Patterns of IgE responses to multiple allergen components and clinical symptoms at age 11 years

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Background: The relationship between sensitization to allergens and disease is complex.

Objective: We sought to identify patterns of response to a broad range of allergen components and investigate associations with asthma, eczema, and hay fever.

Methods: Serum specific IgE levels to 112 allergen components were measured by using a multiplex array (Immuno Solid-phase Allergen Chip) in a population-based birth cohort. Latent variable modeling was used to identify underlying patterns of component-specific IgE responses; these patterns were then related to asthma, eczema, and hay fever.

Results: Two hundred twenty-one of 461 children had IgE to 1 or more components. Seventy-one of the 112 components were recognized by 3 or more children. By using latent variable modeling, 61 allergen components clustered into 3 component groups (CG1, CG2, and CG3); protein families within each CG were exclusive to that group. CG1 comprised 27 components from 8 plant protein families. CG2 comprised 7 components of mite allergens from 3 protein families. CG3 included 27 components of plant, animal, and fungal origin from 12 protein families. Each CG included components from different biological sources with structural homology and also nonhomologous proteins arising from the same biological source. Sensitization to CG3 was most strongly associated with asthma (odds ratio [OR], 8.20; 95% CI, 3.49-19.24; $P < .001$) and lower FEV_1 ($P < .001$). Sensitization to CG1 was associated with hay fever (OR, 12.79; 95% CI, 6.84-23.90; $P < .001$). Sensitization to CG2 was associated with both asthma (OR, 3.60; 95% CI, 2.05-6.29) and hay fever (OR, 2.52; 95% CI, 1.38-4.61).

Conclusions: Latent variable modeling with a large number of allergen components identified 3 patterns of IgE responses, each including different protein families. In 11-year-old children the pattern of response to components of multiple allergens appeared to be associated with current asthma and hay fever but not eczema. (J Allergy Clin Immunol 2015;■■■:■■■-■■■.)

Key words: *IgE, childhood, component-resolved diagnostics, latent variable modeling, allergens, asthma, wheeze, eczema, hay fever*

Although the presence of specific IgE (sIgE) to allergens is a major risk factor for asthma and hay fever, the relationship is inconsistent, and IgE-mediated sensitization is neither necessary nor sufficient for the expression of disease.[1] In clinical practice and research studies patients are usually assigned as being atopic or not based on the results of skin prick or sIgE tests to extracts made from whole allergen sources.[2,3] One potential limitation of using whole-allergen extracts is that the sources used for their preparation contain multiple different allergenic proteins and that a positive result might reflect cross-reactivity consequent to homology between similar proteins in different allergen sources.[4] The advent of molecular allergology has enabled investigators to identify individual proteins within whole allergen sources and to detect sIgE to individual allergen components.[5] In patients with food allergy, measuring sensitization to components is more informative than measuring levels of IgE to whole extracts.[4,6-8] For some allergen sources, there is a dominant component to which most sensitized subjects will react (eg, Fel d 1 is positive in almost all subjects with IgE to cat), but for others, no such dominant allergen exists (eg, sensitization to Can f 1, 2, 3, and 5 identifies less than half of those with IgE to dog).[9]

The commercialization of molecular or component-resolved diagnostics (CRD) has facilitated the development of products in which sIgE to more than 100 allergen components can be measured simultaneously by using small volumes of serum.[10,11] One such technology is the multiplex Immuno Solid-phase Allergen Chip (ImmunoCAP Immuno Solid-phase Allergen Chip [ISAC]).[12] We have recently reported that ISAC data might facilitate better assessment of allergic airway diseases.[13]

The role of such "high-resolution" tools in clinical practice and how best to interpret the complex data they generate is a matter of

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Abbreviations used
CG: Component group
CRD: Component-resolved diagnostics
eNO: Exhaled nitric oxide
ISAC: Immuno Solid-phase Allergen Chip
ISU: ISAC standardized units
OR: Odds ratio
sIgE: Serum IgE

some debate.[14,15] Conventional analyses can overaggregate the underlying complexity[16] and do not capture the heterogeneity in patterns of responses to multiple components, and therefore more sophisticated approaches are needed. A particularly appealing framework is that of latent variable models, in which a latent underlying mechanism explains the presence of multiple correlated items.[17]

We hypothesize that distinct patterns of component-specific IgE are associated with different clinical presentations. We propose that latent variable models can be used to identify such patterns and might facilitate better understanding of how data on sIgE to multiple allergen components can be interpreted within individual patients. To address our hypotheses, we measured levels of sIgE to 112 allergen components using a commercially available multiplex array in a population-based birth cohort and used a latent variable model to identify underlying patterns of component-specific IgE responses; these patterns were then related to asthma, eczema, and hay fever.

