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IgE recognition patterns in peanut allergy are age dependent: perspectives of the EuroPrevall study

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Abstract

Background: We tested the hypothesis that specific molecular sensitization patterns correlate with the clinical data/manifestation in a European peanut-allergic population characterized under a common protocol.

Methods: Sixty-eight peanut-allergic subjects and 82 tolerant controls from 11 European countries were included. Allergy to peanut and lowest symptom-eliciting dose was established by double-blind placebo-controlled food challenge in all but anaphylactic subjects. Information of early or late (before or after 14 years of age) onset of peanut allergy was obtained from standardized questionnaires. IgE to peanut allergens rAra h 1–3, 6, 8–9, profilin and CCD was determined using ImmunoCAP.

Results: Seventy-eight percent of peanut allergics were sensitized to peanut extract and 90% to at least one peanut component. rAra h 2 was the sole major allergen for the peanut-allergic population. Geographical differences were observed for rAra h 8 and rAra h 9, which were major allergens for central/western and southern Europeans, respectively. Sensitization to rAra h 1 and 2 was exclusively observed in early-onset peanut allergy. Peanut-tolerant subjects were frequently sensitized to rAra h 8 or 9 but not to storage proteins. Sensitization to Ara h

Abbreviations
CCD, cross-reactive carbohydrate determinant; CRD, component-resolved diagnostics; DBPCFC, double-blind placebo-controlled food challenge; LOAEL, lowest observed adverse effect level; sIgE, specific IgE.

$2 \geq 1.0$ kU_A/l conferred a 97% probability for a systemic reaction ($P = 0.0002$). Logistic regression revealed a significant influence of peanut extract sensitization and region on the occurrence of systemic reactions ($P = 0.0185$ and $P = 0.0436$, respectively).

Conclusion: Sensitization to Ara h 1, 2 and 3 is usually acquired in childhood. IgE to Ara h $2 \geq 1.0$ kU_A/l is significantly associated with the development of systemic reactions to peanut.

Peanut allergy is one of the most important food allergies in industrialized countries, with prevalence rates estimated to be 1–3% in the UK, USA, Canada and Australia (1–4). Most data on prevalence of peanut allergy, however, are collected from selected, mainly Anglo-American paediatric populations.

The EU-funded integrated project EuroPrevall comprised a multidisciplinary partnership aiming to address major issues of food allergy in Europe (5). Cohorts representing the main climatic regions of Europe were established through a birth cohort (6), a community survey in school children and adults (7) and an outpatient clinical study (8). We report here data on peanut allergy elaborated within the outpatient clinical study and the community survey.

All patients in this study from 12 European allergy centres underwent a standardized diagnostic workup that comprised assessment of sensitization to peanut extract and inhalant allergens and confirmation of clinical reactivity by means of standardized double-blind placebo-controlled food challenges (DBPCFC) (9) or by means of a consistently recorded case history of anaphylaxis to peanut. A number of recent studies have used purified allergen components to study IgE antibody responses in peanut-allergic subjects in order to improve diagnostic sensitivity and refine the correlation between *in vitro* diagnosis and clinical parameters (10–17). In our present study, the highly characterized population described above and appropriate control subjects were used to evaluate routine and molecular diagnostic approaches by means of single peanut allergens (rAra h 1, rAra h 2, rAra h 3, rAra h 6, rAra h8, rAra h 9, profilin and CCD), and to compare such results with the severity of the clinical response, the amount of peanut eliciting allergic reaction (threshold dose), the geographical origin and age of the patients. In summary, we tested the hypothesis that allergen-specific IgE testing will lead to an improved sensitivity and specificity of peanut allergy diagnosis and that specific sensitization patterns will correlate with the clinical data/manifestation in an European peanut-allergic population.

Design and methods

Study design and subjects

Twelve allergy clinics in Bulgaria (BG, Sofia), Czech Republic (CZ, Prague), France (FR, Strasbourg), Greece (GR, Athens), Iceland (IS, Reykjavik), Italy (IT, Milan), the Netherlands (NL, Utrecht), Poland (PL, Lodz), Lithuania (LT, Vilnius), Spain (ES, Madrid), Switzerland (CH, Zürich) and UK (Manchester) participated in this prospective multicentre study. LT did not contribute patients fulfilling the inclusion criteria and was not considered for further analysis. The clinical part of the study took place between 2006 and 2009. The study was approved by each centre's local ethical committee, and all patients gave written informed consent before entering the study.

In total, 150 individuals were included into the study. All patients underwent the same clinical evaluation, including an extensive interview (such as on age at the primary manifestation of peanut allergy, last allergic reaction, type of reaction) using a standardized questionnaire, specifically developed for the EuroPrevall study (8). Serum samples of the included patients were stored at −20°C and sent to the Paul-Ehrlich-Institut (Langen, Germany) for further analysis.

Eighty-eight patients were recruited within the cross-sectional study of EuroPrevall in an outpatient clinic population. Of these, 48 were included after volunteering for a DBPCFC for which they were selected on the basis of a suggestive case history of an allergic reaction to peanut, that is the development of symptoms to peanut within two hours after ingestion. The remaining 40 patients were included on the basis of a case history of an anaphylactic reaction, defined as severe life-threatening reaction such as drop of blood pressure, severe bronchospasm, or laryngeal oedema within two hours after ingestion of peanut (18) without performing a food challenge. Inclusion was subject to a prior intercentre review process at three different sites (Madrid by Montserrrat Fernandez-Rivas, Utrecht by Andre Knulst and Zürich by Barbara Ballmer-Weber) and was accepted without food challenge if all three centres agreed. In 17 patients, anaphylactic reaction to peanut occurred within the last year, in five within 2 years, in five between 2–5 years and in the remaining over 5 years before inclusion into the study. In addition, six patients from the EuroPrevall community surveys in adults and school-age children (7) were selected for DBPCFC on the basis of a positive case history and sensitization to peanut. Final inclusion criteria for the group of peanut-allergic patients were a positive DBPCFC or a case history of anaphylaxis. Forty-four atopic controls were recruited from CH ($n = 10$), GR ($n = 12$), NL ($n = 11$) and E ($n = 11$) and 12 nonatopic controls from NL ($n = 5$) and E ($n = 7$). The atopic controls had a convincing history of pollen allergy with a positive SPT and/or positive sIgE test to relevant pollen allergens and were tolerant to peanuts. The nonatopic controls had no history of pollen or food allergy and negative skin prick tests (SPT) and/or sIgE tests to a panel of food and inhalant allergens (see also fig. 7 in the online repository).

Food challenges

Double-blind placebo-controlled food challenge was performed according to a common, harmonized protocol and consisted of the patient ingesting increasing doses of a food matrix with or without [placebo] the addition of peanut. The matrix consisted of the EuroPrevall chocolate dessert (9), except for the top dose of peanut which was delivered in a chocolate bar to maintain blinding. Challenges were performed in nine steps at an interval of 20 min, starting with 3 μg of peanut protein and progressing to a cumulative dose of 4.43 g of peanut protein as recently described by Mackie et al. (19). The active and placebo challenges were performed about 1 week apart. Twenty symptoms were monitored and classified as either objective (blister of the oral mucosa, rhinitis, conjunctivitis, flush, urticaria, cough, angio-oedema, emesis, diarrhoea, laryngeal oedema, bronchospasm, drop of blood pressure, shock), or subjective (oral allergy syndrome (OAS), defined as itch restricted to the oral cavity), localized pruritus of skin, generalized pruritus, dysphagia, nausea, gastric pain and/or burning and dyspnoea). The challenge procedure was stopped if the patient developed either objective (externally observable) symptoms or reported severe and/or persistent (lasting >45 min) subjective symptoms. The cumulative dose of peanut inducing such symptoms, that is the lowest observed adverse effect level (LOAEL) (20), was calculated for each patient. As long as no objective or severe subjective symptoms occurred, the challenge continued until all doses were consumed.

Specific IgE measurements

For all patients and controls, serum IgE to peanut, timothy grass pollen, birch pollen, rAra h 1, rAra h 2, rAra h 3, rAra h 8, rAra h 9, rPhl p 12 (profilin) and CCD was determined by ImmunoCAP tests (Thermo Fisher Scientific, Uppsala, Sweden). IgE to rPru p 3 was determined for all patients with a positive case history of peanut allergy. IgE to rAra h 6 was analysed using experimental ImmunoCAP tests prepared as described (21, 22). All IgE measurements were carried out on an ImmunoCAP 250 instrument at the Paul-Ehrlich-Institut and IgE values ≥0.35 kU_A/l were regarded as positive.

