral peanut challenge; PA, peanut allergy, peanut allergic; PT, peanut tolerance, peanut tolerant; sIgE, specific IgE; SPT, skin prick tests; tIgE, total IgE.

albumins and Ara h 9 sensitization in Mediterranean patients is thus still open and awaiting practical guidelines.

Our aim was to identify peanut CRD profiles and search for CRD-derived predictors of PA in a pediatric clinical population in Marseille, second largest city of France, located on the Mediterranean shore.

Patients and methods

Patients

Local community outpatients referred to allergy departments in the hospitals of the Medical School of Marseille for a history of peanut-induced symptoms underwent PA diagnostic procedures according to EAACI guidelines (4, 5). Peanut CRD was routinely assessed in patients displaying peanut sIgE of 1 kU_A/l or more. 181 patients (124 males, mean age 6.3 yr, range 0–14) fulfilled these criteria from March 2008 through December 2011. Follow-up data for these patients were collected until July 2013. Sixty-eight patients' CRD also comprised ISAC[®] 112 (Thermo Fisher Scientific, Phadia AB, Uppsala, Sweden) testing on the same blood samples.

PA diagnostic procedure

Peanut allergy diagnosis was confirmed or rejected according to EAACI guidelines (4, 5). The association of a convincing clinical history in the past 6 months, positive skin prick tests (SPT) to peanut extract or fresh peanut with a wheal of 4 mm or more, and/or peanut sIgE sensitization allowed PA diagnosis. Convincing clinical history was defined as one or more immediate reaction(s) within 2 h after peanut ingestion, inhalation or direct contact, presenting as local or systemic urticaria, angioedema, laryngeal swelling, immediate vomiting, rhinitis, cough, wheezing, or anaphylaxis. Diagnostic OPC was performed as advised (5, 6), (i) if PA diagnosis outcome was uncertain despite the use of detailed clinical history and sIgE testing, or (ii) if the cause of the suspected food-allergic reaction was uncertain despite allergy testing, for example, reactions following composite meals or occurring in polysensitized/polyallergic children.

Oral peanut challenge followed an open OFC protocol. Children were hospitalized in the day-care unit, next to the intensive care unit, and monitored for 4 h after the last peanut dose. The OFC was stopped if objective symptoms appeared as follows: erythema, urticaria, sneezing, wheezing, cough, vomiting, important abdominal pain, hypotension, loss of consciousness. For diagnostic OPC, fresh peanut was administered in sequential 20-min steps: skin prick testing; labial challenge test; 1/8 peanut (60 mg of peanut protein); 1/4 peanut; 1/2 peanut; one peanut; two peanuts; four peanuts (cumulative maximum challenge dose 3.8 g of peanut protein). For peanut reintroduction, OPC was done with peanut butter (up to doses of 120 mg), Curly[®] (Intersnack France, Vic sur Aisne, France) (1 Curly[®] = 120 mg, 2 Curly[®] = 240 mg), and peanut. The initial dose of 1 mg of peanut was doubled every 20 min up to 480 mg.

Ethics statement

Component-resolved diagnosis with peanut components has become part of the regular medical care since the release of

commercial molecular allergens, due to its potential preventative, diagnostic, and therapeutic value (17). ISAC[®] was performed as part of routine procedures in patients displaying complex clinical conditions. Patients received written laboratory work-up reports and comments on their results. The study was based on a retrospective review of medical charts and laboratory results. The local ethics committee stated that ethical approval and patient consent were not necessary for this type of retrospective, non-interventional study.

Serological analyses

Total and specific serum IgE

Total IgE (tIgE) levels, sIgE to peanut extract and components Ara h 1, 2, 3, 8, 9, Pru p 4 (for profilin testing), and MUXF3 (for plant cross-reacting carbohydrate determinants, CCD) were measured with the ImmunoCAP 250[®] singleplex assay (ThermoFisher Scientific). Measures of tIgE levels are expressed in international units per unit volume (kIU/l, 1 IU equals 2.4 ng). sIgE levels are expressed in allergen units per unit volume (kU_A/l). The measuring range is between 2 and 5000 kIU/l for tIgE and between 0.10 and 100 kU_A/l for sIgE. In case a measure was too high for the measuring range, the serum was diluted and tested at the tenth. The laboratory rates 'ideal' or 'good' with external quality control programs (UK NEQAS, Phadia Quality Club) and is certified to the ISO 15189 standard for sIgE assays.

