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PRINCIPAL INVESTIGATOR: Jonathan M. Spergel, M.D., Ph.D.

CONTRACTING ORGANIZATION: Children's Hospital, Philadelphia
Philadelphia, PA 19104

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Jonathan M. Spergel, M.D., Ph.D.

E-Mail: spergel@email.chop.edu

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14. ABSTRACT
Food Allergies occur in 2-6% of children in the United States. The reactions can be mild to severe and life threatening and potential fatal. No study has been able to identify genetic causes of IgE mediated food allergies. In our previous work, we identified polymorphism in thymic stromal lymphopoietitin region as risk factor for Eosinophilic Esophagitis, a non-IgE mediated disease. This work has examined the genetics of food allergy looking at both IgE and non-IgE mediated food allergy using Genome Wide Analysis (GWAS). We have collected samples from patients at The Children's Hospital of Philadelphia, University of Colorado Children's Hospital, Riley's Children Hospital-San Diego, CA; Packard Children's Hospital-Stanford, CA. The samples and analysis was split into IgE mediated allergies and Eosinophilic Esophagitis. For patients with Eosinophilic Esophagitis, we have collected over 700 samples. Our preliminary results confirmed the importance of TSLP region as a risk factor for Eosinophilic Esophagitis with p value of 10-12. Two additional regions have been identified and now being confirmed using a replicative cohort. For the patients with IgE mediated allergies, we have collected families and done initial GWAS analysis on previous collected samples of 800 patients with no identified regions. Therefore, we have done exon sequencing of 10 family cohorts with normal controls. Multiple areas of interest have been identified and awaiting confirmation via alternative methods. The projects also examined genotype-phenotype relationships. Using novel component analysis, we are able to identify serological markers that identified patients at risk for a severe reaction.

15. SUBJECT TERMS
Food Allergy, Eosinophilic Esophagitis.

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INTRODUCTION:

Approximately 25% of the United States population believes that they have an allergic reaction to foods. The 2008 CDC report from Branum and Luckas indicated 1 out of 4 children have food allergies with nearly 3 million children reporting a food reaction in 2007. The prevalence of food allergies have increased 18% in the last 10 years and over 9500 hospital admission for food allergies were noted from 2004-06[1] making it an urgent medical need. Finally, many food allergies are life-long and are significant risk for adults including potentially military personnel. Food allergies are a broad category and are part of the larger adverse food reaction, which is any reaction to food regardless of the pathophysiology. The food reactions are split into immunologic and non-immunologic entities. Non-immune reactions include jitteriness from caffeine or metabolic disorders such as lactose deficiency[2]. The immune reactions are what physicians consider **food allergies (FA)**. Immune reactions are further divided into IgE- and non-IgE-mediated reactions. Non-IgE-mediated food reactions have been more recently described in the last several years and include food protein-induced enterocolitis syndrome[2]. Some food reactions such as atopic dermatitis and eosinophilic esophagitis (EoE) reactions are considered mixed IgE and non-IgE. To further complicate the scenario, patients often have a combination of different types of food allergies such as anaphylaxis to one food and atopic dermatitis to another.

The understanding that genetics play a role in allergic disease and asthma has been recognized for more than 100 years. This genetic component was suggested through observations that allergic subjects had a significantly higher incidence of family histories of disease as compared with controls[4, 5]. Follow-up studies have shown that if one parent has allergies, a child has a 33% chance of developing allergies and if both parents are allergic that number jumps to a 70% chance[6]. Eventually, it was recognized that allergies and asthma represent complex genetic disorders, defined as disorders that have numerous contributing genes, each having variable degrees of involvement in any given individual. In addition to specific genes, environmental exposures (including allergen exposure, secondhand cigarette smoke, pollutants), low birth weight, infectious agents, and numerous other factors have been recognized to contribute to the development of allergies through their ability to influence gene expression [7]. This will probably apply to food allergy too, where it has been shown that in a prospective study of infants with documented atopic parents (approximately half with 1 and half with 2 atopic parents) confirmed that atopic disease developed in ~ 70% and food allergy in approximately 25% from birth to 7 years of age. An even higher risk (~70%) exists for developing the same disorder as one's parents, given that both parents have the identical disorder. Indeed documented food allergy in a sibling or a parent also increases the likelihood for the development of food allergy in an offspring[8].