Statistics

Geographical differences between the four climatic regions with respect to sensitization to individual peanut allergens, correlations between sensitization and systemic reactions and differences in sensitization pattern between early- and late-onset groups (first manifestation of peanut allergy before or after 14 years of age) were analysed with Fisher's exact test.

A logistic regression model was applied with clinical severity (occurrence of systemic reactions) as dependent variable and sex, age at onset of peanut allergy (younger/older than 14 years), geographical region (eastern (BG, CZ, PL), northern (IS, UK), southern (IT, ES, GR), western/central (F, CH, NL) Europe) and sensitization to peanut components (below/above threshold 0.35 kU_A/l) as independent variables.

Results

Patients' characteristics and food challenges

Hundred and fifty patients from the EuroPrevall allergy centres were enrolled in this study. Twenty-eight patients (15 males, 13 females, age 28 ± 12 years) were included on the basis of a positive DBPCFC (DBPCFC+ve); 26 patients (9 males, 17 females, age 29 ± 10 years) passed DBPCFC without symptoms and were included as tolerant but history-positive controls; 40 patients (14 males, 26 females, age 21 ± 10 years) passed intercentre review processing and were included as anaphylactic patients. Furthermore, 44 atopic controls with a history of peanut tolerance and 12 nonatopic controls were analysed. In 18 DBPCFC+ve patients (64%) and in 30 anaphylaxis patients (75%), the onset of peanut allergy was before their 14th birthday (≤ 13 years), at 4 ± 3 years (average ±SD) in both groups. Ten DBPCFC+ve patients and 10 anaphylaxis patients acquired peanut allergy after their 14th birthday (≥14 years), at 25 ± 8 years and 20 ± 7 years, respectively. The 68 peanut-allergic patients were recruited in BG (n = 4), CZ (n = 4), FR (n = 3), GR (n = 4), IS (n = 2), IT (n = 2), NL (n = 5), PL (n = 1), ES (n = 3), CH (n = 17) and UK (n = 23). The patients were grouped into four European regions: northern Europe (IS, UK, n = 25), western/central Europe (F, CH, NL, n = 25), eastern Europe (BG, CZ, PL, n = 9) and southern Europe (IT, ES, GR, n = 9).

Twenty-five of the 28 DBPCFC+ve patients (89%) reported OAS as an initial symptom under challenge and 13 of these (52%) additionally developed a systemic reaction. Table 1 summarizes the clinical reactivity in response to DBPCFC with peanut and the cumulative dose of ingested peanuts leading to subjective or objective symptoms (lowest observed adverse effect level, LOAEL). Methods and more information on LOAEL values in peanut-allergic patients will be published elsewhere.

Sensitization to peanut extract and individual allergens

Tables 2–3 (see online repository) summarize the IgE results of DBPCFC+ve and anaphylactic patients, their age at inclusion into the study and at onset of peanut allergy. Table 4 (see online repository) provides the corresponding information for the peanut-tolerant controls, who had a positive case history of peanut allergy but a negative challenge. Figure 1 shows the percentage of patients and challenge-negative but history-positive controls with sensitization to extract and components and Fig. 2 the concentrations of specific IgE antibodies. The overall sensitivity of IgE measurement to peanut extract in all allergic patients was 78%.

Among the 15 peanut-allergic patients without detectable IgE to peanut extract, five were sensitized to rAra h 8, three to rAra h 9, one to rAra h 3, whereas seven were negative to all components tested. Thus, the inclusion of rAra h 8, rAra h 9 and rAra h 3 into the serological investigation of peanut-allergic patients increased the sensitivity to 90%.

rAra h 2 was recognized by IgE of 56% of the peanut allergics and was therefore the sole major allergen among these

Table 1 Symptoms under DBPCFC and lowest observed adverse effect level (LOAEL) for objective and subjective symptoms

Patient ID	Symptoms (DBPCFC)	LOAEL subjective (μg protein)	LOAEL objective (μg protein)
610183*	OAS. AE, F, C, G,	33.333	433.333
610195*	OAS, AE, U, F, C, Dph, G, Lpru	3.333	1433.333
610223	OAS	0.033	
610262*	OAS, AE, Bl	33.333	33.333
610266*	OAS, AE	0.003	4283.333
610271	OAS	4433.333	
610279	OAS	433.333	
610309*	OAS, tght, C, G, N	0.333	0.633
710083*	OAS, erythema, E	4323.333	4323.333
710157	OAS, Bl, perioral erythema	33.333	883.333
2010002	OAS	4433.333	no
2010069*	OAS; Bl, AE	0.003	33.333
2010102*	OAS, U, F, G, N	0.003	0.033
2010160	OAS	0.333	no
2010209	OAS	0.333	no
3810088	OAS	3.333	no
4710161*	OAS, U, C, G	0.333	433.333
4810055*	OAS, F	133.333	133.333
610012	OAS	33.333	no
610078	OAS	433.333	no
610301	OAS	433.333	no
710134*	U	4433.333	no
3210255*	U, AE, G	no	433.333
3710114*	AE, G, itching	433.333	433.333
3710134	OAS	1433.333	1433.333
4510001	OAS	3.333	no
4710154	OAS	0.333	no
4810002	OAS	3.333	no

LOAEL, lowest observed adverse effect level; subj, subjective; obj, objective; AE, angioedema; B, blisters of oral mucosa; C, conjunctivitis; E, emesis; Dph, dysphagia; F, flush; G, gastrointestinal pain; Lpru, localized pruritus of the skin; N, nausea; OAS, oral allergy syndrome; tght, tightness of the chest; U, urticarial.
*Patients with systemic reaction

European patients. Of the 30 rAra h 2-negative patients with peanut allergy, two were sensitized to rAra h 1 and two to rAra h 3. All patients sensitized to rAra h 6 were also sensitized to rAra h 2 and the concentrations of IgE to Ara h 2 and Ara h 6 were strongly correlated (r = 0.861, Fig. 3). We observed statistically significant differences between anaphylactic and DBPCFC+ve patients in regard to concentration of IgE to peanut extract (median value 7.4 kU_A/l *vs* 0.7 kU_A/l; $P = 0.0009$) and rAra h 2 (median value 3.9 kU_A/l *vs* 0.3 kU_A/l; $P = 0.0283$).

Higher level of IgE to rPru p 3 than to rAra h 9

rAra h 9 was recognized by IgE of 27% of the peanut allergics and rPru p 3 by 37%. All patients with IgE to rAra h 9 were also positive to rPru p 3 and the concentrations of IgE were on average three times higher ($P < 0.0001$) to rPru p 3 than to rAra h 9 (correlation: $r = 0.758$, Fig. 4). Only two allergics displayed higher levels of IgE to rAra h 9 than to rPru p 3.

Sensitization profile in atopic and nonatopic controls

All nonatopic controls tested negative to peanut extract and peanut components. Ten of 44 atopic controls (23%) with the history of peanut tolerance were sensitized to peanut extract. The sensitization comprised sIgE to rAra h 8 ($n = 3$, 7%), rAra h 9 ($n = 1$, 2%), profilin ($n = 4$, 9%) and CCD ($n = 3$, 7%). In addition, 4 atopic controls without detectable IgE to peanut extract were sensitized to rAra h 8 and one to profilin. Of the 26 controls with the history of peanut allergy but negative challenge (Table 4), 14 (54%) were sensitized to peanut extract. This sensitization was due to sIgE to rAra h 9 ($n = 9$, 64%), rAra h 8 ($n = 2$, 14%) and to profilin ($n = 3$, 21%). In six of the 12 subjects of this control group, with no detectable IgE to peanut extract, three were sensitized to rAra h 8 and three to rAra h 9. No IgE to the peanut storage proteins or to CCD was detected in any of the peanut-tolerant controls, with or without the history of peanut allergy.

Geographical differences in sensitization pattern

Table 5 summarizes the sensitization pattern of patients from the four geographical regions to the extract and the single components. Particularly, prominent differences were observed for sensitization to rAra h 9, which was significantly higher in southern Europe than in other parts of Europe and for rAra h 8, which was highest in western/central Europe (52%).

