

Allergy or tolerance in children sensitized to peanut: Prevalence and differentiation using component-resolved diagnostics

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Background: Not all peanut-sensitized children develop allergic reactions on exposure.

Objective: To establish by oral food challenge the proportion of children with clinical peanut allergy among those considered peanut-sensitized by using skin prick tests and/or IgE measurement, and to investigate whether component-resolved diagnostics using microarray could differentiate peanut allergy from tolerance.

Methods: Within a population-based birth cohort, we ascertained peanut sensitization by skin tests and IgE measurement at age 8 years. Among sensitized children, we determined peanut allergy versus tolerance by oral food challenges. We used open challenge among children consuming peanuts ($n = 45$); others underwent double-blind placebo-controlled challenge ($n = 34$). We compared sensitization profiles between children with peanut allergy and peanut-tolerant children by using a microarray with 12 pure components (major peanut and potentially cross-reactive components, including grass allergens).

Results: Of 933 children, 110 (11.8%) were peanut-sensitized. Nineteen were not challenged (17 no consent). Twelve with a convincing history of reactions on exposure, IgE ≥ 15 kU/L and/or skin test ≥ 8 mm were considered allergic without challenge. Of the remaining 79 children who underwent challenge, 7 had ≥ 2 objective signs and were designated as having peanut allergy. We estimated the prevalence of clinical peanut allergy among sensitized subjects as 22.4% (95% CI, 14.8% to 32.3%). By using component-resolved diagnostics, we detected marked differences in the pattern of component recognition between children with peanut allergy ($n = 29$; group enriched with 12 children with allergy) and peanut-tolerant children ($n = 52$). The peanut component Ara h 2 was the most important predictor of clinical allergy.

Conclusion: The majority of children considered peanut-sensitized on the basis of standard tests do not have peanut

allergy. Component-resolved diagnostics may facilitate the diagnosis of peanut allergy. (*J Allergy Clin Immunol* 2010;125:191-7.)

Key words: Peanut allergy, oral food challenge, component-resolved diagnostics, Ara h 2, microarray, birth cohort

Peanut allergy is one of the most common food allergies, affecting $\sim 1\%$ of the population,^{1,2} with recent reports suggesting that it is increasing in prevalence.³⁻⁵ Unlike other food allergies, which present early in life and are usually outgrown by school age (eg, cow's milk, egg), peanut allergy tends to be lifelong.⁶ Avoidance of peanut remains the mainstay of management⁵ but is difficult to achieve because of peanut's widespread use in prepared foods and its popularity as a cheap and nutritious food.⁷ Consequently, accidental exposures are common and may be life-threatening, reducing the quality of life among patients with peanut allergy and their families.^{8,9}

Given the impact of peanut allergy, accurate diagnosis is critically important. The double-blind placebo controlled food challenge (DBPCFC) is the gold standard for diagnosis of peanut allergy.^{1,10,11} However, it is time-consuming and expensive, and there is a risk of severe reaction. In practice, diagnosis is usually based on a suggestive clinical history after exposure to peanut, supported by positive specific serum IgE (sIgE) and/or skin prick test (SPT).^{1,12} However, both these tests detect the presence of allergen-specific antibodies (sensitization), which does not equate to the presence of allergic symptoms after exposure to the sensitizing allergen (clinical allergy).¹³ One of the reasons current tests differentiate asymptomatic sensitization from peanut allergy relatively poorly may be that they are based on crude natural peanut extracts that contain both allergenic and nonallergenic molecules.^{14,15} Because some of these molecules may cross-react with pollen or other allergens,¹⁶⁻¹⁸

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Abbreviations used

CRD: Component-resolved diagnostics
 DBPCFC: Double-blind placebo-controlled food challenge
 MWD: Mean wheal diameter
 OFC: Oral food challenge
 sIgE: Serum IgE
 SPT: Skin prick test
 UK: United Kingdom

peanut sensitization may not equate to clinical peanut allergy.^{3,19,20}

Recent progress in molecular biology and biochemistry has led to recombinant production of individual allergenic proteins for several allergens including peanut.^{14,21-25} Ara h 1 to 3 are considered major peanut allergens.²⁶ These molecules can be used in component-resolved diagnostics (CRD) tests, which may be more accurate diagnostic tools for the assessment of food allergy.²⁷⁻²⁹

Within a population-based birth cohort, we established the proportion of children with peanut sensitization by using standard SPT and sIgE. Among sensitized children, we determined the presence or absence of clinical peanut allergy by using oral challenge tests. We then investigated the utility of a novel CRD test by using microarray technology to correctly identify those children with clinical peanut allergy.

METHODS**Study design, setting, and participants**

The Manchester Asthma and Allergy Study is an unselected population-based birth cohort.³⁰ Participants were recruited prenatally and followed prospectively, attending review clinics at ages 1, 3, 5, and 8 years. The study is registered as ISRCTN72673620 and approved by the Local Research Ethics Committee (04/Q1403/45). Written informed consent was obtained from all parents and children gave their assent. For detailed *Methods*, see this article's Online Repository at www.jacionline.org.

Definition of variables

At age 8-year review, we collected detailed information on exposure and reactivity to peanut by using a validated questionnaire.³¹

Assessment of peanut sensitization. We ascertained peanut sensitization by SPT (Hollister-Stier, Wash) and measurement of peanut-specific sIgE (ImmunoCAP; Phadia, Uppsala, Sweden). We defined sensitization as mean wheal diameter (MWD) at least 3 mm greater than the negative control and/or peanut sIgE ≥ 0.2 kU/L.

Assessment of peanut allergy. Children who were classified as peanut-sensitized ($n = 110$) were invited for a detailed assessment of their clinical reactivity to peanut. This included further validated questionnaires,^{12,20} skin tests to peanut and tree nuts, and sIgE measurement (if not already available). We considered subjects with a convincing history of reaction on exposure to peanut combined with sIgE ≥ 15 kU/L and/or SPT ≥ 8 mm as having peanut allergy without challenging.^{12,32,33} All other children underwent oral food challenge (OFC; details in the *Methods* section in the Online Repository). We used open OFCs among children consuming peanuts; these were performed in 4 stages (10 mg, 100 mg, 1 g, and 5 g peanut protein in brownies) at 30-minute intervals. Three children with milk/egg allergy had open OFC with roasted peanuts (1/8-15 peanuts). All others underwent DBPCFCs; these were performed in 10 stages (1 mg, 10 mg, 100 mg, 1 g, and 5 g peanut protein in brownies) and lasted ~ 8 hours (including the 2-hour observation period after the administration of the final dose). Challenge was considered positive after development of at least 2 objective signs

indicating allergic reaction (eg, skin rash, sneezing, vomiting, cough, wheeze, $>20\%$ fall in FEV₁).

Definition of outcomes

Peanut allergy was defined as sensitized (positive SPT or sIgE) and positive OFC or a convincing history of reaction on exposure to peanut and peanut sIgE ≥ 15 kU/L and/or SPT ≥ 8 mm.

Peanut tolerance was defined as sensitized (positive SPT or IgE) but negative OFC.

Asthma, hay-fever, eczema, and food allergies were defined as parentally reported symptoms and medication use (from interviewer-administered validated questionnaires at each follow-up).

CRD

We proceeded to compare sensitization profile of children with peanut allergy with those peanut-tolerant by using CRD. Because the number of subjects with peanut allergy within the cohort with available blood sample was small ($n = 17$), we enriched the peanut-allergic group with 12 children (6 boys) age 7 to 14 years with confirmed peanut allergy recruited from a local allergy clinic (convincing history of reaction on exposure to peanut and sIgE ≥ 15 kU/L and/or SPT ≥ 8 mm). All subjects who underwent assessment by CRD had detectable peanut sIgE (≥ 2 kU/L).

