Original Article

Association of Clinical Reactivity with Sensitization to Allergen Components in Multifood-Allergic Children

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What is already known about this topic? It is estimated that 6% to 8% of children suffer from food allergy, and of these, 30% are multifood allergic. Multifood-allergic children frequently suffer from nut allergies and can exhibit varied reactions to their food allergens.

What does this article add to our knowledge? This study of multifood-allergic children is the largest and most comprehensive of its kind, providing a systematic analysis of associations among different food allergies based on clinical double-blind, placebo-controlled food challenge as well as serological (IgE levels) data.

How does this study impact current management guidelines? Cashew/pistachio and walnut/pecan/hazelnut allergies show significant clinical associations, suggesting that this be considered when giving avoidance advice.

BACKGROUND: Thirty percent of children with food allergies have multiple simultaneous allergies; however, the features of these multiple allergies are not well characterized serologically or clinically.

OBJECTIVE: We comprehensively evaluated 60 multifood-allergic patients by measuring serum IgE to key allergen components, evaluating clinical histories and medication use, performing skin tests, and conducting double-blind, placebo-controlled food challenges (DBPCFCs).

METHODS: Sixty participants with multiple food allergies were characterized by clinical history, DBPCFCs, total IgE, specific IgE, and component-resolved diagnostics (IgE and IgG4) data. The food allergens tested were almond, egg, milk, sesame, peanut, pecan, walnut, hazelnut, cashew, pistachio, soy, and wheat.

RESULTS: Our data demonstrate that of the reactions observed during a graded DBPCFC, gastrointestinal reactions occurred more often in boys than in girls, as well as in individuals with high levels of IgE to 2S albumins from cashew, walnut, and hazelnut. Certain food allergies often occurred concomitantly in individuals (ie, cashew/pistachio and walnut/pecan/hazelnut). IgE testing to components further corroborated serological relationships between and among these clustered food allergies.

CONCLUSIONS: Associations of certain food allergies were shown by DBPCFC outcomes as well as by correlations in IgE reactivity to structurally related food allergen components. Each of these criteria independently demonstrated a significant association between allergies to cashew and pistachio, as well as among allergies to walnut, pecan, and hazelnut. © 2017 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2017;■:■-■)

Key words: *Multifood allergy; Component-resolved testing; IgE; Double-blind, placebo-controlled food challenge*

It has been estimated that approximately 8% of children suffer from food allergy, and of those, 30% are allergic to multiple

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This work is supported by NIH U19 AADCRC-10F, U19 AI104209, the Sean N. Parker Center for Allergy and Asthma Research at Stanford University, and the Department of Pathology, Stanford University.

Conflicts of interest: S. Andorf has received research support from the National Institutes of Health (NIH). M. P. Borres is employed by Thermo Fisher Scientific and Uppsala University. J. B. Bollyky has received research support from NIH/National Institute of Allergy and Infectious Diseases (NIAID); and is employed by Stanford University. J. Lidholm and J. E. Jones are employed by Thermo Fisher Scientific. S. J. Galli has received research support from the NIH (grants: 5U19AI104209, R01AR067145); is a member of the NIAID Advisory Council. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication September 29, 2016; revised January 18, 2017; accepted for publication January 26, 2017.

Available online ■■

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2213-2198

http://dx.doi.org/10.1016/j.jaip.2017.01.016

Abbreviations used
au- Approximately unbiased
CRD- Component-resolved diagnostics
DBPCFC- Double-blind, placebo-controlled food challenge
FDR- False discovery rate
GI- Gastrointestinal
LTP- Lipid transfer protein
sIgE- Specific IgE
SPT- Skin prick test

foods.[1,2] However, little information is available regarding the clinical and serological characteristics associated with multiple food allergies, and such information could advance our understanding of phenotypes and endotypes, respectively, of food allergy.[3] Clinically, it has been well recognized that certain food allergies occur concurrently. For example, studies have indicated that approximately 92% of patients with cow's milk allergy are also allergic to goat's milk,[4] and individuals with shrimp allergy are often allergic to other shellfish. Among tree nuts, allergies to pistachios have been found more commonly in individuals with cashew nut allergy[5] and individuals with walnut allergy are often allergic to pecan nuts.[6] Studies by Goetz et al[7] and Masthoff et al[8] found that there was little cross-reactivity between proteins found in peanuts and tree nuts suggesting that concurrent sensitization between these foods is likely due to independent sensitization to both peanuts and tree nuts. Clinical associations between allergens are more commonly seen among allergens that are closely related botanically like tree nuts[7]; however, links between allergens from very different taxonomical groups have also been observed.[9] Many foods that are closely related share homologous proteins, and sensitization to one food is therefore likely to result in positive tests or clinical reactivity to related foods. In addition, sensitization to proteins that are particularly highly conserved can result in associated allergies to foods that are not closely related. Therefore, we set out to test if individuals with multifood allergies have clinically relevant food allergies to proteins belonging to similar food categories and/or to similar protein classes.