Age dependency of the sensitization pattern to peanut allergens

Figure 5 summarizes the prevalence of sensitization to peanut components in peanut-allergic patients (anaphylaxis and DBPCFC+ve) who acquired peanut allergy before (early onset, $n = 48$) or after (late onset, $n = 20$) their 14th birthday. All patients with late-onset peanut allergy were negative for all peanut storage proteins except one patient with sensitization to rAra h 3. Eighty-five per cent of patients with early-onset peanut allergy had elevated sIgE to peanut storage proteins. Differences in sensitization to peanut storage proteins between early- and late-onset patients were statistically significant for patients with anaphylaxis as well as for those with a positive DBPCFC ($P < 0.01$ and $P < 0.05$, respectively). Furthermore, prevalence of positive IgE measurement to peanut extract was significantly lower in late-onset peanut allergy than in early-onset allergy (45% *vs* 92%, $P < 0.0001$). This was particularly evident in DBPCFC+ve patients with late-onset allergy, among which sensitization to peanut extract was detected only in 20%. In 60% of these latter patients ($n = 10$), however, IgE specific to

Table 2 Peanut-allergic patients with a positive DBPCFC: age at inclusion into the study, age at onset of peanut allergy and IgE antibody concentrations (kU_A/l)

Patient ID	Age at inclusion	Age at onset	peanut extract	Ara h 1	Ara h 2	Ara h 3	Ara h 6	Ara h 8	Ara h 9	CCD	Profilin	Pru p 3	birch extract	phleum extract
Patients with first manifestation of peanut allergy < 14 years														
CH-610183	14	1	15.2	6.5	6.6	2.7	2.0	0.64	<0.1	<0.1	<0.1	<0.1	38.7	>100
CH-610195	31	6	50.0	4.3	33.5	9.1	nd	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	8.6
CH-610223	26	11	<0.1	<0.1	0.15	<0.1	<0.1	0.21	<0.1	<0.1	<0.1	<0.1	2.5	0.20
CH-610262	25	4	49.2	19.1	15.9	6.2	3.1	<0.1	<0.1	<0.1	<0.1	0.17	<0.1	7.7
CH-610266	32	10	1.0	<0.1	0.27	<0.1	<0.1	4.0	0.27	<0.1	<0.1	<0.1	16.4	0.43
CH-610271	30	4	0.41	<0.1	0.31	<0.1	0.30	<0.1	<0.1	<0.1	<0.1	<0.1	0.38	1.5
CH-610279	41	3	1.4	0.44	0.71	0.13	0.65	0.60	<0.1	<0.1	<0.1	<0.1	5.4	0.15
CH-610309	11	3	37.6	4.32	25.0	0.31	14.8	0.53	<0.1	<0.1	0.58	0.53	32.7	100.0
E-710083	1.25	1	2.3	<0.1	3.6	<0.1	1.03	<0.1	<0.1	<0.1	<0.1	0.78	<0.1	<0.1
E-710157	7	3	0.79	0.22	0.92	<0.1	0.53	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
NL-2010002	36	3	2.2	<0.1	0.87	<0.1	2.3	0.31	<0.1	<0.1	<0.1	0.46	2.4	18.2
NL-2010069	17	2	>100	78.0	>100	61.8	17.1	6.4	0.81	<0.1	0.27	10.7	36.3	65.2
NL-2010102	36	1	1.2	1.3	0.29	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.10	<0.1
NL-2010160	22	1	0.27	<0.1	0.10	<0.1	0.11	6.4	5.2	<0.1	<0.1	0.19	>100	17.3
NL-2010209	29	3	1.5	0.44	1.5	<0.1	1.8	0.80	<0.1	<0.1	<0.1	0.11	13.1	6.2
CZ-3810088	6	1	1.3	<0.1	0.78	<0.1	0.11	<0.1	<0.1	<0.1	<0.1	<0.1	0.29	0.15
F-4710161	17	5	5.1	2.8	2.5	<0.1	3.1	<0.1	<0.1	2.1	<0.1	0.19	6.7	5.5
IS-4810055	29	6	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.11	<0.1	4.0
>0.35 kU/l*			83	50	67	22	59	39	11	6	6	22	61	67
Patients with first manifestation of peanut allergy >14 years														
CH-610012	45	35	<0.1	<0.1	0.14	<0.1	<0.1	1.2	<0.1	<0.1	<0.1	<0.1	10.5	0.17
CH-610078	23	19	0.69	0.22	0.30	0.18	0.28	0.74	0.12	0.13	1.3	0.11	6.1	51.5
CH-610301	41	15	<0.1	<0.1	0.13	<0.1	0.24	<0.1	<0.1	<0.1	<0.1	0.40	0.30	0.13
E-710134	45	28	0.34	<0.1	<0.1	<0.1	<0.1	<0.1	0.36	<0.1	<0.1	9.2	0.13	<0.1
PL-3210255	39	37	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
BG-3710114	43	30	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
BG-3710134	19	17	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
I-4510001	42	22	0.31	<0.1	<0.1	<0.1	<0.1	<0.1	0.56	<0.1	<0.1	3.09	<0.1	<0.1
F-4710154	29	20	0.61	<0.1	<0.1	<0.1	<0.1	1.6	<0.1	0.19	<0.1	0.23	16.9	23.8
IS-4810002	28	24	0.21	<0.1	<0.1	1.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.13	0.24
>0.35 kU/l*			20	0	0	10	0	30	20	0	10	3	3	2

*%positive

Table 3 Peanut-allergic patients with an anaphylactic reaction and onset of peanut allergy (a) < 14 and (b) >14 years: age at inclusion into the study, age at onset of peanut allergy, and IgE antibody concentrations (kU_A/l)

Patient ID	Age at inclusion	Age at onset	Extract	Ara h 1	Ara h 2	Ara h 3	Ara h 6	Ara h 8	Ara h 9	CCD	Profilin	Pru p 3	birch extract	phleum extract
(a)														
CH-610129	25	4	>100	>100	>100	33.7	22.7	64.2	31.9	1.7	0.38	0.78	>100	56.2
CH-610166	18	4	>100	>100	>100	9.6	0.15	1.5	<0.1	5.0	0.13	0.41	27.4	88.2
CH-610209	20	2	>100	52.6	74.5	7.3	39.2	<0.1	<0.1	0.48	<0.1	<0.1	0.17	0.62
CH-610213	22	10	1.3	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	1.3	<0.1	3.8	7.9
CH-610240	22	2	4.7	0.46	4.0	<0.1	3.5	<0.1	<0.1	<0.1	0.82	0.21	2.5	7.9
GR-1710148	1	0.7	19.1	0.91	6.3	1.2	9.0	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
UK-3310002	37	4	1.9	2.1	0.76	<0.1	0.60	0.10	<0.1	<0.1	<0.1	0.15	4.7	3.7
UK-3310012	34	6	17.7	5.9	14.4	0.34	12.0	4.5	0.56	0.24	4.6	2.8	24.7	61.4
UK-3310026	13	2	>100	>100	>100	>100	>100	0.41	1.3	0.50	0.18	1.3	0.64	85.8
UK-3310043	20	5	>100	>100	>100	>100	>100	0.10	0.12	0.13	1.5	0.17	0.55	38.0
UK-3310049	24	4	11.4	5.6	8.0	0.33	5.8	<0.1	<0.1	<0.1	<0.1	0.16	<0.1	7.1
UK-3310060	46	5	8.3	1.2	8.1	0.13	3.1	<0.1	<0.1	<0.1	<0.1	0.11	<0.1	<0.1
UK-3310061	23	0.5	20.8	21.9	3.8	0.17	2.9	0.13	1.9	1.8	0.27	17.5	2.1	51.4
UK-3310067	21	0.6	0.11	<0.1	<0.1	<0.1	0.12	1.8	<0.1	<0.1	<0.1	0.41	10.5	9.3
UK-3310069	19	10	6.3	<0.1	0.29	7.0	0.11	<0.1	<0.1	<0.1	<0.1	0.25	0.17	9.0
UK-3310072	31	11	39.7	18.3	26.1	8.6	16.6	<0.1	<0.1	<0.1	0.31	<0.1	0.41	2.3
UK-3310074	15	0.9	17.4	8.3	11.8	0.39	6.3	1.4	<0.1	<0.1	<0.1	<0.1	22.3	4.2
UK-3310083	15	1	>100	66.8	77.4	16.4	41.0	<0.1	<0.1	<0.1	<0.1	0.15	<0.1	3.6
UK-3310090	18	3	2.9	<0.1	1.5	<0.1	1.5	4.5	<0.1	<0.1	1.2	2.0	16.2	18.7
UK-3310107	19	3	0.59	0.45	0.12	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
UK-3310108	11	6	45.0	7.0	47.2	<0.1	25.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	2.2
UK-3310112	12	5	7.4	<0.1	6.1	0.13	5.3	<0.1	<0.1	<0.1	<0.1	0.13	2.0	3.0
UK-3310113	15	2	1.6	<0.1	1.2	<0.1	0.14	<0.1	<0.1	<0.1	1.1	0.47	0.60	30.5
UK-3310117	13	2	6.8	<0.1	6.7	<0.1	3.2	0.23	<0.1	<0.1	<0.1	0.24	2.6	3.9
UK-3310119	11	5	162.7	79.3	95.3	11.2	55.7	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	1.1
BG-3710021	8	1	55.5	1.2	45.6	<0.1	61.6	0.67	1.5	1.3	<0.1	2.8	3.9	68.9
CZ-3810011	19	6	75.3	22.7	42.3	0.24	43.9	1.4	7.0	<0.1	<0.1	16.2	11.2	10.1
CZ-3810018	11	1	7.4	<0.1	2.6	<0.1	7.4	3.5	<0.1	<0.1	<0.1	0.13	32.0	1.4
CZ-3810039	2	1	4.7	<0.1	3.2	<0.1	1.2	<0.1	0.58	<0.1	3.3	0.94	6.5	2.5
F-4710016	17	2	28.7	11.6	18.8	2.6	12.4	1.7	<0.1	<0.1	<0.1	<0.1	4.4	<0.1
>0.35kU/l*			97	70	87	40	80	37	23	20	27	37	70	87
(b)														
CH-610230	22	21	1.2	<0.1	<0.1	<0.1	<0.1	0.15	<0.1	0.23	1.0	<0.1	2.6	69.7
GR-1710022	22	22	8.8	<0.1	0.29	<0.1	<0.1	<0.1	43.9	<0.1	<0.1	69.5	0.52	0.41
GR-1710038	30	27	0.66	<0.1	<0.1	<0.1	<0.1	<0.1	1.5	<0.1	<0.1	5.3	0.32	0.15
GR-1710143	19	15	1.5	<0.1	0.12	<0.1	<0.1	<0.1	5.6	0.12	<0.1	9.2	0.44	1.2