The proposed work has two aims:

Specific Aim 1. To perform a whole genome scan to test for association of EoE and FA with single nucleotide polymorphism (SNP)s, SNP haplotypes or copy number variations using high-throughput tag-SNP arrays.
Specific Aim 2. To determine the expression of candidate genes in Esophageal tissue or peripheral blood monocytes

BODY:
The Statement of Work was divided into 4 quarters for each year.

First Quarter:

1) *Submit DOD application for Human Subjects experimentation*

IRB approval was obtained at The Children's Hospital of Philadelphia (CHOP), Univ of Colorado's Children Hospital, Riley's Children's Hospital in San Diego and Packard Children's Hospital in Stanford, CA

2) *Begin work on Aim 1*

For the Eosinophilic Esophagitis cohort. DNA Samples were collected 378 samples from CHOP, 72 from San Diego, 78 from Colorado, 36 from Stanford and 181 from Cincinnati for the entire first year.

For the IgE mediated food allergy cohort, 90 samples were collected from genetic related CHOP including 18 family cohorts.

Second quarter

1) *Initial Analysis of Eosinophilic Esophagitis (EoE) Cohort*

We have performed initial analysis of the EoE cohort by GWAS analysis and pathway analysis.

The initial Genome Wide Array Studies (GWAS) confirmed the importance of TSLP. Our initial studies found a p value of 10^{-8} when we used a total of approximate 300 samples. Using the current analysis of over 700 samples, the p value was increased to 10^{-12}. This result is important as it emphasizes the importance of this locus. We have identified a novel region. We are doing the replicate analysis (planned to be done in 1st quarter of the second year, consistent with original statement of work).

2) *Continue to collect DNA samples for EoE and Food Allergy Cohorts*

We have continued to collect samples (Current number of samples are listed in 1st quarter statement).

Third quarter

1) Initial Analysis for Food Allergy Cohort

We have done the initial GWAS analysis on our previously identified cohort of patients with IgE mediated allergy. Unfortunately, the GWAS analysis did not identified any significant loci. Therefore, we are examining a specific cohort of patients with families. We are doing both GWAS and next generation whole exon sequencing from 18 families with normal and 2 affected

children to identify potential loci. When these loci are identified, we will re-examine the GWAS to be a replicative cohort.

As part of this analysis, we did phenotyping of the patients with IgE mediated food allergy. We preformed microarray analysis (ImmunoCap ISAC). Microarray components analyzed were particular for milk (Bos d 4, Bos d 5, Bos d 8, Bos d lactoferrin), egg (Gal d 1, Gal d 2, Gal d 3, Gal d 5), sesame (Ses i 1), and peanut (Ara h 1, Ara h 2, Ara h 3, Ara h 6). Positivity for each component was set as >0.3 standardized units.

We found that the odds of having related food allergy reaction was significantly increased but to a lower degree using cumulative sum of peanut or milk components or Ara h 1, Ara h 2, Ara h 6, and Bos d 4 individually as the dependent variable. Sensitivity and specificity for relevant food reaction history was high for combined number of positive peanut components (100%; 96%) and Ara h 1 alone (100%; 98%), respectively. Combined number of positive components for milk, egg, and sesame had specificities of 96%, 94%, and 96% and sensitivities of 80%, 50% and 70%, respectively. Sensitivities for combined number of positive milk and egg components were higher than individual positive components, with exception of Bos d 6 (95%). Median cumulative sum of food components was higher when the food allergic reaction involved 2 or more organ systems compared to only 1 system.

2) *Begin to collect DNA samples for EoE from other sites (Walter Reed Medical Center, and Alaska Allergy)*

Contract and IRB approval from Walter Reed Medical Center is still ongoing.

KEY RESEARCH ACCOMPLISHMENTS:

- Confirmation of TSLP locus as a genetic risk factor for Eosinophilic Esophagitis
- Identification of novel locus as a genetic risk factor for Eosinophilic Esophagitis
- Identification of multiple elements of component assays to predict severity of food reactions

REPORTABLE OUTCOMES:

- Abstract submitted and abstract as oral presentation at the American College of Asthma Allergy and Immunology Annual Meeting, 2012
- Work was the recipient of the Von Pirquet Award at the American College of Asthma Allergy and Immunology
- Database for genotype and phenotype of 740 patients with Eosinophilic Esophagitis
- Database of 18 family cohorts with IgE mediated food allergies
- Database of 90 patients with Component testing.