Microarray assays for the determination of specific IgE binding to allergenic components were carried out by using microspot multiplex technique (see this article's *Figs E1* and *E2* in the Online Repository at www.jacionline.org). Briefly, pure components from peanut (Ara h 1-3 and 8), grass (Phl p 1, 4, 5b, 7, and 12) and potentially cross-recognizing components (Bet v 1, Pru p 3, and cross-reactive carbohydrate determinants [CCD]) were immobilized in triplicates on a capillary-flow membrane-based microarray. We measured the fluorescence intensity, and after preprocessing the resulting data, fold-change quantities were calculated as

$$\log_2 \left(\frac{\text{expression level estimate for a particular component and sample}}{\text{expression level estimate of the same component in the negative control pool}} \right).$$

This implies that a fold-change of, for example, 3 corresponds to having an expression level $2^3 = 8$ times higher than the negative control pool of nonatopic subjects.

Statistical analysis

Analysis was carried out by using the R system for statistical computing version 2.8.1³⁴ (Vienna, Austria) and SPSS for Windows 15.0 (Chicago, Ill). SPT MWD and levels of peanut sIgE had skewed distributions and are reported as medians and ranges. Prevalence estimation of outcomes is reported as proportions and 95% CIs.

The nonparametric random forest method was used to investigate the components' ability to correctly discriminate between subjects with peanut allergy and peanut-tolerant subjects.³⁵ The random forest permutation importance measure, based on each predictor's contribution to accurate discrimination (including multivariate interactions with other predictors), was used to judge the importance of each component (details in the *Methods* section in the Online Repository).

To ease interpretation of the graphical displays, a suitable ordering of the components was found by using seriation.³⁶

RESULTS

The study profile is shown in *Fig 1*. Of 1085 children born into the cohort, 1029 were reviewed at age 8 years (follow-up rate, 94.9%); 17 (1.6%) had parentally reported history of peanut allergy (details in the *Results* section in the Online Repository).

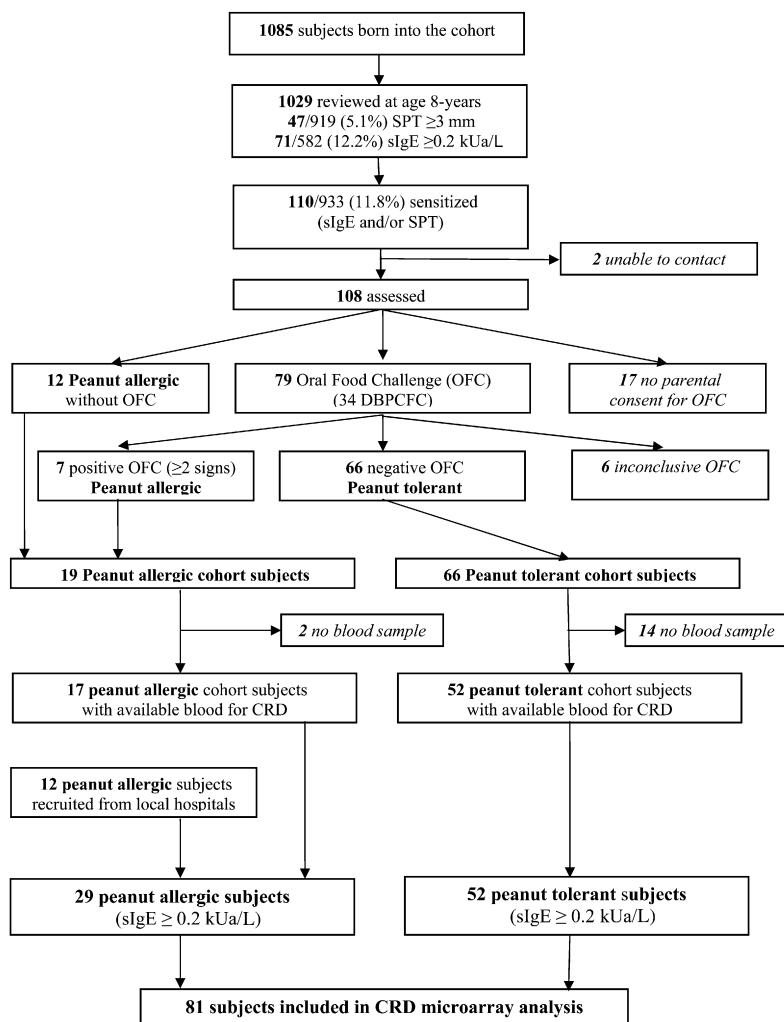


FIG 1. The study profile.

Sensitization to peanut

Of 1029 children, 919 (89.3%) were skin-tested. Forty-seven (5.1%) had a positive SPT to peanut (MWD, median [range], 5 mm [3-13 mm]). Ten children were monosensitized; sensitization to grasses was observed in 28 (59.6%) of peanut-sensitized subjects.

Of 582 children who agreed to venepuncture, 71 (12.2%) had detectable peanut sIgE (median kUa/L [range], 1.11 [0.24-443.44]). When using the 0.35 kUa/L cutoff level, 54 children (9.3%) were sensitized to peanut. Sensitization to grasses was observed in 67 (94.4%) of peanut-sensitized subjects.

Of 933 children with either SPT or sIgE data available, 110 (11.8%) were considered sensitized to peanut.

There were no significant differences in demographics between children who were skin-tested and venepunctured and those who were not (see this article's Table E1 in the Online Repository at www.jacionline.org).

Clinical peanut allergy and tolerance among peanut-sensitized children

Of 110 sensitized subjects, 108 (98%) were assessed for peanut allergy; 17 did not consent to OFC. Of the remaining 91 children,

12 had a convincing history of reaction to peanut and sIgE ≥ 15 kUa/L and/or SPT ≥ 8 mm and were designated as having peanut allergy without OFC. We carried out OFCs in 79 children (45 open, 34 DBPCFC). Of these, 66 had no symptoms, and 13 developed symptoms during OFC. Seven children had 2 or more objective signs (all on DBPCFC) and were designated as having peanut allergy. Six subjects had only 1 sign and/or subjective symptoms (2 open OFC); these were considered inconclusive challenges and excluded from further analysis. Details on the type of reactions developed on OFC are presented in this article's Table E2 in the Online Repository at www.jacionline.org. We did not observe any reactions to placebo brownies. Thus, of 85 peanut-sensitized children with unequivocal outcome, 66 were peanut-tolerant and 19 had peanut allergy, with the estimate of the proportion of children with peanut allergy among those sensitized to peanut being 22.4% (95% CI, 14.8% to 32.3%).