Table 3 (continued)

Patient ID	Age at inclusion	Age at onset	Extract	Ara h 1	Ara h 2	Ara h 3	Ara h 6	Ara h 8	Ara h 9	CCD	Profilin	Pru p 3	birch extract	phleum extract
UK-3310039	29	16	0.48	<0.1	<0.1	<0.1	0.13	<0.1	1.2	<0.1	<0.1	1.3	<0.1	<0.1
UK-3310042	39	16	0.71	<0.1	0.17	<0.1	<0.1	<0.1	1.8	<0.1	<0.1	2.9	0.34	0.27
UK-3310045	40	35	0.17	<0.1	0.10	<0.1	<0.1	17.7	<0.1	<0.1	<0.1	0.1	32.2	2.4
UK-3310127	18	16	0.32	<0.1	<0.1	<0.1	<0.1	14.9	<0.1	<0.1	<0.1	0.1	48.9	3.1
BG-3710058	17	14	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	nd	<0.1	0.34
I-4510017	25	20	3.0	<0.1	0.13	<0.1	<0.1	<0.1	8.4	<0.1	<0.1	10.8	<0.1	31.6
>0.35kU/l*			70	0	0	0	0	20	60	0	10	67	50	60

*%positive.

individual peanut components was detectable. IgE to peanut extract was positive in 70% of late-onset anaphylactic patients (n = 10), whereas 90% of these patients had IgE to at least one of the peanut components tested.

Are there any predictive parameters for systemic reactions to peanut?

All patients with a history of anaphylaxis to peanut and the 13 patients with objective symptoms under DBPCFC such as urticaria, flush, angio-oedema, gastrointestinal or respiratory symptoms were included into the group of patients with systemic reactions. Systemic reactions were observed more frequently in the northern and eastern regions (96% and 89%, respectively) than in the southern and western/central regions (67% and 60%, respectively). No pairwise comparisons were performed because of statistical limitations due to the relatively low number of patients per region. Logistic regression revealed a significant influence of peanut extract sensitization and region on the occurrence of systemic reactions (P = 0.0185 and P = 0.0436, respectively). Gender, age at onset of peanut allergy and sensitization to peanut components (≥0.35 kU_A/l) all showed no influence on clinical severity ($P > 0.05$). However, a level of IgE to rAra h 2 ≥ 1.0 kU_A/l conferred a 97% probability for a systemic reaction (P = 0.0002; Fisher's exact test) (Fig 6).

The risk of developing systemic reactions was significantly higher for peanut extract-sensitized patients (≥0.35 kU_A/l) than for nonsensitized patients (odds ratio (OR): 16.7; 95% confidence interval: 1.6 – 167, P = 0.0185). Results for global and univariate regression logistic modelling, the odds ratios, their 95% confidence intervals and the P values are summarized in Table 6 (online repository).

For rAra h 2-sensitized patients (≥0.35 kU_A/l), the risk was increased, although not significantly (OR 2.6; 95% confidence interval: 0.4–17.5; P = 0.3219).

Comparison of threshold dose and sensitization pattern

The threshold dose of peanut eliciting symptoms during challenge (LOAEL) among the 28 DBPCFC+ve patients (18 early onset, 10 late onset) was analysed in relation to their quantitative IgE test results. Some patients with higher level of IgE to rAra h 2 (or extract) showed lower LOAEL for subjective symptoms and vice versa, but no clear correlation could be detected (Spearman's rank correlation coefficient: -0.077 and -0.135, respectively). The same was observed for IgE level to rAra h 2 or extract and LOAEL for 'any (i.e. subjective and objective) symptoms' (correlation: -0.195 and -0.264, respectively).

Discussion

This study represents a comprehensive CRD analysis quantifying IgE concentration to peanut allergens by the ImmunoCAP method in 150 European individuals recruited within two EuroPrevall cohorts, the outpatient clinical study (8) and the community survey (7). The full data set is presented in

Table 4 Tolerant control subjects with a negative DBPCFC: age at inclusion into the study and IgE antibody concentrations (kU_A/l)

Patient ID	Age at inclusion	Age at onset	peanut extract	Ara h 1	Ara h 2	Ara h 3	Ara h 6	Ara h 8	Ara h 9	CCD	Profilin	Pru p 3	birch extract	phleum extract
Patients with a positive case history of first manifestation of peanut allergy < 14 years														
CH-610004	22	10	4.3	<0.1	<0.1	<0.1	<0.1	1.5	9.1	<0.1	<0.1	10.2	3.3	15.6
E-710098	30	10	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.19	<0.1	<0.1	0.28	<0.1	28.5
E-710273	8	8	1.5	<0.1	<0.1	<0.1	<0.1	2.3	<0.1	<0.1	<0.1	<0.1	11.3	35.6
BG-3710098	14	11	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.23	<0.1
CZ-3810068	18	12	0.20	<0.1	0.23	<0.1	<0.1	<0.1	0.79	<0.1	<0.1	1.2	<0.1	<0.1
IS-4810001	19	5	1.7	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	2.1	<0.1	1.7	14.6
Patients with a positive case history of first manifestation of peanut allergy > 14 years														
CH-610217	28	16	0.25	<0.1	<0.1	<0.1	<0.1	<0.1	0.24	<0.1	<0.1	0.94	0.12	5.3
CH-610295	30	20	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	11.7	0.43
E-710088	23	19	1.7	<0.1	<0.1	<0.1	<0.1	<0.1	0.28	0.30	4.18	1.4	6.5	>100
E-710123	35	32	0.79	<0.1	0.10	<0.1	<0.1	<0.1	3.8	<0.1	<0.1	11.9	1.3	8.6
GR-1710007	31	22	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.79	<0.1	<0.1	1.5	<0.1	<0.1
GR1710025	43	27	0.0	<0.1	<0.1	<0.1	<0.1	2.8	<0.1	<0.1	<0.1	11.9	0.43	4.9
GR-1710053	24	15	0.41	<0.1	<0.1	<0.1	<0.1	<0.1	0.61	<0.1	<0.1	nd	<0.1	0.33
GR-1710099	26	24	0.27	<0.1	0.22	<0.1	<0.1	<0.1	0.62	<0.1	<0.1	0.73	<0.1	0.18
GR-1710170	30	23	4.0	<0.1	<0.1	<0.1	<0.1	<0.1	9.1	<0.1	<0.1	28.0	<0.1	0.38
GR-1710186	24	24	0.35	<0.1	<0.1	<0.1	<0.1	<0.1	1.4	<0.1	<0.1	11.0	<0.1	40.3
NL-2010074	47	46	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
PL-3210140	32	32	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
PL-3210212	52	52	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
PL-3210308	32	28	6.3	<0.1	0.33	<0.1	<0.1	<0.1	13.4	<0.1	<0.1	14.5	<0.1	0.43
F-4710159	34	30	0.30	<0.1	<0.1	<0.1	<0.1	3.6	0.17	<0.1	<0.1	0.63	27.4	7.5
IS-4810029	42	38	0.85	<0.1	0.12	<0.1	<0.1	<0.1	6.4	<0.1	<0.1	<0.1	0.13	0.29
IS-4810031	32	20	3.0	<0.1	<0.1	<0.1	<0.1	<0.1	9.9	<0.1	<0.1	<0.1	6.3	1.5
Patients with no records on the age at first manifestation of peanut allergy														
GR-1710152	31	nk	7.3	<0.1	<0.1	<0.1	<0.1	<0.1	19.0	0.29	<0.1	27.0	0.92	1.9
NL-2010078	23	nk	0.37	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.30	<0.1	<0.1	1.6	0.64
IS-4810093	23	nk	1.0	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	1.5	<0.1	2.5	27.8
>0.35 kU/l*			54	0	0	0	0	19	46	0	12	56	42	65

*%positive; nk, not known.