CONCLUSION:

We are able to recruit and enroll patients for the genetics study of allergy. The project split the population into 2 separate cohorts as they appear to have distinct types of food allergy.

Patients with Eosinophilic Esophagitis, an unique food allergy with symptoms related to esophageal dysfunction with eosinophils depositing in the esophagus. We confirmed the importance of TSLP loci as a genetic risk factor. In addition, we identified a novel locus that is be confirmed using a replicative cohort. This new locus has the potential to identify key elements in the pathogenesis and affect long-term treatment of the disease.

For the IgE mediated cohort, GWAS analysis did not identified any particular region. But, exon sequencing of related families with affected and non-affected children have identified multiple regions. These regions are now being confirmed by additional genomic analysis.

As part of the genotype-phenotype analysis, we performed microarray analysis. This analysis showed that patients had multiple positive components for a food, there were more likely to have a significant food allergy. This data was seen for milk, egg and peanut food allergies. If this can be replicated on a larger scale, it can have an important impact in the treatment of food allergy to help identified which patients might be true allergic and which may not be.

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APPENDICES:

Abstract submitted to American College of Allergy Asthma and Immunology
Data submitted for Von Pirquet Award from the American College of Allergy Asthma and Immunology

SUPPORTING DATA: Not applicable

2012 American College of Asthma Allergy and Immunology Abstract

Microarray component testing in association with IgE mediated food allergy: a retrospective analysis assessing performance of multiple versus individual components

I. Fung, J. Kim and JM Spergel

Introduction: By assessing multiple recombinant allergen components, protein microarray may be informative for food allergy diagnosis. Here, we assess if combination of positive ISAC components for a specific food correlated better with reported food allergy reaction than individual components alone.

Methods: Families with food allergic children were identified from a Jewish community in Lakewood, New Jersey. Approval was obtained from the Children's Hospital of Philadelphia Institutional Research Board and written informed consent obtained from all research subjects. Histories of food reactions and blood samples for protein microarray analysis (ImmunoCap ISAC) were collected from family members. Microarray components analyzed were particular for milk (Bos d 4, Bos d 5, Bos d 8, Bos d lactoferrin), egg (Gal d 1, Gal d 2, Gal d 3, Gal d 5), sesame (Ses i 1), and peanut (Ara h 1, Ara h 2, Ara h 3, Ara h 6). Positivity for each component was set as >0.3 standardized units.

Results: Of 73 subjects from 23 families, 27 (37%) reported food allergy. The most common reported food allergies were to milk (n=20), sesame (n=10), egg (n=10) and peanut (n=6). The odds of having had a related food allergy reaction was significantly increased when using the combined number of positive microarray component to that culprit food as a dependent variable. Odds of having related food allergy reaction was significantly increased but to a lower degree using cumulative sum of peanut or milk components or Ara h 1, Ara h 2, Ara h 6, and Bos d 4 individually as the dependent variable. Other individual components did not reach significance (**Table 1**). Sensitivity and specificity for relevant food reaction history was high for combined number of positive peanut components (100%; 96%) and Ara h 1 alone (100%; 98%), respectively. Combined number of positive components for milk, egg, and sesame had specificities of 96%, 94%, and 96% and sensitivities of 80%, 50% and 70%, respectively. Sensitivities for combined number of positive milk and egg components were higher than individual positive components, with exception of Bos d 6 (95%). Median cumulative sum of food components was higher when the food allergic reaction involved 2 or more organ systems compared to only 1 system.

Conclusion: Measuring the number of multiple food allergen components performs better than cumulative sum of measurement of individual components in characterizing food allergy reactions.

Von Piquet Award Application- To be received at American College of Allergy Asthma and Immunology Meeting, 2012

Microarray component testing in association with IgE mediated food allergy: a retrospective analysis assessing performance of multiple versus individual components

Fung, I, Kim JS, and Spergel, JM.