ISAC[®] IgE allergen microarray

Sera from 68 patients were assayed with both singleplex and multiplex sIgE determination. The ISAC[®] 112 allergen microarray consists of recombinant or purified allergenic proteins, including Ara h 1-3, 6, 8, and 9, profilins, and CCD MUXF3. Proteins are spotted in triplicate onto a functionalized glass surface. Bound sIgE are revealed by means of an Alexa-FITC coupled secondary antibody. Proprietary software MIA[®] (ThermoFisher Scientific) converts fluorescence intensity into semi-quantitative results with a detection threshold of 0.3 ISU (ISAC[®] standardized unit). Technical comparison between singleplex and microarray assays was detailed elsewhere (18, 19).

Data processing and statistical analysis

For each patient, levels of tIgE and sIgE were expressed as previously described. For patient groups, levels of tIgE and sIgE were expressed as the median and the range of observed values. Ratios of component to peanut sIgE were calculated for each individual patient before statistical processing.

Prevalences of component sensitization in different groups were compared using the Mann–Whitney test. sIgE measures and ratios in different groups were compared with two-sample *t*-tests (Kruskal–Wallis). Correlation studies were carried out by calculating Pearson's correlation coefficient. Odds ratios were calculated as previously described (20). *p*-values of 0.05 or less were considered as statistically significant. ROC analyses were performed with the XLSTAT software (Addinsoft, Paris, France).

Results

PA diagnosis and follow-up

Peanut allergy diagnosis was confirmed in 117 patients and rejected in 64 others. Diagnostic OPC was necessary in 28 patients, of whom 10 failed and 18 passed. Among the 64 peanut tolerant (PT) patients, 23 were nut allergic (hazelnut, chestnut, almond, cashew, pistachio, or pine tree nut). Among the 117 PA patients, 80 were also allergic to one or more nuts.

Peanut allergic patients were advised peanut avoidance regimens and follow-up with biannual reexamination, SPT, and serum analysis. In 21 patients, reintroduction OPC was performed in order to determine peanut traces tolerance or the feasibility of peanut reintroduction.

For 63 patients, clinical and laboratory follow-up data were available for at least 4 yr after the initial PA diagnosis and were used for prognostic CRD markers analysis.

Prevalence of peanut components, profilin, and MUXF3 IgE as a function of PA or PT

In PA children, 2S albumins Ara h 6 and 2, LTP Ara h 9 and vicillin Ara h 1 were major allergens, with sIgE prevalences of 64%, 63%, 62%, and 60%, respectively (Table 1). sIgE to Ara h 6, Ara h 2, and Ara h 1 were significantly more frequent in

PA than in PT children ($p \leq 0.008$ in both cases). Almost one of two PT children (43%) was sensitized to Ara h 9.

Minor allergens included the PR-10 protein Ara h 8 and profilin (25–37%). CCD reactivity was frequent in both PA (36%) and PT (50%) patients. CRD profiles of patients finally categorized as PT are presented in Table S1.

Prevalence of component sIgE as a function of age

Peanut allergic patients molecular profile at diagnosis fluctuated with age (Table 2). Cross-sectional data showed that both storage protein and LTP reactivity were present in infants younger than 1 yr. Ara h 2 sIgE prevalence peaked in 5-yr-olds. Ara h 9 sensitization presented a minor peak around 5 yr and reached maximum prevalence in PA teenagers.

Molecular profile complexity

The complexity of molecular profile of each patient was evaluated according to three criteria: prevalence of sIgE to storage proteins (two or more), sIgE to Ara h 2 and Ara h 9, and extended sensitization, defined as detectable sIgE to three or more components (Table 3). PA children showed more complex sensitization profiles ($p < 0.04$).