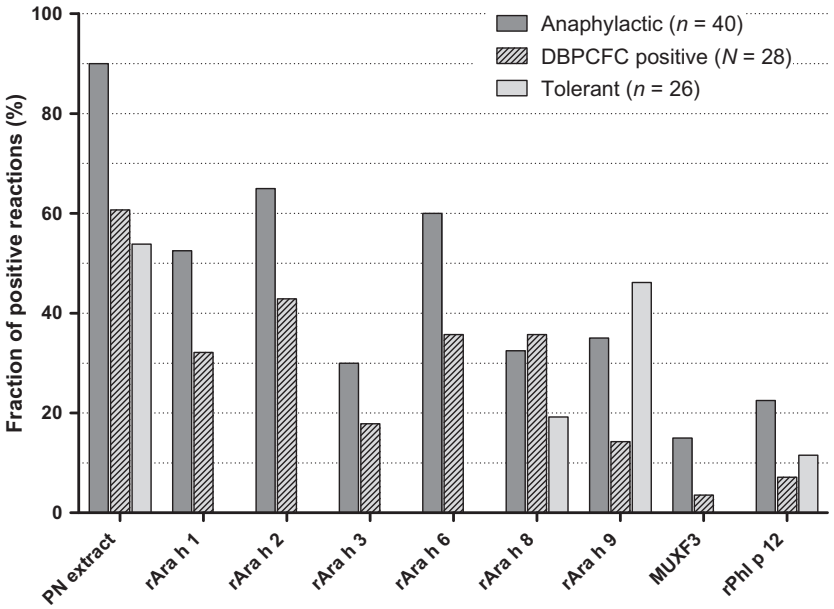

Figure 1 Percentage of patients and controls with sensitization to peanut extract and to the different components in peanut-allergic patients (DBPCFC+ve plus anaphylaxis patients) and in tolerant controls with a negative DBPCFC but a positive case history.

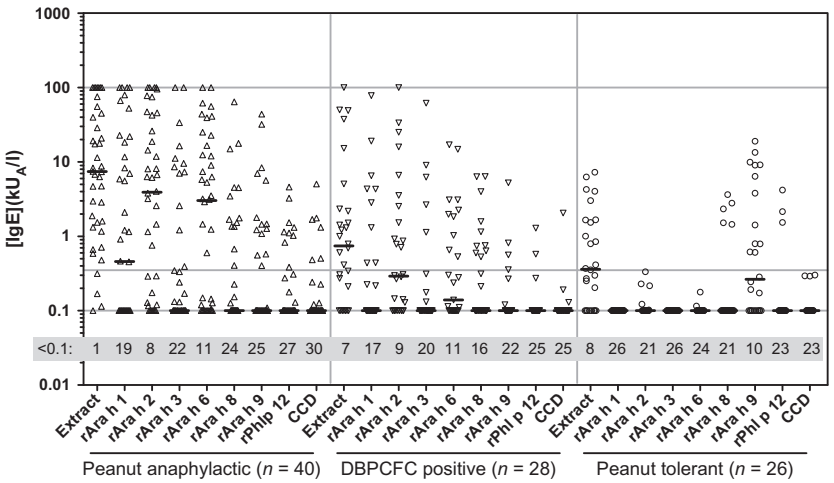

Figure 2 Concentration of IgE antibodies (kU$_A$/l) to extract and to different components in peanut-allergic patients (A: DBPCFC+ve plus anaphylaxis patients) and in controls with a negative DBPCFC but a positive case history (B).

detail in Tables 2–4. Sixty-eight patients with a confirmed peanut allergy were included, 48 of whom acquired the disease before and 20 patients after the age of 14. The population comprised 13 children (aged <14 years) and 55 adolescents or adults (aged ≥14 years). A subgroup of the study population appeared to have not a primary but a pollen-related (PR-10) or LTP-related peanut allergy, with their IgE response directed to cross-reactive peanut protein present in low amounts in peanut extract. Some patients with such a cross-reactive sensitization tested negative to peanut extract. The overall sensitivity of IgE measurement to peanut extract was 78% in this study, indicating that almost one quarter of mainly adult European peanut-allergic patients may be missed by conventional testing with peanut extract. Similar results with a sensitization rate to peanut extract of 80% were obtained in challenge-positive children from the UK with a mean age of 7 years (23). In contrast, 96% of a population of peanut-allergic paediatric patients from the United States showed positive IgE test results to peanut extract (24).

Sera of all patients and controls in this study were analysed for IgE to the majority of peanut allergens identified so far, including the four storage proteins, rAra h 1 (7S globu-

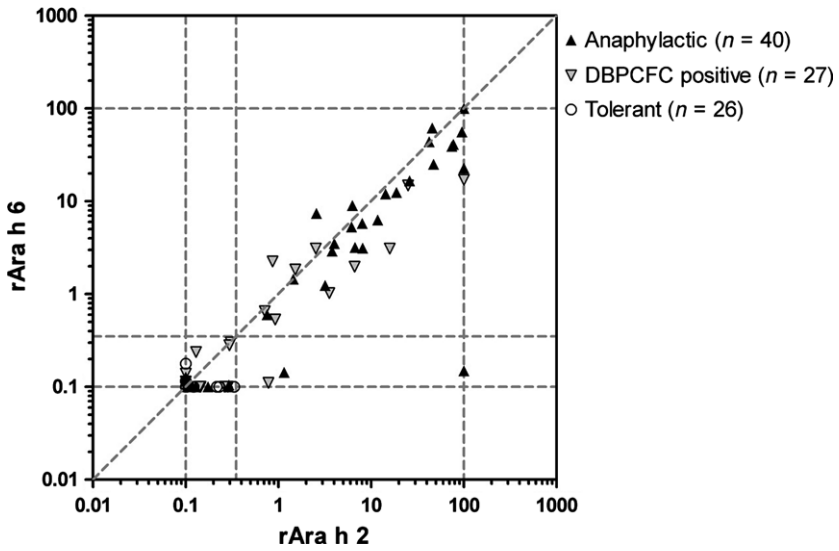

Figure 3 Concentration of IgE antibodies (kU_A/l) to rAra h 2 and rAra h 6 in peanut allergics (DBPCFC+ve and anaphylaxis) and in tolerant but history-positive controls.

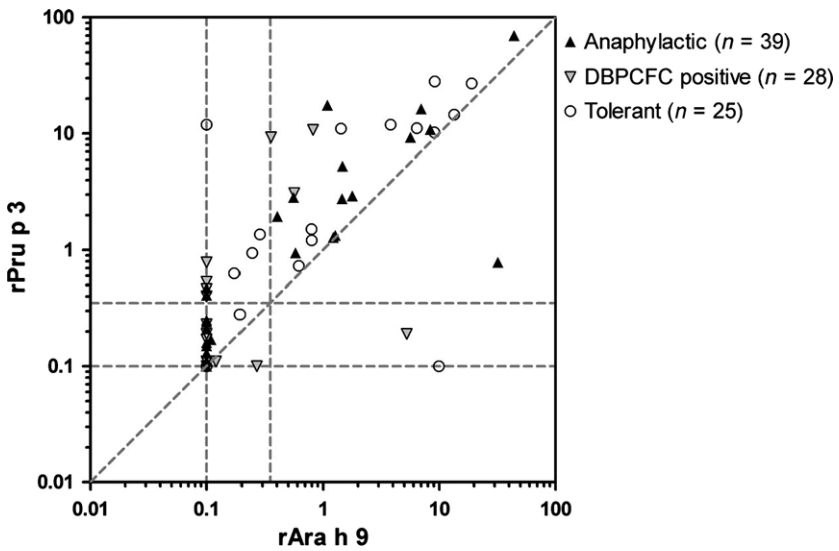

Figure 4 Concentration of IgE antibodies (kU_A/l) to rAra h 9 and to rPru p 3 in peanut allergics (DBPCFC+ve and anaphylaxis) and in tolerant but history-positive controls.

lin), rAra h 2 and 6 (2S albumins) and rAra h 3 (11S globulin), as well as rAra h 8 (Bet v 1 homologue or PR-10 protein), rAra h 9 (lipid transfer protein, LTP) and additionally, IgE to profilin (rPhl p 12) and cross-reactive carbohydrate determinants (CCD). For reasons of availability, relevance or redundancy, no measurements of IgE to Ara h 7, a low-abundant 2S albumin related to Ara h 2 and Ara h 6 (25), Ara h 4, reclassified by the WHO/IUIS Allergen Nomenclature Subcommittee as an isoform of Ara h 3 (26), and peanut oleosins Ara h 10 and Ara h 11 (27,28) were performed. The use of molecular allergens in this study population led to an increase in diagnostic sensitivity from 78% to 90%, mostly due to rAra h 8 and rAra h 9 and less prominently to rAra h 3.