CRD

Serum was available in 29 children with peanut allergy and 52 peanut-tolerant children (Fig 1). Asthma, eczema, and food allergies were more common among subjects with peanut allergy, whereas hay fever was more common in peanut-tolerant children

TABLE I. Characteristics of children included in the CRD microarray study

Characteristic	Peanut-sensitized tolerant (n = 52)	Peanut-sensitized allergic (n = 29)	P value
Male sex, no. (%)	32 (62)	18 (62)	.96
Breast-fed, no. (%)	38 (73)	21 (72)	.95
Maternal allergic disease, no. (%)	25 (48)	18 (62)	.23
Paternal allergic disease, no. (%)	32 (61)	19 (68)	.57
Current asthma/wheeze, no. (%)	20 (39)	18 (62)	.04
Current hay fever, no. (%)	41 (79)	15 (52)	.01
Current eczema, no. (%)	16 (31)	17 (59)	.01
Other known food allergies, no. (%)	4 (8)	9 (31)	.006
Serum specific IgE (CAP) (kU/L), median (range)			
Peanut	0.96 (0.24-43.86)	26.47 (0.20-1249.00)	<.001
Grass	74.70 (0.20-1594.00)	6.93 (0.20-574.00)	<.001
Peanut >15 kU/L	1 (2)	16 (55)	<.001
Peanut SPT MWD >8 mm	1 (2)	17/28 (61)	<.001

(Table I). Peanut-tolerant children had lower peanut sIgE and higher grass sIgE. Among children with peanut allergy, 16 (55%) had peanut sIgE ≥ 15 kU/L and 17 (61%) SPT MWD ≥ 8 mm. When using the peanut specific IgE level > 15 kU/L cutoff for discriminating between subjects with peanut allergy and peanut-tolerant subjects, the overall misclassification rate was 17.3% (95% CI, 9.8% to 27.3%); for SPT MWD ≥ 8 mm, the overall misclassification rate was 15% (95% CI, 7.9% to 24.4%). The estimated sensitivity, specificity, and positive predictive value for various cutoff points for sIgE and skin tests in the cohort are presented in this article's Tables E3 to E6 in the Online Repository at www.jacionline.org.

The pattern of component recognition clearly differed between subjects with peanut allergy and peanut-tolerant subjects (Fig 2). Subjects with peanut allergy tended to have higher fold-change values to the major peanut components Ara h 1 to 3, whereas the peanut-tolerant subjects had higher values to CCD and grass components Phl p 1, Phl p 4, and Phl p 5b. The groups did not seem to differ for Ara h 8, Bet v 1, Pru p 3, Phl p 7, and Phl p 12. Ara h 2 appeared to offer the best discrimination: the groups were well separated over the whole fold-change range. As an example, the median fold-change for Ara h 2 was 6.06 and 0.28 in the subjects with peanut allergy and the peanut-tolerant subjects, respectively. This corresponds to expression levels $66.7 (= 2^{6.06})$ and $1.2 (= 2^{0.28})$ times higher than the negative control pool, with peanut-tolerant subjects differing only slightly from nonsensitized control subjects. We also noted that several components were pairwise associated, and that Ara h 2 offered good discrimination irrespective of the other components' values (see this article's Fig E3 in the Online Repository at www.jacionline.org). However, a large interindividual variation was observed (see this article's Fig E4 in the Online Repository at www.jacionline.org).

Discriminating peanut allergy from peanut tolerance. Using all components simultaneously, we used the random forest method to develop a model for discriminating between subjects with peanut allergy and peanut-tolerant subjects. The resulting model misclassified 2 (6.9%) subjects with peanut allergy and 4 (7.7%) peanut-tolerant subjects. The overall misclassification rate was 7.4% (95% CI, 2.8% to 15.4%).

As shown in Fig 3, the variable importance measure indicated that Ara h 2 contributed incomparably to accurate discrimination. The mean change in accuracy for Ara h 2 was 0.20, whereas all other components had a value less than 0.02.

To explore whether the discriminative accuracy could be improved if relevant clinical information was added, we developed a model simultaneously using all components, sex, current eczema, hay fever, asthma or other known food allergy, maternal and paternal history of allergic disease, and siblings' history of peanut allergy. Adding clinical information did not improve the discriminative accuracy, with this model misclassifying 3 (9.8%) subjects with peanut allergy and 5 (10.7%) peanut-tolerant subjects. The overall misclassification rate was 10.1% (95% CI, 4.5% to 19.0%).

DISCUSSION

Principal findings

We used OFCs to establish that the majority of children with positive SPT or measurable sIgE to peanut do not have clinical peanut allergy; approximately 10% of 8-year-old children in the United Kingdom (UK) are sensitized, but only ~2% have peanut allergy. CRD using microarray revealed marked differences in the pattern recognition between subjects with peanut allergy and peanut-tolerant subjects, suggesting that IgE response to Ara h 2 may prove a clinically useful tool in predicting peanut allergy.

Strengths and limitations

A strength of our study is the setting, a population-based birth cohort with high follow-up rate. We confirmed peanut allergy objectively in a large number of children by OFC, allowing accurate estimations of sensitization and peanut allergy which is generalizable to the population of 8-10 year old children in the UK.

We did not perform open challenge in children with negative DBPCFC.¹⁰ However, the final dose of peanut allergen in the DBPCFC was high (~15 whole peanuts).

We enriched the peanut-allergic group with 12 children recruited from a local clinic. However, we recruited children of approximately the same age and applied identical procedures as for cohort children.

We used 0.2 kU/L as a cutoff to diagnose peanut sensitization, rather than the more commonly used (but also arbitrary) 0.35 kU/L level. Using the higher cutoff point did not materially alter the results. The lower sIgE level was used because of the reports that reactions to peanut can occur in patients with sIgE < 0.35 kU/L.^{5,12}