Table 1 Prevalence of component sIgE in peanut allergic vs. peanut tolerant patients

sIgE prevalence (%)	Ara h 1	Ara h 2	Ara h 3	Ara h 6	Ara h 8	Ara h 9	Pru p 4	CCD
PA	60*	63*	46	64*	25	62	37	36
PT	34	30	32	4	33	43	31	50

CCD, cross-reacting carbohydrate determinants.

Data are shown in bold characters when scoring over 50% (major allergens). sIgE to Ara h 1, Ara h 2, and Ara h 6 were significantly more prevalent in peanut allergic (PA) children compared with peanut tolerant (PT) ones ($p < 0.008$, asterisks). sIgE determination was performed with the ImmunoCAP® 250 system (detection threshold 0.1 kU_A/l), except for sIgE to Ara h 6 (ISAC® 112 allergen microarray, detection threshold 0.3 ISU).

Table 2 Peanut component-resolved diagnosis as a function of age in peanut allergic patients. Prevalences of sIgE are presented as the percentage of positive patients in each age group. sIgE were determined at diagnosis (singleplex ImmunoCAP® 250, detection threshold 0.1 kU_A/l)

Age (years)	Number	Prevalence of sIgE to components (%)						
		Ara h 1	Ara h 2	Ara h 3	Ara h 8	Ara h 9	Pru p 4	CCD
0	5	40	40	40	0	20	67	33
1	9	63	56	63	0	33	13	0
2	7	71	57	29	14	29	29	50
3	10	50	67	50	38	56	25	43
4	7	57	43	0	50	71	67	50
5	11	82	91	46	46	73	55	55
6	8	50	75	25	0	38	13	14
7	13	42	83	33	8	42	36	25
8	8	63	63	75	38	63	38	57
9	15	60	87	60	36	60	57	40
10	9	50	57	43	14	57	40	20
11	4	33	75	0	0	25	0	0
12	6	33	67	33	33	67	17	20
14	5	80	80	40	40	80	60	40

CCD, cross-reacting carbohydrate determinants.

Table 3 Complexity of molecular sensitization profile in peanut allergic (PA) vs. peanut tolerant (PT) patients. PA children displayed more complex patterns of sensitization than PT counterparts ($p < 0.04$ in all cases)

Diagnosis	sIgE to at least 2 storage proteins (%)	sIgE to at least 3 protein components (%)	sIgE to Ara h 2 and Ara h 9 (%)
PA	53*	56*	37*
PT	33	38	23

Data for PA patients are shown in bold characters when significantly different from PT data ($p < 0.04$, asterisks).

Quantitative analysis of sIgE to components

Peanut allergic patients displayed higher sIgE to peanut extract ($p \leq 0.05$), but levels of component sIgE did not differ from PT patients (Table S2). In contrast, when component sIgE were normalized to peanut sIgE in each patient ('component/peanut sIgE ratio'), higher Ara h 2/peanut sIgE ratio (median 50%), and lower Ara h 3/peanut and CCD/peanut sIgE ratios ($p = 0.002$, 0.02 and 0.0004 , respectively) were found in PA patients (Table 4).

ROC analysis of components

ROC analysis was performed with components Ara h 1, 2, 3, 6, 8, 9, and profilin. Ara h 6 and 2 sIgE were the best predictors of PA, with positive predictive values (PPV) of 96% and 86%, respectively, for cutoff levels of $0.13 \text{ kU}_A/\text{l}$ (Ara h 2) and 0.5 ISU (Ara h 6) (Table 5). The performance of sIgE to Ara h 2 as a PA predictor slightly improved when raw measures of sIgE to Ara h 2 were substituted with the Ara h 2/peanut sIgE ratio (PPV 89%). Adjustment for tIgE levels did not improve diagnostic accuracy of the tests (not shown).

Table 4 Comparison of the ratio component/peanut sIgE in peanut allergic (PA) vs. peanut tolerant (PT) patients

Diagnosis	Ara h 1/peanut	Ara h 2/peanut	Ara h 3/peanut	Ara h 8/peanut	Ara h 9/peanut	Pru p 4/peanut	CCD/peanut
PA	9% (0.6–96)	50%* (0.8–295)	4%* (0.1–113)	13% (0.03–591)	35% (0.2–294)	9% (0.04–1093)	3%* (0.1–73)
PT	10% (0.2–81)	8% (6–79)	19% (0.1–188)	8% (0.06–555)	21% (0.1–179)	19% (0.1–288)	26% (0.04–118)

CCD, cross-reacting carbohydrate determinants.