The frequency of sensitization to rAra h 2 and rAra h 6 among the patients studied here was 56% and 50%, respectively, making peanut 2S albumins the only major allergen for these European patients. This finding is consistent with those of several other studies (10, 11, 14–16). Concentrations of IgE to rAra h 2 and rAra h 6 correlated strongly in this study (Fig. 3). Even though a recent case report described a patient with an isolated sensitization to Ara h 6 (29), our results show such an extensive overlap in IgE reactivity to these two 2S albumins that Ara h 6 appears not to be an essential part of

Table 5 Prevalence of sensitization to peanut extract and components in different climatic regions in the peanut allergic population

	All patients $n = 68$ (%)	North $n = 25$ (%)	West/Central $n = 25$ (%)	East $n = 9$ (%)	South $n = 9$ (%)	*P*-value*
Allergens (sIgE ≥0.35 kU/l)						
peanut extract	78	80	84	56	78	0.3838
rAra h 1	44	52	56	22	11	0.0505
rAra h 2	56	64	56	56	33	0.4971
rAra h 3	25	32	32	0	11	0.1495
rAra h 6	50	60	48	44	33	0.5758
rAra h 8	34	28	52†	33	0	0.0286
rAra h 9	26	24	12	33	67†	0.0159
Profilin	16	16	24	11	0	0.4852
CCD	10	8	16	11	0	0.7865
≥1 component	90	96	92	56	100	0.0140

**P*-values for comparison of overall differences among all 4 climatic regions, Fisher's exact test.
Significant differences (*P*-values adjusted for multiple comparisons according Bonferroni–Holm) between single climatic regions.
†Marks significant differences between West/Central *vs* South, placed at the highest value.

the component-based diagnostic work-up in peanut allergy. Being available for IgE testing in any routine laboratory, rAra h 2 is clearly the most important screening tool for the detection of sensitization to peanut storage proteins.

While sensitization to multiple peanut storage proteins has been linked to higher probability of symptoms (10, 30), measurement of IgE to rAra h 1 and rAra h 3 does not contribute significantly to diagnostic sensitivity, and in the present study, only four peanut-allergic patients were sensitized to rAra h 1 or rAra h 3 in the absence of IgE to rAra h 2. Our finding of a high rate of co-sensitization to rAra h 1 and rAra h 3 in rAra h 2-positive patients might to some extent be explained by the presence of IgE-binding peptides with similar sequences in these three otherwise nonhomologous peanut allergens (31), potentially conferring some degree of cross-reactivity.

Most importantly, all of the 70 atopic controls, with or without the history of peanut allergy, and the nonatopic controls were negative to all four peanut storage proteins tested. Thus, sensitization to storage proteins was 100% specific for clinical peanut allergy in this study. Similar results have been published recently. In French patients with peanut allergy, a cutoff level of sIgE to Ara h 2 of 0.29 kU_A/l was 100% specific for peanut allergy (10), whereas cutoff levels of 1.63 kU_A/l in a Danish population (11) and 0.55 kU_A/l in a British population (14) were required to obtain a specificity of 100%.

Of the 40 patients with a history of anaphylactic reactions to peanut, two were negative to all allergen components tested, and four had detectable IgE only to components unlikely to cause severe reactions (3/40 to Ara h 8 und 1/40 to profilin). We speculate that peanut oleosin (27, 28, 32), which was not included in our allergen panel, may have caused the severe reactions in these patients. Most importantly, sensitization to storage proteins is not a prerequisite to develop an anaphylactic reaction.

A particularly important finding in this study was that the vast majority of the patients (85%) with early-onset peanut allergy were sensitized to storage proteins, whereas all but one patient with a late-onset peanut allergy were negative to all four storage proteins. Patients in the latter group were instead predominantly sensitized to peanut components cross-reactive to pollen (e.g. Ara h 8) or plant-derived food (Ara h 9). Our results indicate that sensitization to storage proteins mainly occurs in childhood and is rarely acquired in adolescence and adulthood. These findings promote the speculation that an affected intestinal barrier and an increased intestinal permeability as observed in young children genetically predisposed to allergic diseases are prerequisites for the onset of sensitization towards storage proteins.

We observed significant differences in the sensitization pattern of the atopic controls, depending on the presence or absence of a positive case history of peanut allergy. Of those with a positive case history and a negative challenge, 77% were sensitized to peanut extract and/or components, as compared to only 23% of the controls with a reported tolerance to peanut. Among the DBPCFC-negative controls with a history of peanut allergy and sensitization to peanut extract and/or components, IgE to rAra h 9 was detected in 60% and to rAra h 8 in 25%. It is possible that these individuals have previously been peanut allergic but unknowingly developed tolerance, that they suffer from other food allergies and falsely perceive allergy also to peanut, or that they need a cofactor to trigger the allergic reaction.

Even though allergic symptoms to peanut are associated with isolated sensitization to Ara h 8 or Ara h 9 in many patients across Europe, the majority of individuals sensitized to these allergens tolerate peanut. In our study population, IgE antibodies to LTP were present in 27% of all peanut-allergic patients and in 19% of all atopic controls. Thus, sensitization to rAra h 9 or Ara h 8 did not discriminate between peanut-allergic and tolerant subjects in this study. In the absence of other diagnostic criteria, IgE to Ara h 8 or Ara h 9 is therefore not predictive of clinical reactivity to peanut.

All subjects with IgE to rAra h 8 were positive to birch pollen extract, and all subjects with sIgE to rAra h 9 were