Background

Food allergy is a common disease that affects between 2 to 10% of the population [1]. The gold standard for diagnosis is an oral food challenge (OFC), preferably a double-blind, placebo-controlled food challenge (DBPCFC). However, this is not always done due to resource unavailability and potential reaction risk. More commonly, a food allergy diagnosis is made based on clinical history combined with skin prick test (SPT) and/or fluorescence enzyme immunoassay (FEIA). These investigations have their limitations. The specificity of SPT for food allergy is approximately 50% [2,3]. FEIA usually has a specificity of >50%, and can yield a 95% positive predictive value in some foods (reviewed in [3]). In spite of this, as whole food extracts are used in FEIA, results may be positive if the subject generates IgE antibodies against cross-reactive antibodies within the food which do not cause an allergic reaction. In patients with atopic dermatitis (AD) test interpretation is even more difficult. Approximately 40-90% of infants with moderate-to-severe AD will have a positive SPT to 1 or more common food allergens, but only 27-60% of these children will have reaction on OFC. Likewise, only 30-40% of children with at least moderate AD and a positive IgE result will react to a particular food under DBPCFC (reviewed in [4]).

Peptide microarray immunoassay is a novel method for analyzing food allergy. A specific panel of purified or recombinant proteins components for each food extract is provided within a microarray, and detection for host specific IgE antibody to these proteins is identified. Microarray assessment also permits quantitative data to be collected in a robust fashion, which may help better characterize food allergy. Already, microarray studies have shown that epitope diversity in testing correlates with reaction severity of patients with food allergies to peanut [5-7], milk [8-10], and egg [11,12]. An increase in specific individual components is predictive of OFC outcome [13-15]. However, patterns of detectable microarray components vary among different patients [16,17], likely because of population heterogeneity [16]. Thus, we wondered if assessing the sum of a set of components for a culprit food would be more helpful than identification of individual causative components. As well, at least one prior study included subjects with food-exacerbated AD within their food-allergic cohort [18], which could cloud results given non-IgE mediated mechanisms in AD [14,19]. We thus separated out this subset in our analysis.

The purpose of our study was to assess what manner of interpreting peptide microarray results best related to subject-reported 1) food allergy and 2) food-triggered AD in a monogenic population. First, we analyzed for odds risk ratio using self-reported food reaction to milk, egg, and peanut as the independent variables. Dependent variables were 1) number of detectable components, 2) sum of component values, and 3) individual detectable components. Second, we looked at how well these variables related to reported milk-, egg- and peanut-related atopic dermatitis, using the same method. Third, to investigate the relationship between food component values and severity of reported allergic reaction, we evaluated the median sum of component values and number of components detected in relation to the number of organs involved in the reported allergic reaction.

Objective

The aim of this study was to characterize associations between food microarray component test results and self-reported food allergy reactions.

Methods

Families with food allergic children were identified from a Jewish community in Lakewood, New Jersey. Approval was obtained from the Children's Hospital of Philadelphia Institutional Research Board and written informed consent obtained from all research subjects. Food reaction histories and blood samples for protein microarray analysis (ImmunoCAP ISAC) were collected from food allergic and non-food allergic family members. Food allergy histories were adjudicated by an allergy fellow (IF) to determine plausibility for an IgE-mediated reaction, and number of organs affected in such a reaction was recorded. Sets of microarray components analyzed were to milk (Bos d 4, Bos d 5, Bos d 8, Bos d lactoferrin), egg (Gal d 1, Gal d 2, Gal d 3, Gal d 5), and peanut (Ara h 1, Ara h 2, Ara h 3, Ara h 6). Positivity for each component was set as >0.3 standardized units. Sum of components referred to the sum of standardized units for each component set. Summary statistics and analyses were performed using STATA12 statistical software (StataCorp. College Station, TX). *P* value of 0.05 or less was considered significant.

Results

Seventy-three subjects from 23 families were recruited. Reported atopic conditions included asthma (40%) food allergy (37%), allergic rhinitis (29%), and atopic dermatitis (29%). Culprit foods in reported IgE-mediated allergy included milk (n=20), egg (n=10), and peanut (n=6). These foods were also implicated in atopic dermatitis (milk, n=10; egg, n=7; peanut, n=2).

The odds of having had a related food allergy reaction were significantly increased when considering the total number of positive microarray components (0-4) to that culprit food (Table 1: odds ratio peanut-10.2, p<0.004; milk 38.7, p<-0.0001; egg-3.34, p<0.003). When the sum of individual components are used the odds of having related food allergy reaction were significantly increased but to a lower degree using the sum of peanut components (range: 0 – 199.42, p<0.006)) or milk components (range 0 – 31.73, p<0.008) or individually positive Ara h 1, Ara h 2, Ara h 6, and Bos d 4 as the dependent variable. Sum of egg components and other individual components did not reach significance. In general, combined and individual components had high specificities but lower sensitivities (Table 2) . For peanut, combined specificity and sensitivity was highest for total number of positive peanut components and Ara h 1 alone. Sensitivity of the total number of positive milk and egg components was higher than individual positive components.