For each patient, component sIgE levels were expressed as a percentage of the peanut sIgE level (ratio of component sIgE to peanut sIgE). Median values and range of the ratio component/peanut are presented in bold characters with asterisk when statistically significant. Ara h 2/peanut, $p = 0.002$; Ara h 3/peanut, $p = 0.02$; CCD/peanut, $p = 0.0004$.

Table 5 Predictive values and cutoff levels for sIgE to 2S albumins Ara h 6 and Ara h 2 and for LTP Ara h 9. ROC curves were calculated as a function of sIgE to Ara h 6, Ara h 2, Ara h 9, or the ratio Ara h 2/peanut sIgE

	Cutoff	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)	AUC
Ara h 6 sIgE (ISU)	0.5	96	62	57	96	0.776
Ara h 2 sIgE (kU_A/l)	0.130	86	54	67	78	0.735
Ara h 2/peanut sIgE ratio	0.113 (11.3%)	89	48	56	86	0.740
Ara h 9 sIgE (kU_A/l)	0.23	79	39	46	74	0.569

AUC, area under the curve; PPV, positive predictive value; NPV, negative predictive value; LTP, nonspecific lipid transfer protein.

sIgE to components in persistent vs. outgrown PA

During PA follow-up, 63 patients were identified as having outgrown PA ($n = 10$) or not ($n = 53$). At diagnosis, patients who were to outgrow PA were younger (median age 1 yr, 0–7) than those who were not (median age 6 yr, 0–14) and showed less complex molecular profiles (Fig. 1). Ara h 2 sensitization at diagnosis was not discriminant with respect to PA evolution. In contrast, sensitization to Ara h 1, 8, 9, and complexity markers previously defined (Table 3) were associated with persistent PA, with an odds ratio of 4.5–7.7. These results were checked through comparison of age-matched outgrown vs. persistent PA patients, which yielded identical results (not shown).

Comparison of peanut CRD with ImmunoCAP® 250 and ISAC® microarray

Results of ISAC® 112 and ImmunoCAP® 250 for peanut components, profilin, and MUXF3 were compared in 68 patients (Table S3). Results were best correlated for sIgE to PR-10 protein Ara h 8 (Pearson's correlation coefficient 0.978), while sIgE to Ara h 2 and Ara h 9 showed the poorest correlation (0.5). Quantitative comparison showed that microarray results represented about 1/10 to 1/5 of the levels measured with ImmunoCAP® 250, with the exception of sIgE to profilin sIgE and CCD, which were comparable.

Discussion

In this study, we addressed molecular sensitization as a function of age and PA in a clinical population of 181 pediatric patients referred for PA assessment and follow-up, 117 of whom received PA confirmation.

Storage proteins Ara h 1, 2, and 6 were major allergens, albeit with unusually low prevalence figures. 2S albumin Ara h

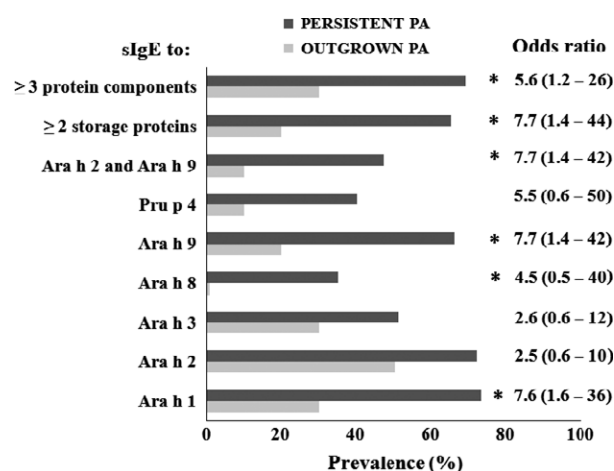


Figure 1 Molecular sensitization patterns associated with peanut allergy (PA) persistence in children during a 4-yr follow-up. Prevalence and odds ratio are shown for children who outgrew PA during the study ($n = 10$, light gray bars) and for those who did not ($n = 53$, dark gray bars). Significant differences are indicated by an asterisk ($p < 0.02$).