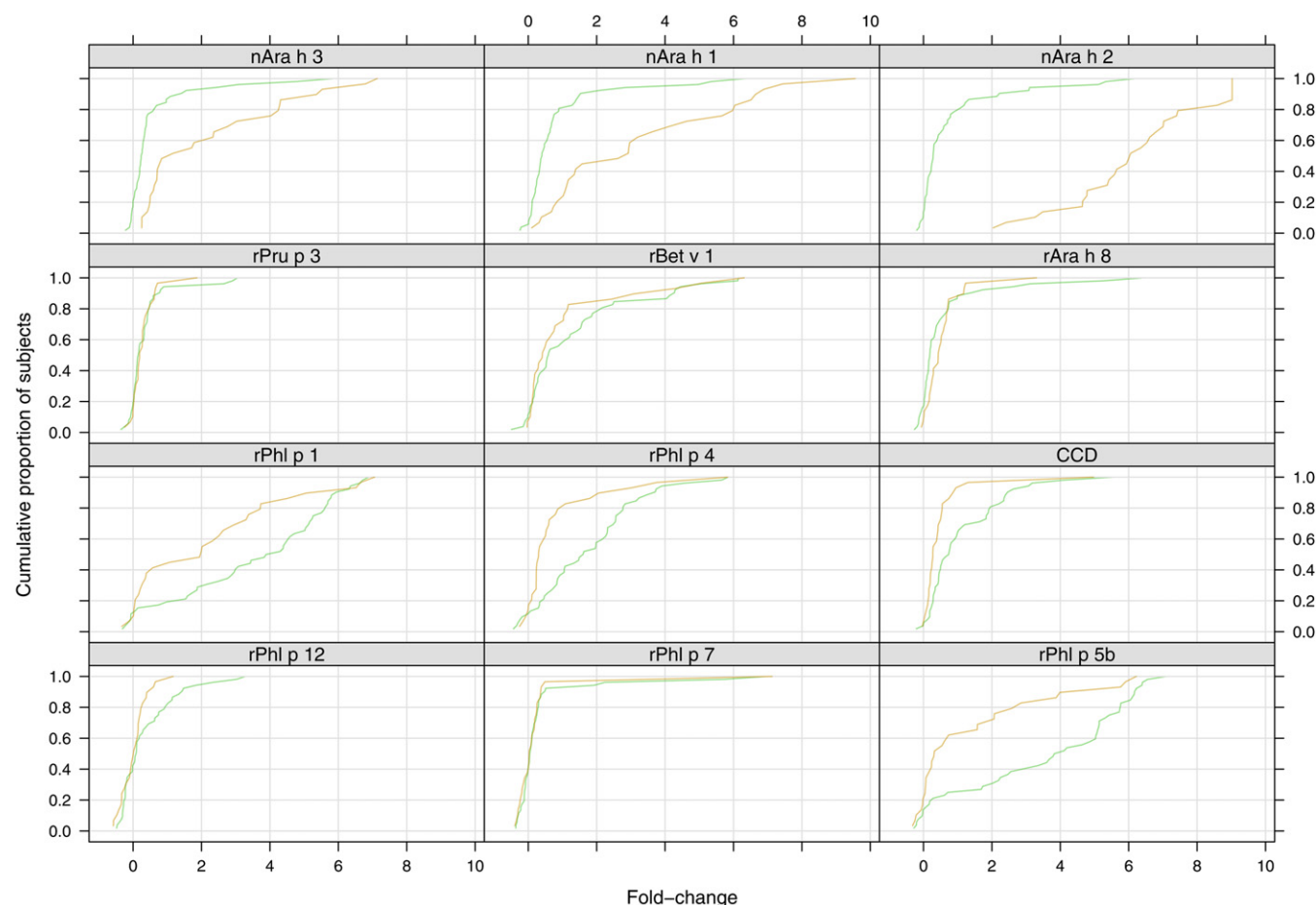


FIG 2. Empirical cumulative distribution functions. Green and yellow lines represent peanut-tolerant subjects and subjects with peanut allergy, respectively. *n*, Native; *r*, recombinant.

We decided *a priori* to use a conservative definition of positive OFC (>2 objective signs) to be as specific as possible in the absence of a consensus on what constitutes a positive challenge¹¹ and to exclude children with an inconclusive challenge from the analysis.

Our findings are derived from a relatively small group of subjects with peanut allergy and need replication. However, we wish to emphasize that this is one of the first studies to compare patients with peanut allergy with OFC-confirmed peanut-tolerant subjects.^{37,38}

Interpretation

We have confirmed that the prevalence of peanut allergy is substantially lower than peanut sensitization.^{3,12,19,20}

Despite models (based on high-risk children) indicating that peanut sIgE level ≥ 15 kUA/L and/or SPT wheal size ≥ 8 mm have a 95% positive predictive value for clinical peanut allergy,^{12,32,33} the accurate diagnosis of peanut allergy for many remains problematic, particularly in the absence of suggestive clinical history. Parents are often anxious to find out whether their child diagnosed with another food allergy will react to peanut, or whether siblings of their child with peanut allergy have peanut allergy. The recent development of pharmacists untrained in allergy providing over-the-counter allergy diagnosis (advertised direct-to-consumer)

based on IgE to whole peanut extract, often without regard to clinical history, is of concern. The lack of specificity of this test when used in isolation indicates many patients will be given the diagnosis inappropriately. Tests to discriminate subjects with peanut allergy from peanut-tolerant subjects without the need to perform OFC would be very useful.

We have demonstrated that current methods of assessment of peanut sensitization do not accurately predict peanut allergy. Reagents (based on whole peanut extracts) contain nonallergenic molecules that have sequence homology with pollen allergens. IgE directed against pollen allergens may bind to components of the whole peanut extract, resulting in a false-positive test for peanut allergy. We observed that peanut-tolerant subjects had higher grass sIgE and lower peanut sIgE compared with those with peanut allergy. In addition, they were more likely to have hay fever and less likely to have asthma, eczema, or other food allergies.

Beyer et al³⁸ demonstrated differences in IgE-binding epitope recognition between subjects with peanut allergy and peanut-tolerant subjects, and proposed that determination of specific epitope recognition may be an additional tool in the diagnosis of peanut allergy. The majority of subjects with peanut allergy showed IgE binding to 3 immunodominant epitopes on Ara h 2 that were recognized by <10% of tolerant individuals. However, epitope recognition among individuals with peanut allergy showed

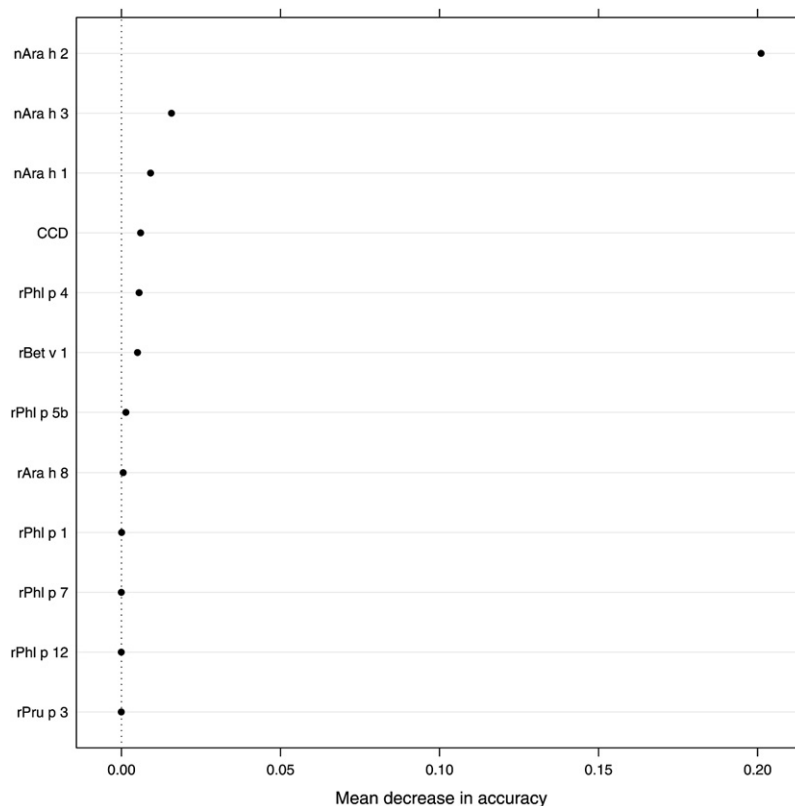


FIG 3. Variable importance. High positive values of the importance measure indicate a high variable importance, whereas small positive or negative values indicate that a variable is irrelevant for discrimination. *n*, Native; *r*, recombinant.

marked heterogeneity.³⁹ Although SPT with recombinant Ara h 1 to 3 discriminated subjects with peanut allergy from healthy controls, peanut-sensitized tolerant subjects were not tested in a study by Astier et al.³⁷

We followed a different approach and used microarray technology, which allows detection of IgE to individual allergens (components) of peanut and other potentially cross-reacting allergens including grass pollen. This revealed marked differences in the pattern of component recognition between the 2 groups: subjects with peanut allergy had a higher response to Ara h 1 to 3, whereas peanut-tolerant children had higher responses to grass components and CCD. Both subjects with peanut allergy and peanut-tolerant subjects recognized major peanut allergens; however, the majority of tolerant subjects had low response levels for these allergens, whereas the levels were high among subjects with peanut allergy.

The random forest method was used to develop a model for discrimination of subjects with peanut allergy and peanut-tolerant subjects, resulting in a relatively low misclassification rate (~7%). The variable importance measure revealed Ara h 2 as the most important component for accurate discrimination. All other components had small importance values, indicating that both individually and in interaction with each other, their contribution to accurate discrimination was negligible. Thus, when Ara h 2 is measured, the other components are of only slight interest. We informally investigated this further through a random forest using only Ara h 2, resulting in a model with discriminative ability identical to that using all 12 components.

The importance of Ara h 2 has been suggested in other studies.^{25,37,40} Our data indicate that Ara h 2 may be useful in predicting reactivity or tolerance to peanut in subjects with a wide range of peanut sIgE values. Furthermore, it is of note that among children with peanut allergy in our study, only 55% had sIgE and 61% had SPT MWD above the proposed clinical decision points for 95% prediction of clinical reactivity. Results from our study suggest that using specific IgE response to Ara h 2 may be a useful tool in predicting clinical peanut reactivity in sensitized individuals, therefore significantly reducing the need for oral food challenges (a risky, costly, and time-consuming procedure). However, our findings would need to be replicated in a larger population before general application as a useful diagnostic tool.