Milk and egg-associated AD was also assessed as an independent variable, while peanut-associated AD was not because there were only 2 patients reported to have the latter affliction. For both milk and egg-associated AD, the number of positive components reached statistical significance as a dependent variable (milk: OR 1.63; 95% CI 0.92 – 2.87, p=0.09; egg: OR 2.21; 95% CI 1.07 – 4.56; p=0.03), while sum of components and individual components did not. In general, individual components and combined number of components had high specificity both for milk (range: 75% – 97%) and egg (range: 91-98%), but had relatively lower sensitivities (milk component range: 0-38%, egg component range 0-38%). The specificity and sensitivity of the combined number or milk components (75%; 38%) or egg components (91%; 38%) was not higher than individual components alone.

For milk and egg IgE mediated allergy, both the median number of components and median total sum of food components was higher when the food allergic reaction involved 2 or more organ systems compared to only 1 system. As only 1 subject had peanut allergy affecting 1 organ

system, and the remainder had 2 or more systems affected, we did not have an adequate sample size to perform this analysis.

Discussion

We were able to use a relatively large sample population within a monogenic population to characterize microarray immunoassay performance. Our results suggest that the number of food allergen components performs well in indicating food IgE-mediated allergy and food-associated AD. Moreover, the number of components performs better than individual components or the sum of component values. In general, microarray results had high specificities and lower sensitivities in relation to reported food reactions. Accordingly, microarray analysis may be a helpful adjunct test to SPT and FEIA, which have high sensitivities but low specificities [3].

A major limitation to our study is that OFC were not performed to substantiate food allergy histories. Such testing is necessary to validate our findings. As well, because we studied a homogenous population, our results may not be generalizable to other groups. These factors may explain why Ara h 2 did not have higher specificity compared to other peanut components, as has been found previously in a pediatric population from the United Kingdom [20,21]. Nonetheless, our findings are in keeping with prior studies, in demonstrating that immunoassay is a useful additional tool for food allergy diagnosis [20,22]. Future studies should be done to validate efficacy of the microarray immunoassay in clinical practice.

Conclusion: Measuring a diversity of food allergen components performs better than the sum of components or individual components in characterizing food allergy reactions.

Table 1. Logistic regression analysis evaluating the association between microarray component outcomes and hisory of reported IgE-mediated food allergy reaction

	Odds ratio	95% Confidence interval	p-value
Peanut			
Ara h 1	1.15	1.03-1.28	0.02
Ara h 2	1.29	1.08-1.52	0.003
Ara h 3	--	--	--
Ara h 6	1.16	1.03-1.31	0.01
Sum of components	1.06	1.02-1.11	0.006
# of positive components	10.16	2.08-49.75	0.004
Milk			
Bos d 4	9.21	1.96-43.4	0.005
Bos d 5	--	--	--
Bos d 8	--	--	--
Bos d lactoferrin	1.41	0.25-7.80	0.69
Sum of components	9.61	1.81-51.16	0.008
# of positive components	38.67	6.05-247.24	0.0001
Egg			
Gal d 1	1.17	0.96-1.41	0.11
Gal d 2	1.60	0.86-2.96	0.14
Gal d 3	1.03	0.71-1.48	0.89
Gal d 5	--	--	--
Sum of components	1.07	0.97-1.20	0.16
# of positive components	3.34	1.50-7.43	0.003

-- inadequate sample size available to perform logistic regression

	Sensitivity	Specificity	PPV	NPV

Table 2. Sensitivities and specificities of microarray components in predicting reported IgE-mediated food allergy

Peanut				
Ara h 1	100	98	86	100
Ara h 2	83	96	71	98
Ara h 3	33	100	71	98
Ara h 6	83	96	71	88
# of positive components	100	96	100	96
Milk				
Bos d 4	65	98	93	88
Bos d 5	60	100	100	87
Bos d 8	45	100	100	83
Bos d lactoferrin	5	98	50	73
# of positive components	80	96	89	93
Egg				
Gal d 1	40	96	67	90
Gal d 2	20	98	67	90
Gal d 3	30	94	50	8
Gal d 5	20	100	100	87
# of positive components	50	94	62	91

PPV: Positive predictive value; NPV: Negative predictive value

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