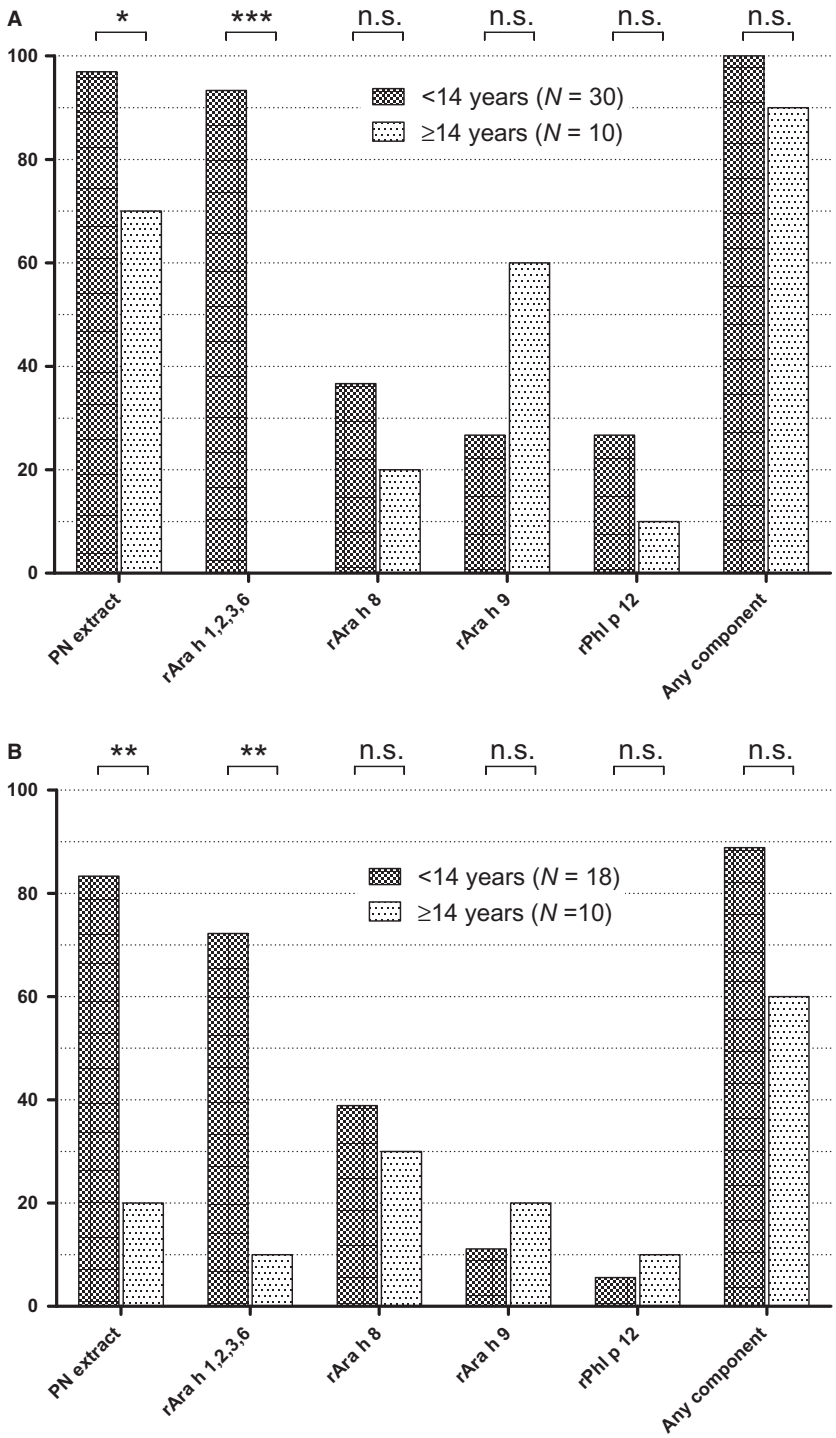

Figure 5 Age dependency of sensitization pattern to peanut extract and to peanut components in anaphylactic patients (A) and in DBPCFC+ve patients (B). *< 0.05; **<0.01; ***<0.001

positive to rPru p 3. The IgE concentrations were significantly higher to rPru p 3 than to rAra h 9 ($P < 0.0001$) in all but five patients, three of which were peanut tolerant. Our findings suggest that primary sensitization to LTP had in most cases been induced by peach and that IgE binding to peanut LTP occurred due to cross-reactivity with Pru p 3. Similar findings have been published for patients with hazelnut allergy and IgE to Cor a 8, the LTP of hazelnut (33).

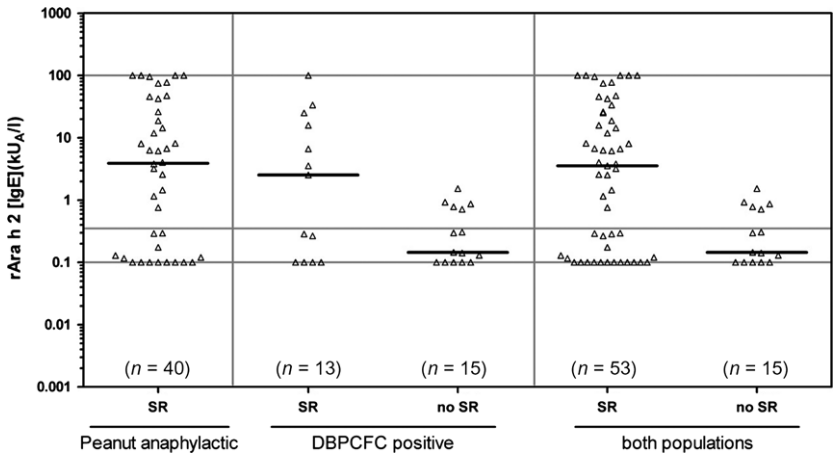

Figure 6 Concentration of IgE to rAra h 2 in peanut-allergic patients with anaphylaxis and with positive DBPCFC analysed according to the severity of clinical reaction (SR: systemic reaction; no SR: no systemic reaction).

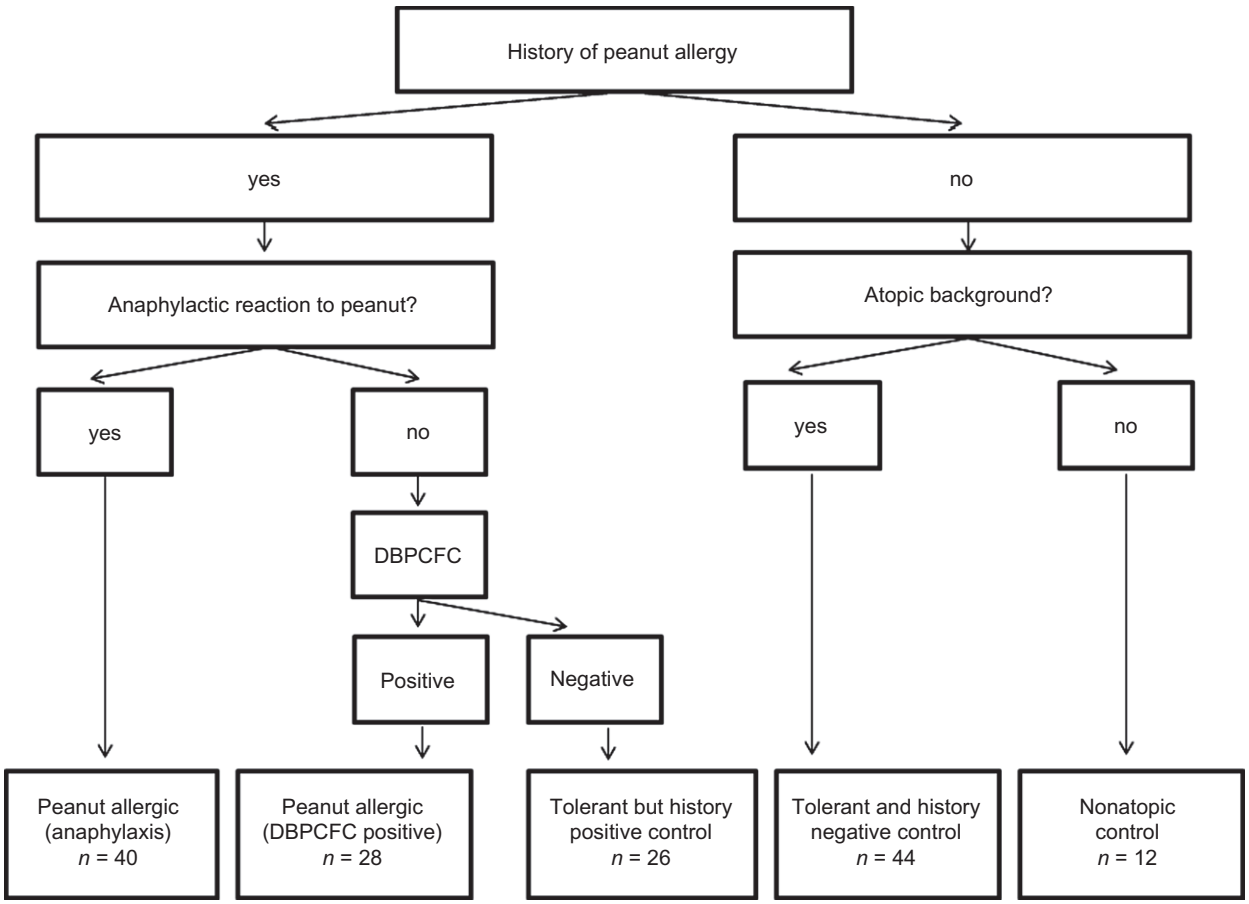

Figure 7 Flow chart for the grouping of patients and controls.

To compare the sensitization pattern across Europe, we attributed our patients to four climatically different regions: northern Europe (IS, UK), western/central Europe (F, CH, NL), eastern Europe (BG, CZ, PL) and southern Europe (IT, ES, GR). Significant differences were only detected between western/central Europe and southern Europe. As a

Table 6 (a) Global logistic regression model for systemic reactions ($n = 68$ subjects) sensitization against extract, (b) Univariate logistic regression model for systemic reactions ($n = 68$ subjects), (c) Global logistic regression model for systemic reactions ($n = 68$ subjects), sensitization against rAra h 2

Effect	Comparison	OR	95% CI	*P* value
(a)				
Gender	female *vs* male	2.068	0.420–10.174	0.3714
Age group	<14 years *vs* ≥14 years	1.867	0.268–12.983	0.5282
Region	East *vs* West/Central	32.884	1.403–770.812	0.0436
	North *vs* West/Central	39.374	2.435–636.730	
	South *vs* West/Central	1.880	0.212–16.688	
Extract	≥0.35 *vs* <0.35	16.667	1.605–166.67	0.0185

Effect	Comparison	*N*	OR	95% CI	*P* value
(b)					
Gender	female *vs* male	68	1.742	0.549–5.522	0.3461
Age group	<14 years *vs* ≥14 years	68	2.692	0.818–8.866	0.1034
Region	East *vs* West/Central	34	5.333	0.575–49.477	0.5684
	North *vs* West/Central	50	15.995	1.855–137.888	0.0572
	South *vs* West/Central	34	1.333	0.269–6.606	0.1704
Extract	≥0.35 *vs* <0.35	68	4.926	1.393–17.544	0.0134

Effect	Comparison	OR	95% CI	*P* value
(c)				
Gender	female *vs* male	2.417	0.546–10.696	0.2448
Age group	<14 years *vs* ≥14 years	2.362	0.276–20.237	0.4328
Region	East *vs* West/Central	7.498	0.636–88.384	0.0693
	North *vs* West/Central	14.646	1.581–135.686	
	South *vs* West/Central	2.010	0.212–14.531	
r Ara h 2	≥0.35 *vs* <0.35	2.611	0.391–17.544	0.3219

consequence of the overall high sensitization rate to birch pollen in western/central Europe (34), rAra h 8 was the major allergen for peanut-allergic patients from France, Switzerland and the Netherlands. In contrast, rAra h 9 was the major allergen in peanut-allergic patients from Spain and Greece.