The majority of children within the general population with positive skin test or measurable serum IgE to peanut do not have clinical peanut allergy. Based on the data derived from a population-based birth cohort study maintaining high follow-up rates (> 90%), approximately 1 in ten 8-year-old children in the UK are sensitized to peanut, but only ~1 in 50 have peanut allergy. CRD may facilitate the accurate diagnosis, and IgE response to Ara h 2 may prove a useful tool in predicting clinical peanut allergy.

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Clinical implications: Measurement of IgE response to major peanut allergen Ara h 2 is more useful in predicting clinical peanut allergy than currently used skin or blood tests based on whole extract.

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METHODS

Study design

The Manchester Asthma and Allergy Study is a population-based birth cohort study specifically designed to investigate the development of asthma and allergic diseases.^{E1}

Study was approved by the Local Research Ethics Committee. Informed consent was obtained from all parents, and children gave their assent when appropriate.

Setting

The setting was the maternity catchment area of Wythenshawe and Stepping Hill Hospitals, composed of 50 square miles of South Manchester and Cheshire, UK, a stable, mixed urban-rural population.

Participants

All pregnant women were screened for eligibility at booking antenatal visits (eighth to tenth week of pregnancy). The study was explained to the parents, and informed consent for initial questionnaires and skin testing was obtained. Both parents completed a questionnaire about the history of asthma and allergic diseases and smoking habits.

When informed consent had been obtained, pregnant women and their partners were skin-tested. If the partner was not present at the antenatal visit, an invitation was sent for him to attend an open-access evening clinic at Wythenshawe Hospital for skin testing and questionnaire. Once both parents had completed questionnaires and skin testing, a full explanation of the proposed future follow-up for the child was given.

Of the 1499 couples who met the inclusion criteria (<10 weeks of pregnancy, maternal age >18 years, questionnaire and skin test data available for both parents), 288 declined to take part in the study. Of 1211 couples who initially agreed to take part, 1085 had a successful full-term pregnancy (>36 weeks gestation) and gave consent to a further follow-up.

Follow-up

The children have been followed prospectively, and attended review clinics at age 1, 3, 5, and 8 years (within 4 weeks of a birthday). Clinical assessment included the administration of validated questionnaires focusing on respiratory and allergic symptoms, assessment of sensitization by skin tests and serum IgE, and measurement of lung function.^{E2} The follow-up rate during the first 8 years of life was high (~90%).

A study on peanut allergy within the cohort

Within the birth cohort, we designed a study to estimate the prevalence of sensitization and clinical reactivity to peanut and investigate the predictors of peanut allergy. A pilot study carried out during follow-up at age 5 years revealed that 42 of 590 (7.1%) of the tested children had detectable peanut-specific IgE (>0.2 kU/L). This was a considerably higher rate of sensitization compared with the previous reports from the UK; we therefore further explored peanut allergy at the 8-year follow-up.

At age 8 years, participants were investigated for their peanut sensitization status. We administered questionnaires to collect the information on food allergy and exposure and reactivity to peanut. Sensitized children were invited for a further assessment of their clinical reactivity to peanut. Assessment included the following procedures.

Detailed allergy questionnaire. We used modified version of a standard questionnaire used in previous studies in the UK.^{E3,E4} Parents provided information on their child's consumption of peanuts, the symptoms developed, and the management of any allergic reactions after exposure to peanuts. We collected the information on maternal exposure to peanuts before and during pregnancy and while breast-feeding. A detailed family history of allergies was obtained.

SPTs. Skin prick tests were performed by the prick method on the volar surface of the forearm by using standardized allergen preparations (Hollister-Stier Laboratories). Subjects were tested to peanut, tree nuts, grass, milk, and egg. Histamine dihydrochloride 10 mg/mL and 50% glycerin served as

positive and negative controls. Test sites were inspected and reaction recorded after 15 minutes. Wheal size at least 3 mm greater than the negative control was regarded as a positive response.

Specific IgE measurements. Specific peanut and grass IgE measurements were carried out by using ImmunoCAP (Phadia, Uppsala, Sweden). We defined sensitization to peanut as peanut sIgE ≥ 0.2 kU/L.

Based on the information obtained from the questionnaire, the levels of peanut sIgE, and the peanut SPT mean wheal diameter, subjects were invited to undergo a controlled OFC, either open or DBPCFC, for the confirmation or exclusion of their clinical reactivity to peanut.

OFCs

Open challenge inclusion criteria.

- Positive SPT and/or detectable peanut-specific IgE, eating peanuts (freely)

DBPCFC inclusion criteria.

- Positive SPT and detectable sIgE with or without a history of reaction
- Positive SPT, no sIgE available, no history of reaction for more than 2 years
- Positive SPT, no sIgE available, does not eat nuts
- Detectable sIgE, no SPT available, no history of reaction for more than 2 years
- Detectable sIgE, no SPT available, does not eat nuts
- Discordance between SPT and sIgE, history of reaction

OFC exclusion criteria.

- Anaphylactic reaction after contact with peanut + peanut SPT ≥ 8 mm and/or peanut specific IgE ≥ 15 kU/L
- Parental consent refused
- Peanut DBPCFC within the last 2 years with available results
- Current poorly controlled asthma
- Unwell on the day of the challenge

Recipe for peanut and placebo brownies. We adopted a standardized recipe to prepare the challenge material used in previous studies in the UK.^{E3} Active and placebo brownies were baked on separate days, with separate utensils, to avoid cross-contamination. They were identical in appearance, smell, taste, viscosity, texture, structure, and volume. Sensory evaluation by individuals not participating in the study confirmed that no differences between placebo and active brownies could be detected. The peanut flour was provided by the Golden Peanut Company (Alpharetta, Ga) and was constituted of partially defatted (12% fat) light-roasted peanut flour from runner-type peanuts (product code 521271).

Three subjects in the study had cow's milk and/or egg allergy, and we were unable to challenge them with brownies prepared by using the above recipe. Although we made efforts to prepare brownies for them by using milk and egg substitutes, we were unsuccessful, because such brownies had an unacceptable taste and the children were unable to eat them. We therefore offered these children an open challenge using roasted peanuts (1/8-15 whole peanuts).

Procedures. All peanut challenges were carried out at the Paediatric Day Care Unit at the University Hospital of South Manchester by the same research personnel (N.N. and G.K.).

Participants with asthma were asymptomatic on the week before the challenge and had not used any bronchodilators in the last 24 hours before challenge. Subjects with seasonal allergic rhinoconjunctivitis were challenged outside the pollen season. Antihistamines were withheld for at least 72 hours before commencement of the challenge. On the challenge day, detailed medical history, physical examination, and baseline observations ensured that the subject was fit, and parental consent was obtained before proceeding with the challenge. Children were closely observed and monitored during the challenge, and an action plan for the management of potential allergic reactions was prepared. Medication and equipment for resuscitation including oxygen and injectable adrenaline were readily available. Challenges were not discontinued on the basis of subjective symptoms, but were stopped after development of 2 objective signs indicating an allergic reaction. Appropriate treatment was administered to those who had reacted. If no reaction had

occurred, subjects were discharged after an observation period of 2 hours after the last given dose with specific information and satisfactory arrangements for care if a late reaction occurred. Children who reacted were observed for at least 2 hours after their symptoms had resolved completely and were provided with a tailored management plan before discharge. In addition, they were contacted by phone in the evening and the following day by the study doctor to confirm that they were well and that no late-phase reactions had occurred.