Understanding the patterns of cross-reactivity between and among foods may aid the clinician in evaluating the risk of food reactions as well as reactions to multiple foods. Moreover, the application of food allergy diagnostics, using knowledge of relevant protein relationships, along with clinical history, could enable more precise allergen avoidance strategies to avoid unnecessarily eliminating entire food groups from the diet. Also, recent data indicate that multifood-allergic patients carry a higher risk for developing asthma and other comorbid conditions compared with those with single-food allergies, which further underscores the need to test relationships of cross-reactivity in multifood-allergic patients.[10]

There has been great interest in developing novel *in vitro* tests that can more accurately predict food allergies. One of these approaches is based on component-resolved diagnostics (CRD) in which native or recombinant allergens are used to test IgE sensitivity to individual allergen proteins.[11] At the present time, CRD tests are mainly performed in research settings. However, Food and Drug Administration-approved milk, egg, peanut, and tree nut CRD tests are available for clinical use in the USA.

In this study, our goal was to comprehensively evaluate the characteristics of patients with multiple food allergies. We focused especially on the association of multifood allergies with the phenotype (for our study, clinical symptoms during a double-blind, placebo-controlled food challenge [DBPCFC] and comorbidities) and endotype (for our study, the component IgE levels) of the participants. We analyzed the baseline data of a study in which the participants were screened and eligible only if they had a high likelihood of reactions to more than one food allergen in separate DBPCFCs. Accordingly, the study was not aimed at evaluating the ability of new diagnostic tools to predict negative versus positive reactions to DBPCFCs. Instead, we focused on testing whether there were associations between the extent and type of food challenge reactions and relatedness of allergen proteins. To our knowledge, this is the first study that systematically investigates multiallergic participants with the aim of identifying associations among food challenge outcomes, component testing, and levels of whole allergen-specific IgE (sIgE).

TABLE I. Demographics

Number participants	60
Female, n (%)	30 (50%)
Age, y (median, range)	8 (4-15)
With atopic dermatitis, n (%)	46 (77%)
With allergic rhinitis, n (%)	44 (73%)
With asthma, n (%)	30 (50%)
DBPCFCs performed	311
Positive DBPCFCs, n (%)	273 (88%)
No. of DBPCFCs performed for participant (one per food) (median, range)	5 (2-8)

DBPCFC, Double-blind, placebo-controlled food challenge.

METHODS

The protocol for this study was reviewed and approved by the Institutional Review Board of Stanford University.

Study population

Sixty pediatric participants allergic to multiple foods were included in this study. Their demographic characteristics are summarized in Table I. Information about participant selection (screening criteria, DBPCFC, skin prick test [SPT], food flours) can be found in this article's Online Repository at www.jaci-inpractice.org.

Antibody measurements

Total IgE and allergen-sIgE and IgG4 antibody concentrations were determined using the ImmunoCAP 250 assay (Thermo Fisher Scientific, Portage, Mich). Antibodies to the following foods and allergen components were measured: peanut (Ara h 1, Ara h 2, Ara h 3, Ara h 8, Ara h 9), hazelnut (Cor a 1, Cor a 8, Cor a 9, Cor a 14), walnut (Jug r 1, Jug r 3), cashew (Ana o 3), egg white (Gal d 1, Gal d2, Gal d 3), cow's milk (Bos d 4, Bos d 5, Bos d 8), soy (Gly m 4, Gly m 5), wheat (Tri a 14, Tri a 19), and birch (Bet v 1).

Statistical analysis

Differences between nonparametric unpaired variables were assessed using a 2-sided Mann-Whitney *U* test. *P* values were adjusted for multiple comparisons using the approach by Benjamini and Hochberg[12] to control the false discovery rate (FDR) and the corrected values were noted as *Q* values.

To characterize the multiallergic character of the participants, for each allergen combination, the number of participants who were allergic against both allergens in DBPCFC tests was determined. To