6 and Ara h 2 sensitization peaked at 64% and 63%, respectively, followed by Ara h 1 (60%) and Ara h 3 (46%). These figures contrast with the 90% prevalence of Ara h 2 sensitization commonly reported (2, 9, 11, 12, 14, 21), but are very similar to those reported in Spanish PA children, that is, 60%, 72%, and 44% prevalence of sIgE to Ara h 1, 2, and 3, respectively (13). These and our results suggest that storage protein sensitization may be lacking in a considerable proportion of Mediterranean PA children.

Variations in reported prevalence figures of sIgE to peanut components may be linked to different study designs. Some studies (11) addressed selected PA patients with peanut sIgE higher than 14 kU_A/l or peanut SPT ≥ 8 mm, while others chose a real-life approach (13, 22, 23) in patients referred to allergy clinics for PA suspicion. The positivity threshold for sIgE detection may be set at 0.35 kU_A/l (24), although values of 0.10–0.35 kU_A/l have been in use since 2006 and proved relevant (9). Singleplex vs. microarray assays may also yield different results (17, 22, 24, 25).

In line with preliminary results (26), LTP Ara h 9 behaved as a major allergen in our PA patients, with a prevalence of 62%, midway between the 90% and 46% figures reported in Spanish and Italian PA patients (15, 16). sIgE to Ara h 9 was also frequent in PT patients (43%), suggesting an environmental imprint that might be confusing during PA work-up.

Quantitative analysis showed that raw measures of levels of sIgE to components did not differ between PA and PT patients, but the ratio component/peanut sIgE did. Median level of Ara h 2/peanut sIgE ratio was 50% in PA patients, but only 8% in PT counterparts. This finding suggests that peanut sensitization may be seen as the sum of multiple molecular responses, not all of which are associated with true PA. This view received support from the negative correlation of PA with Ara h 3/peanut sIgE or CCD/peanut sIgE ratios, as such findings might

correspond either to another allergy (nut 11S globulins) or to an environmental imprint (plant carbohydrates).

sIgE to Ara h 6 and to Ara h 2 were the best predictors of PA in children, with PPV of 96% and 86% for cutoff values of 0.5 ISU or 0.13 kU_A/l, respectively. Ara h 2/peanut sIgE ratio was also a good predictor. Optimal cutoff levels in our study were in the low range of previous literature results of 0.1–1 kU_A/l (9–11, 27), but close to those reported in similarly designed studies (13, 23). The clinical usefulness of Ara h 6 and Ara h 2 as PA predictors in our population resided in good PPV, while negative predictive value were low, in keeping with the absence of 2S albumin sensitization in almost 40% of PA patients.

Together with heterogeneous study designs, cutoff levels discrepancy and lower prevalence of storage protein sIgE may reflect distinct sensitization processes between northern and southern environments. The pathophysiology of selective storage protein sensitization may be altered by geographic, ethnic, and dietary factors (2, 28). Indeed, our population displayed rich molecular profiles, with sIgE prevalences relatively high for LTP and minor allergens. In particular, Ara h 8 prevalence was surprisingly high (25%), given that children were usually born and presently living in an area where birch pollen is virtually lacking.

With respect to PA prognostic issues, we found that Ara h 2 sensitization at diagnosis was not discriminant for PA evolution, while sensitization to Ara h 1, 8, 9, or a complex molecular profile were preferentially associated with persistent PA. Therefore, patients presenting for PA diagnosis may benefit from an extended molecular profiling. Interestingly, lack of prognostic value for Ara h 2 sensitization at diagnosis is in keeping with findings in Canadian PA children (21).