Open challenge. Open challenges were performed in 4 stages and took approximately 4 hours. The first brownie contained 10 mg, the second 100 mg, the third 1 g, and the last 5 g peanut protein. The interval between stages was 30 minutes. Baseline observations (body temperature, oxygen saturation, pulse rate, blood pressure, and FEV₁) were recorded before the administration of each dose and repeated every 30 minutes for as long as 2 hours after administration of the last dose.

Open challenges with roasted peanuts (1/8 of a peanut to 15 peanuts) followed similar intervals and observation protocol as those performed by using the peanut brownies.

DBPCFC. Double-blind placebo-controlled food challenges were performed in 10 stages (on the same day with 1 hour break between administration of placebo and active brownies) and lasted approximately 8 hours, including the 2-hour observation period after administration of the final dose. The active and placebo brownies were randomly allocated in 2 boxes labeled A and B; the code for unblinding was kept in a sealed envelope. Each box contained 5 brownies, and both the researchers and the child undergoing the challenge were unaware which box contained the active or placebo brownies. The first active brownie contained 1 mg peanut protein, the second 10 mg, the third 100 mg, the fourth 1 g, and the last 5 g peanut protein. The interval between stages and observations recording was the same as in the open challenge. The code was broken after the challenge was completed.

Definitions of oral food challenge outcome. In a negative challenge, no symptoms were observed on OFC. Children with negative OFCs were considered peanut-tolerant.

In a positive challenge, 2 or more objective signs constituted a positive challenge, and these children were considered to have peanut allergy. Objective signs included flushing, pruritus, urticaria, angioedema, abdominal tenderness with increased bowel sounds, vomiting, diarrhea, sneezing, rhinorrhea, cough, hoarse voice, stridor, wheeze, >20% fall in FEV₁, >30% drop in blood pressure, and loss of consciousness.

We used this more conservative definition of positive OFC because there is no consensus on the standardization of OFC methodology.^{E5}

In an inconclusive challenge, there was 1 objective sign or only subjective symptoms on OFC. These children were excluded from further analysis to ensure that those included in the microarray study unequivocally had peanut allergy or were peanut-tolerant.

Microarray

Microarray assays for the determination of specific IgE-binding to allergenic components were carried out by using the microspot multiplex technique.

Laboratory methods. Purified components from peanut (Ara h 1-3 and 8), grass (Phl p 1, 4, 5b, 7, and 12), and potentially cross-reacting components (Bet v 1, Pru p 3, and cross-reactive carbohydrate determinants [CCD]), 0.1 to 0.15 ng/spot, were immobilized in triplicate by using a Nano Plotter NP2 (Gesellschaft für Silizium-Mikrosysteme mbH, Großberkmannsdorf, Germany) on nitrocellulose membranes attached to glass slides as previously described.^{E6} Purified human IgE was spotted at similar concentrations as purified proteins and was used as a position marker.^{E7} The spotted microarrays were prewashed with 30 μ L assay buffer (phosphate buffer, pH 7.5) and incubated with 30 μ L of subjects' sera. After washing with 30 μ L assay buffer, bound IgE antibodies were detected with 20 μ L fluorophore conjugated anti-IgE antibodies, and fluorescence intensity in each spot was measured at a wavelength of 635 nm (GenePix 4000B Axon Instruments; Molecular Devices, Sunnyvale, Calif).

The pattern of component spots on the microarray membrane is shown in Fig E1. Characteristic microarray fluorometric results from 4 subjects with distinct clinical phenotypes are presented in Fig E2.

No background correction was applied to the data. Where present, we identified and compensated for a decrease in expression levels in the direction of the flow of the assay. This was done separately for each component, using linear regression estimates of the expression levels by their positions. Subsequently, for each component and each sample in the experiment, the expression level was estimated through a linear mixed model by using the least-squares method.^{E8} The random effect was a result of the microspot strip.^{E9} Finally, fold-change quantities were calculated as follows:

$$\log_2 \left(\frac{\text{expression level estimate for a particular component and sample}}{\text{expression level estimate of the same component in the negative control pool}} \right)$$

This implies that a fold-change of, for example, 3 corresponds to having an expression level $2^3 = 8$ times higher than the negative control pool.

Statistical analysis

We used the nonparametric random forest method^{E10} to investigate the components' ability to discriminate correctly between subjects with peanut allergy and peanut-tolerant subjects. Random forests provide a permutation-based measure of variable importance useful for detecting predictors relevant for accurate discrimination. One advantage of the random forest variable importance measure, compared with univariate screening methods (eg, significance tests), is that it captures the impact of each predictor including its multivariate interactions with other predictors.

We used the random forest implementation in the Party^{E11} add-on package for R system for statistical computing.^{E12} This version of the random forest method uses conditional inference trees^{E13} in combination with subsampling without replacement, guaranteeing unbiased variable selection when predictors have different scales of measurement, or when the number of levels among categorical predictors varies.^{E14} A conditional permutation variable importance measure^{E15} was used to suppress the effect of correlated predictor variables.

Each forest consisted of 10,000 trees to ensure that sufficiently stable variable importance estimates were obtained. The unscaled variable importance was reported because of its superior statistical properties.^{E16} All estimates of the misclassification error were based on the out-of-sample observations for each tree, and the corresponding exact (Clopper-Pearson) CIs are presented.

RESULTS

Parental reports on food allergy

Of the 1029 parents who completed the study questionnaire, 17 (1.6%) answered yes to the question, "Has your child had any problems/symptoms when eating peanuts?" Regarding the question, "Does your child avoid peanuts?" 192 of 1029 (18.7%) gave a positive response.

Peanut allergy was the second most common food allergy, accounting for one quarter (26.2%) of all food allergies reported. Allergy to egg (2.3%), milk (1.5%), tree nuts (1.0%), and fish (0.5%) were the other common food allergies reported by the parents.

Peanut sensitization

Sensitization to peanut was ascertained by either IgE measurement (n = 582) or SPT (n = 919) in 933 of 1029 subjects reviewed at age 8 years. In total, 110 of 933 (11.8%) were considered sensitized to peanut.

There were no differences in sex, parental history of allergic disease, personal history of wheeze, eczema or hay fever, and pattern of SPT sensitization between children who did (n = 582) and did not give blood (n = 447). However, children who gave blood were more likely to have been breast-fed and less likely to have had a history of any food allergy than the children without a blood test (75% vs 66% and 4% vs 8%, respectively).

Of the 582 subjects who had blood tests performed, 71 (12.2%) had detectable peanut specific IgE (>0.2 kUA/L). In those 71 peanut-sensitized subjects, the values of peanut sIgE ranged from 0.24 to 443.44 kUA/L (median, 1.1 kUA/L). The 25th and 75th percentiles were 0.39 kUA/L and 3.71 kUA/L, respectively.

There were no significant differences in demographics between children who were skin-tested and those who were not (Table E1).

We had both skin tests and IgE measurements in 567 children. Of these, 19 were sensitized on both IgE and SPT, 49 by IgE only, and 5 by SPT only.

OFCs

Clinical reactivity to peanut was assessed by OFC in 79 sensitized subjects. Sixty-six (84%) OFCs were negative, and 13 subjects developed symptoms on challenge.

Eight (62%) of those who reacted on OFC had no previous history of reaction to peanut. However, 4 of those children had tried peanuts in the past but did not like the taste and therefore avoided peanut in their diet, 3 children have had small amount of peanut in their diet without any problem (but never as much as a handful), and 1 child had never knowingly been exposed to peanuts because of multiple food allergies. Four subjects (29%) developed oral symptoms with the first dose given (1 mg), and 3 reacted only after the final dose (5000 mg). Oral symptoms (eg, itchy tongue or mouth, tingling in oral cavity) were the most commonly developed (71% of subjects) followed by nasal (64%), abdominal (50%), and skin (29%) symptoms. All reactions were successfully managed with antihistamines; 1 child who developed wheeze was treated with nebulized salbutamol. None of the subjects required adrenaline administration.