METHODS

Study population

The Manchester Asthma and Allergy Study is a population-based birth cohort.[18-22] Subjects were recruited prenatally and followed prospectively. The study was reviewed by the local institutional review board, and parents provided written informed consent. We used data collected at follow-up at age 11 years for this study. Validated questionnaires were interviewer administered to collect information on parentally reported symptoms, physician-diagnosed diseases, and treatments received.[23] We performed spirometry and measured exhaled nitric oxide (eNO) levels and assessed airway hyperreactivity in a 5-step methacholine challenge test.[24]

Definition of clinical outcomes

Current wheeze was defined as a positive answer to the following question: "Has your child had wheezing or whistling in the chest in the last 12 months?"[25]

Current asthma was defined as a positive answer to 2 of 3 of the following questions: "Has the doctor ever told you that your child had asthma?"; "Has your child had wheezing or whistling in the chest in the last 12 months?"; and "Has your child had asthma treatment in the last 12 months?"[26]

Current hay fever was defined as a positive answer to the following question: "Does your child have hay fever now?"[27]

Current eczema was defined as a positive answer to the following question: "Has your child had an itchy rash that comes and goes in the last 12 months?"[25]

Lung function was recorded as FEV_1 and forced vital capacity values.[28] Data were expressed as percent predicted FEV_1[29] and the FEV_1/forced vital capacity ratio.

Airway hyperreactivity was defined as a greater than 20% decrease in FEV_1 by the final stage of the challenge (16 mg/mL). We also calculated he dose-response slope to analyze the data as a continuous variable.[19]

eNO was recorded as a continuous variable in parts per billion.

CRD

We measured levels of sIgE to 112 allergenic molecules (components) from 51 sources using ImmunoCAP ISAC (Thermo Fisher Scientific, Uppsala, Sweden). Levels of component-specific IgE antibodies were reported in ISAC standardized units (ISU). We transformed (discretized) sIgE data using a binary threshold of 0.3 ISU into 4 categories, according to the manufacturer's guidelines: no (<0.3 ISU), low (0.3-1 ISU), medium (1-15 ISU), and high (>15 ISU) sensitization.

Statistical grouping of allergen components

Our statistical model assumed that there exist clusters of allergen components to which subjects have a similar IgE responses (ie, either being sensitized or not to most of the components within the same cluster). We refer to these clusters as component groups (CGs). We restricted our analysis to 71 components for which there were at least 3 children with positive test results. Components to which fewer than 3 children were sensitized are listed in Table E1 in this article's Online Repository at www.jacionline.org. We modeled different patterns of IgE response in 221 children with any positive test results.

We inferred allergen clusters and child sensitization to each cluster using Expectation Propagation,[30] an algorithm for approximate Bayesian inference (see a detailed description in Fig E1 in this article's Online Repository at www.jacionline.org). We relied on the Expectation Propagation implementation (available in Infer.NET http://research.microsoft.com/infernet), a Microsoft-owned library for large-scale Bayesian inference freely available for research purposes. Inference was performed based solely on IgE responses without using any information about protein structure or function or clinical phenotypes. We assigned a component to a CG if the posterior cluster membership probability was greater than 0.7 and characterized each child as sensitized to a CG if the posterior sensitization probability was greater than 0.5. Robustness and reproducibility of the results were assessed by repeating the analysis on 20 random subsets of 200 subjects.

Sequence, structure, and function of allergen components within CGs

We then investigated the sequence, structure, and functional properties of components clustered within the inferred CGs. We compiled an allergen sequence database (available on request) using allergens listed in the ALLFAM database (http://www.meduniwien.ac.at/allergens/allfam; release 2011-09-12)[31] downloaded from the UniProt database (http://www.uniprot.org). Allergen sequences were grouped by Pfam clan/family designation and aligned with ClustalW2[32] by using default settings. Dendrograms (average distance, BLOSUM62) were drawn by using Jalview, version 2.7. Dendrograms were colored and annotated by using Figtree, version 1.3.1 (http://tree.bio.ed.ac.uk/software/figtree). Within each protein family within a CG where 3 or more family members were present, we used a Venn diagram to map the distribution of sensitization.

Associations between CGs and clinical outcomes

Each child was assigned a probability of being sensitized to each CG on a scale of 0 to 1. We then modeled the quantitative CG scores for each participant as a latent variable predictor of clinical outcome using multivariate logistic regression tests. In addition, we assigned each child as sensitized or not to each of the CGs by using a posterior cutoff threshold of 0.5 or greater, noting that each child could be sensitized to any combination of CGs, to quantify the prevalence of sensitization to each CG and provide descriptive statistics of the characteristics of children sensitized to each CG. Analyses were performed with Stata 12.1 and SPSS 20 software (IBM, Armonk, NY).

RESULTS

Participant flow and demographic data

Among 1184 children born into the cohort, 822 attended follow-up at age 11 years; of these, CRD data were obtained for