Conflicting previous reports (18, 22, 24, 25, 29) prompted us to address technical aspects of singleplex ImmunoCAP[®] 250 vs. microarrayed ISAC[®] results for sIgE to peanut components. Correlation was good except for Ara h 2 and Ara h 9, which is a matter of concern given the importance of these two components in PA diagnosis. Moreover, microarray results were underestimated by a factor of 5–10 when compared with singleplex assays. These findings underscore that quantitative aspects are optimally addressed through singleplex assays, which are entirely automated, very stable, and subjected to international quality controls, while microarray analysis, which is semi-quantitative, manual, and more variable (30), is mostly advised when a large-field view is needed (17).

In conclusion, we showed here that (i) in Mediterranean children from Southern France, despite relatively low prevalences, Ara h 6 and Ara h 2 are major allergens and the best PA diagnostic predictors; (ii) molecular complexity assessment may provide helpful information for both diagnostic and prognostic evaluation; (iii) quantitative aspects are best addressed through singleplex assays.

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Conflict of interest

The authors have no conflict of interest to declare in this work.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Peanut component-resolved diagnosis in PT patients as a function of nut allergy or tolerance.

Table S2. Levels of peanut and component sIgE in PA vs. PT patients.

Table S3. Comparison of sIgE levels to components as measured by ImmunoCAP[®] 250 singleplex method and by ImmunoCAP[®] ISAC 112 allergen microarray in 68 patients.

Suppl Table E1. Peanut component-resolved diagnosis in PT patients as a function of nut allergy or tolerance. Prevalences of serum specific IgE (ImmunoCAP® 250, detection threshold 0.1 kU_A/L) are presented in bold characters when scoring over 50%. Nut allergic patients reacted to at least one of the following: hazelnut, chestnut, almond, cashew, pistachio, and pine tree nut allergy.

Final diagnosis	Ara h 1 (%)	Ara h 2 (%)	Ara h 3 (%)	Ara h 8 (%)	Ara h 9 (%)	Pru p 4 (%)	CCD (%)
Nut allergic, peanut tolerant (n = 23)	20	24	29	23	41	24	42
Peanut and nut tolerant (n = 41)	44	34	35	41	47	39	57

Suppl Table E2. Levels of peanut and component sIgE in PA versus PT patients. Median levels of sIgE (ImmunoCAP® 250, detection threshold 0.10 kU_A/L) and ranges are presented, together with total IgE levels (median and range, kUI/L). No statistically significant difference was found between PA and PT results.

	Median sIgE (kU _A /L) to :								Total IgE level (kUI/L)
	peanut	Ara h 1	Ara h 2	Ara h 3	Ara h 8	Ara h 9	Pru p 4	CCD	
Peanut allergy	7.6 (0.11 – 985)	1.8 (0.10 – 296)	3.7 (0.10 – 915)	0.9 (0.12 – 282)	0.6 (0.12 – 71.3)	2.2 (0.10 – 78.9)	0.6 (0.10 – 87.4)	0.6 (0.10 – 5.69)	638 (2 – 8982)
Peanut tolerance	1.8 (0.10 – 560)	0.6 (0.11 – 152)	0.7 (0.10 – 195)	0.5 (0.12 – 137)	0.4 (0.11 – 96)	1 (0.12 – 26.7)	0.6 (0.15 – 32.5)	0.5 (0.11 – 73.7)	407 (58 – 11416)

Suppl Table E3. Comparison of sIgE levels to components as measured by ImmunoCAP® 250 singleplex method and by ImmunoCAP® ISAC 112 allergen microarray in 68 patients. The correlation between results obtained through concomitant assays with both methods is shown, as well as the quantitative relationship between microarray results (y) and individual tests (x). Poor correlation coefficients for Ara h 2 and Ara h 9 IgE results are shown in bold characters.

	Ara h 1	Ara h 2	Ara h 3	Ara h 8	Ara h 9	profilin	MUXF3
Pearson's correlation coefficient	0.885	0.490	0.761	0.978	0.541	0.858	0.782
Quantitative relationship (y = microarray results)	$y = 0.2x + 1$	$y = 0.1x + 8$	$y = 0.1x$	$y = 0.2x$	$y = 0.2x$	$y = 1.1x - 1$	$y = 1.5x$