DBPCFC

Thirty-four children were tested by DBPCFC; 11 (32%) developed symptoms on challenge. We observed 2 or more objective signs (positive challenge) in 7 children. Four subjects had only 1 sign and/or subjective symptoms (inconclusive challenge). Of the 4 with inconclusive challenges, 1 subject felt only tingling in his mouth; the second felt itchy mouth, sore throat, and had sneezing; the third developed only sneezing; and the last complained of abdominal pain and nausea and had increased bowel sounds. These 4 subjects were not included in microarray analysis. The remaining 23 DBPCFC challenges were negative.

Open peanut OFC

Forty-five children were tested by open peanut food challenge. Forty-three (95%) open challenges were negative. The outcome was inconclusive in 2 open OFCs. One child had 1 sign and subjective symptoms (hoarse cough once and complained of swollen throat), and the other only subjective symptoms (itching in the back of throat). The first child had open challenge with roasted peanuts. Although the second subject was invited for DBPCFC, the parents did not consent.

Details on the type of reactions developed on OFC are presented in Table E2.

Decision points for IgE and skin tests in the general population

sIgE. Of 582 children within the cohort with peanut sIgE measurement available (those for no consent for challenge and

inconclusive OFC excluded), 12 subjects had peanut sIgE levels >15 kUA/L. Eleven of these subjects were considered to have peanut allergy (stringent criteria), and 1 child was peanut-tolerant. Of the 570 subjects with peanut <15 kUA/L, 563 were peanut-tolerant, and 8 had peanut allergy.

The estimated sensitivity, specificity, and positive predictive value (PPV) for the 15kUA/L cutoff point in the cohort are presented in Table E3.

Table E4 lists the sensitivity, specificity, and positive and negative predictive values and likelihood ratios for various cutoff points of peanut-specific IgE.

SPTs. Of 899 children within the cohort with peanut SPT available (those for no consent for challenge and inconclusive OFC excluded), 7 subjects had peanut SPT MWD >8 mm. Six of these subjects were considered to have peanut allergy (stringent criteria), and 1 child was peanut-tolerant. Of the 892 subjects with peanut SPT <8 mm, 879 were peanut-tolerant, and 13 had peanut allergy.

The estimated sensitivity, specificity, and PPV for the SPT 8-mm cutoff point in the cohort are presented in Table E5.

Table E6 lists the sensitivity, specificity, and positive and negative predictive values and likelihood ratios for various cut-off points of SPTs.

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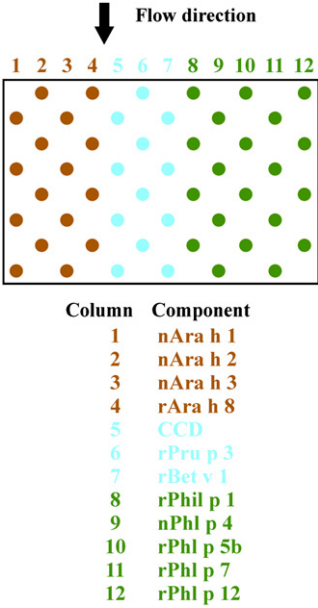


FIG E1. Pattern of component spots on microarray membrane. Pure components (*n*, Native; *r*, recombinant) from peanut (Ara h 1-3 and 8), grass (Phil p 1, 4, 5b, 7, and 12) and others (Bet v 1, Pru p 3, and CCD) were immobilized in spots on a capillary flow membrane.

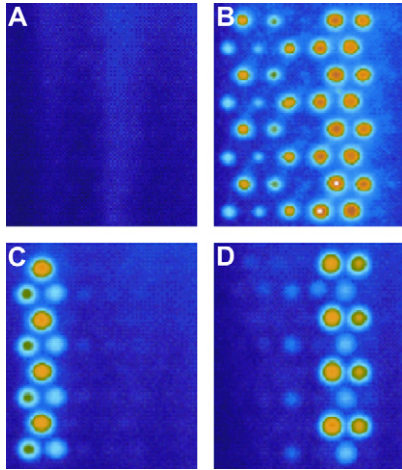


FIG E2. Characteristic fluorometric results of microarrays. Pictures of fluorometric component recognition patterns in subjects with distinct clinical phenotypes. **A**, Subject not recognizing any components (negative control). **B**, Subject recognizing all components (with peanut allergy and hay fever). **C**, Subject recognizing only major peanut components (with peanut allergy). **D**, Subject recognizing only grass and cross-reacting components (with hay fever and asymptomatic peanut sensitization).

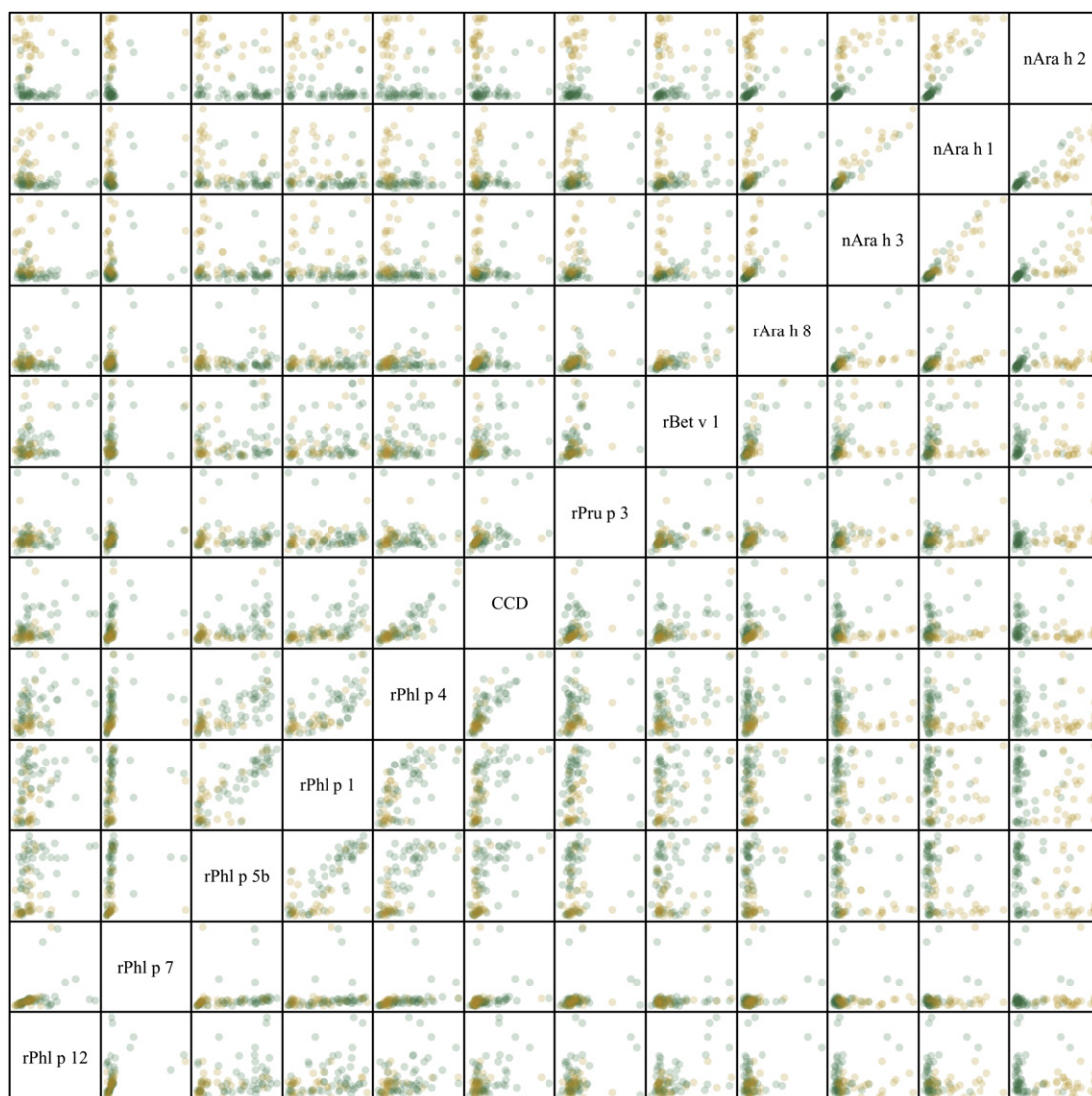


FIG E3. Scatter plot matrix contains all the pairwise scatter plots of the components' fold-change values. Green and yellow dots represent peanut-tolerant subjects and subjects with peanut allergy, respectively. *n*, Native; *r*, recombinant.