These findings are consistent with a retrospective analysis of paediatric subjects from the United States, Spain and Sweden. Sensitization to one or more of the peanut storage proteins was more prevalent in Swedish and US children (up to 74% and 90%, respectively), whereas children from Spain were primarily sensitized to Ara h 9 (60%) (17).

To identify risk factors for systemic reactions in our peanut-allergic population, a logistic regression model was applied with clinical severity as dependent variable (incidence of systemic reactions) and sex, age at onset of peanut allergy (younger/older than 14 years), geographical region and sensitization to peanut extract or components (below/above threshold 0.35 kU_A/l) as independent variables. Logistic regression revealed a significant influence of peanut extract sensitization and region on the occurrence of systemic reactions ($P = 0.0185$ and $P = 0.0436$, respectively). Gender or age at the onset of peanut allergy showed no influence on clinical severity ($P > 0.05$). Furthermore, the severity of clinical reactions was not associated with the presence of IgE (≥0.35 kU_A/l) to any of the single peanut components tested. This was also true for rAra h 2 sensitization (≥0.35 kU_A/l) which was not indicative of the type of clinical manifestation. Thus, our results are in line with other studies where a dichotomous readout (positive or negative) of sIgE to rAra h 2 correlated with the presence or absence of peanut allergy but not with the severity of clinical manifestations (10, 11, 14). However, at a level of sIgE ≥1.0 kU_A/l to rAra h 2, there was a 97% probability ($P = 0.0002$) for a systemic reaction among our peanut-allergic subjects. Hence, while the mere presence of detectable IgE to rAra h 2 was not in itself predictive of a severe reaction phenotype in this population, systemic reactions to peanut occurred almost invariably in subjects with high concentrations of IgE to rAra h 2. Apart from Ara h 6, which appeared highly cross-reactive with Ara h 2, this was not the case for any of the other peanut components in this study.

The expected (negative) correlation between the lowest symptom-eliciting dose of peanut protein (LOAEL) and the sensitization pattern (regarding rAra h2 or peanut extract) was only barely detectable.

In summary, we report a study of molecular sensitization patterns in a large and well-characterized peanut-allergic population representing different European regions and in peanut-tolerant atopic and nonatopic controls. Our findings

point towards a higher sensitivity for the CRD approach as compared to peanut extract, underline the importance of IgE to storage proteins, particularly Ara h 2 for the diagnosis of clinical peanut allergy, and indicate that the most commonly occurring sensitization to peanut proteins in individuals who tolerate peanut is directed to Ara h 8 and Ara h 9. According to our results, sensitization to storage proteins is usually not acquired in adolescent and adult patients. Peanut components cross-reactive with pollen or fruit (peach) were also identified as the main IgE-binding components in subjects with late-onset peanut allergy (≥14 years). In a broader perspective, the study illustrates how allergy to a particular food may vary both within and between geographical regions in regard to characteristics and origin of sensitization, demonstrating that the use of diagnostic tools and the interpretation of laboratory results need to be locally adapted. A potential drawback of our study is that we did not have ethical approval to challenge the patients with an anaphylactic reaction to peanut. However, this particular group of patients has been included in a predefined careful review process performed in three centres with particular experience in food allergy on the criterion of a severe life-threatening reaction.

The uniqueness of the study is reflected by the following facts:

1 Patients were prospectively recruited on the sole criterion of a history of peanut allergy and were not preselected on the presence of IgE to peanut which might affect the outcome of CRD analysis.
2 Patients recruited into this multicentre study underwent a completely harmonized diagnostic procedure.
3 This is the first study reporting a comprehensive pan-European characterization of peanut allergy demonstrating differences in sensitization pattern to peanut allergens and a significant impact of region on the severity of manifestation.
4 This study included children and adults and demonstrated a potential age dependency on the acquisition of sensitization to peanut storage proteins.
5 This is the first study suggesting that levels of IgE to Ara h 2 are indicative for the severity of the clinical response.

Author contributions

Barbara K. Ballmer-Weber has contributed to the study design, the clinical results, to the analysis of the results, the writing of the manuscript and has coordinated the EuroPrevall challenges. Montserrat Fernández-Rivas has coordinated the cross-sectional study of EuroPrevall, contributed to the clinical results and reviewed the manuscript. The following authors were part of the EuroPrevall clinical study teams and have contributed to the clinical results: Suranjith Seneviratne, Peter Bures, Colin Summers, André C. Knulst, Thuy-My Le, Nikolaos G. Papadopoulos, Isabel Reig, Simona Belohlavkova, Todor Popov, Frédéric de Blay, Michael Clausen, Marek L. Kowalski, Riccardo Asero and Ruta Dubakiene. Kay-Martin Hanschmann has performed the statistical analysis, Lothar Vogel did the *in vitro* analysis, Clare Mills and Ronald van Ree have coordinated the EuroPrevall project and Ronald van Ree was responsible for the cross-sectional study and did major review contribution. Jonas Lidholm and Stefan Vieths have contributed to the design of the *in vitro* study, to the analysis of the results and writing of the paper.

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Conflict of interest

Dr Lidholm is employed by Thermo Fisher Scientific and received funding from EU project FP6- FOOD-CT-2005-514000. Dr Ballmer-Weber reports grants from EU, Brussels, during the conduct of the study; other from ThermoFisher, outside the submitted work; Dr Fritsche reports personal fees from EU, during the conduct of the study; Dr van Ree reports grants from EU Framework Program, during the conduct of the study; personal fees from HAL Allergy BV, outside the submitted work; Dr Vogel reports grants from European Union Project 'EuroPrevall', during the conduct of the study; Dr Fernandez-Rivas reports grants from European Commission, during the conduct of the study; Dr PAPADOPOULOS reports grants from GSK, grants from NESTLE, grants from MERCK, personal fees from ABBVIE, personal fees from SANOFI, personal fees from MENARINI, personal fees from MEDA, personal fees from GSK, personal fees from ABBVIE, personal fees from NOVARTIS, personal fees from MENARINI, personal fees from MEDA, personal fees from ALK-ABELLO, personal fees from NOVARTIS, personal fees from ALLERGOPHARMA, personal fees from URIACH, personal fees from GSK, personal fees from STALLERGENS, personal fees from MSD, outside the submitted work; Dr Knulst reports grants from the EC, for conducting the study. Prof. Dr S. Vieths reports grants from European Union, during the conduct of the study; personal fees from Food Allergy Resource and Research Program, Lincoln, NE USA, personal fees from Medical University of Vienna, Austria, grants from Monsanto Company, personal fees from American Academy of Asthma, Allergy and Immunology, personal fees from Deutsche Dermatologische Gesellschaft, personal fees from Spanish Society of Allergy and Clinical Immunology, personal fees from Westdeutsche Arbeitsgemeinschaft für pädiatrische Pneumologie und Allergologie e.V., Köln, Germany, personal fees from Gesellschaft für pädiatrische Allergologie und Umweltmedizin, personal fees from Ärzteverband Deutscher Allergologen, personal fees from Schattauer Allergologie Handbuch, personal fees from Elsevier Nahrungsmittelaller-gien und Intoleranzen, non-financial support from German Research Foundation, non-financial support from Federal Institute for Risk Assessment, non-financial support from Austrian Society for Allergology and Immunology, non-financial support from European Directorate for the Quality of Medicines and Health Care, non-financial support from European Academy of Allergy and Clinical Immunology, non-financial support

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[Correction added on 26 March 2015. The COI statement for Dr. Mills was omitted in the print version while it has been corrected in the online version.]

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