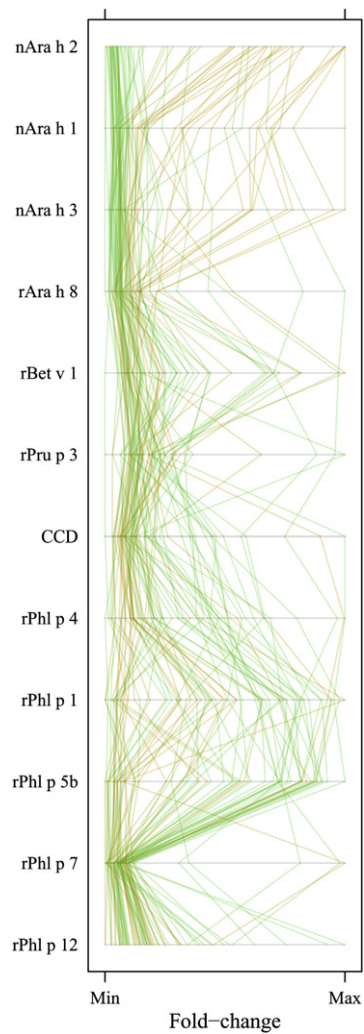


FIG E4. Parallel coordinate plot. Each *line* represents a subject's fold-change values across all components. *Green and yellow lines* represent peanut-tolerant subjects and subjects with peanut allergy, respectively. *Max*, Maximum; *Min*, minimum; *n*, native; *r*, recombinant.

TABLE E1. Characteristics of children with and without peanut SPT at age 8 years

Characteristic	Peanut SPT at age 8 y		P value
	No n/T (%)	Yes n/T (%)	
Male sex	69/110 (63)	497/919 (54)	.09
Maternal asthma	19/110 (17)	181/919 (20)	.54
Paternal asthma	14/110 (13)	132/918 (14)	.64
Maternal hay fever	37/110 (34)	243/918 (27)	.11
Paternal hay fever	24/110 (22)	218/918 (24)	.65
Maternal eczema	18/110 (16)	149/919 (16)	.97
Paternal eczema	8/110 (7)	100/918 (11)	.24
Breast-fed	69/101 (68)	634/884 (72)	.39
Attended nursery	69/97 (71)	607/864 (70)	.86
Cat in home first year of life	19/106 (18)	186/919 (20)	.57
Cat in home at age 8 y	27/107 (25)	190/919 (21)	.27
Dog in home first year of life	12/107 (11)	162/919 (18)	.09
Dog in home at age 8 y	21/107 (20)	177/919 (19)	.93
Ever wheeze	39/105 (37)	369/918 (40)	.54
Ever hay fever	15/109 (14)	90/916 (10)	.20
Ever eczema	45/109 (41)	359/912 (39)	.70
Eczema in first year of life	27/107 (25)	190/919 (21)	.27
Current wheeze at age 8 y	13/106 (12)	171/918 (19)	.11
Current hay fever at age 8 y	21/102 (21)	151/866 (17)	.42
Current eczema at age 8 y	26/109 (24)	217/914 (24)	.98
Any food allergy at age 8 y	11/110 (10)	52/919 (6)	.07

n, Number of children with characteristic; *T*, total number of children with available data for characteristic.

TABLE E2. Details on the type of reactions developed on OFC

OFC	Symptoms/signs	ED (mg)
Open	OS, swelling throat, hoarse cough	5000
Open	OS, itchy throat	1000
DBPCFC	OS, abdominal, nausea, vomit	1
DBPCFC	OS, nasal (sneeze)	10
DBPCFC	Nasal (streaming), skin (pruritus, flushing)	100
DBPCFC	OS (itchy mouth)	1
DBPCFC	OS, nasal, abdominal, wheeze, hoarseness, FEV ₁ drop, agitated	10
DBPCFC	OS, abdominal, nasal, itchy eyes, flushing, vomit, drowsiness	1
DBPCFC	OS, nasal (sneeze), generalized pruritus	100
DBPCFC	Abdominal, nasal, agitated, vomit, diarrhea, drowsiness	5000
DBPCFC	Abdominal, nausea, increased bowel sounds	5000
DBPCFC	Nasal (sniffing)	10
DBPCFC	OS, nasal, abdominal, cough	10

ED, Eliciting dose for symptoms; OS, oral symptoms.

TABLE E3. Sensitivity, specificity, and PPV with 95% CI for sIgE
15 kUA/L

Sensitivity, % (95% CI)	57.9 (36.3-76.9)
Specificity, % (95% CI)	99.8 (99.0-100.0)
PPV, % (95% CI)	91.7 (64.6-98.5)

PPV, Positive predictive value.

TABLE E4. Sensitivity, specificity, PPV, NPV, and LR for different peanut sIgE cutoff values in cohort

Peanut sIgE	Sensitivity	Specificity	PPV	NPV	LR
0.2 kUA/L	94.7	90.4	25.0	99.8	9.9
0.35 kUA/L	94.7	92.9	31.0	99.8	13.3
1.0 kUA/L	89.5	95.2	38.6	99.6	18.6
2.0 kUA/L	78.9	97.0	46.9	99.3	26.1
5.0 kUA/L	73.7	98.9	70.0	99.1	69.1
7.0 kUA/L	73.7	99.5	82.4	99.1	138.8
10.0 kUA/L	63.2	99.5	80	98.8	118
15.0 kUA/L	57.9	99.8	91.7	98.6	325
45.0 kUA/L	20	100	100	76	N/A

PPV, Positive predictive value; NPV, negative predictive value; LR, likelihood ratio; N/A, not applicable.

TABLE E5. Sensitivity, specificity, and PPV with 95% CI
for SPT 8 mm

Sensitivity, % (95% CI)	31.6 (15.4-54.0)
Specificity, % (95% CI)	99.9 (99.4-100.00)
PPV, % (95% CI)	85.7 (48.7-97.4)

PPV, Positive predictive value.

TABLE E6. Sensitivity, specificity, PPV, NPV, and LR for different SPT cutoff values in cohort

Peanut SPT MWD	Sensitivity	Specificity	PPV	NPV	LR
3 mm	78.9	98.1	46.9	99.5	40.3
4 mm	78.9	99.1	65.2	99.5	86.8
5 mm	73.7	99.7	82.4	99.4	216.1
6 mm	47.4	99.8	81.8	98.9	208.4
7 mm	36.8	99.9	87.5	98.7	324.5
8 mm	31.6	99.9	85.7	98.5	277.9
9 mm	8	100	100	74	N/A

MWD, Mean wheal diameter.