

Genetic and Environmental Determinants of Food Allergy

by
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A dissertation submitted to Johns Hopkins University in conformity with the
requirements for the degree of Doctor of Philosophy

Baltimore, Maryland
April 2014

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Abstract

Introduction: This thesis attempts to answer several questions about the epidemiology of pediatric allergy, including (1) the prevalence of pediatric food allergy in the United States and its changes over time, (2) whether variation in serum folate and vitamin D levels is associated with incident sensitization, and (3) whether common genetic variants interact with season of birth to affect the risk of food allergy.

Methods: The thesis follows a three paper format, containing the following sections: (1) a systematic review and meta-analysis/meta-regression of food allergy prevalence in the United States, (2) a nested case-control study of incident mouse allergy in laboratory workers, examining associations with vitamin D and folate, and (3) a genome wide association study looking for possible interactions between fall season of birth and genetic markers influencing risk of food allergy in a family based cohort.

Results: We found the prevalence of self-reported food allergy has increased in the United States in recent decades, and this increase has been most pronounced among Non-Hispanic Blacks; higher folate, but not vitamin D, levels are associated with a higher risk of incident sensitization to a selected allergen; and common genetic markers in or around the PBRM1 gene interact with Fall season of birth to influence the risk of food allergy.

Conclusions: These findings suggest (1) environmental causes should be sought for the increased prevalence of food allergy in the United States,;(2) folic acid supplementation and fortification may be one environmental factor contributing to increased risk of

sensitization; and (3) genetic variants in or around the PBRM1 gene may interact with another environmental factor, fall season of birth, to influence the development of food allergy. Together, these findings suggest several hypotheses for further exploration to understand the genetic and environmental causes of food allergy.

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Acknowledgments

Many people have assisted in the work that went into this thesis. First, I would like to share my deepest gratitude to my advisor Dr. Terri Beaty for support in my PhD study and research. She has helped me at all stages of this project with enthusiasm, insight and patience.

The rest of my thesis committee has also been extremely helpful to my academic development and to that of these projects, including Dr. Xiaobin Wang, Dr. Elizabeth Matsui and Dr. Ingo Ruczinski. A special thanks to Xiaobin and Elizabeth, whose data I used in the second and third papers. My oral examiners also helped me with their constructive questions, and included Drs. Dani Fallin, Rasika Mathias, Priya Duggal and Elizabeth Selvin.

I also want to thank other key co-authors and mentors who have made the specific papers possible, including Drs. Robert Wood, Jessica Savage, Roger Peng, and Xiumei Hong.

I am indebted to the other graduate students in Epidemiology for their support and feedback. Meg Parker was especially helpful in assisting me with genetic analyses.

None of this work would have been possible without funding from the ICTR's KL2 program and a NIAID K23.

Finally, I thank Aaron Burstein for being supportive through yet another training program, and for his willingness to review linear algebra concepts and other technical matters whenever necessary.

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Preface

Allergic conditions are the most common chronic diseases of childhood, collectively affecting 30-50% of children worldwide¹. These conditions include asthma, allergic rhinitis, food allergy and allergic skin disease. Although they each affect different end organs, and may involve different allergens, allergic diseases share the same fundamental process: sensitization to normally innocuous antigens, and the failure of normal tolerance mechanisms. Food allergy, a condition characterized by early failure of tolerance mechanisms to ingested allergens, is thought to be rapidly increasing in prevalence for reasons that remain unclear.

This thesis is broadly focused the phenomenon of rising prevalence of food allergy and the environmental factors that may underlie it. To this end, I employ three different study methods to examine facets of this question. First, I seek to quantify the overall prevalence of food allergy in the United States, and examine temporal trends and racial/ethnic differences. The second paper then looks at possible environmental causes for increased prevalence of allergy in the developed world. Both vitamin D deficiency and folate excess have been hypothesized to partially explain rising rates of allergic diseases, including food allergy. Within the context of a prospective cohort of new employees at a mouse laboratory, this study examines the relationship between serum vitamin D and folate levels and incident mouse sensitization. Although this study is about mouse, not food, sensitization, and is focused on adults, it was chosen because it presented a unique opportunity examine incident sensitization within the context of

new exposure to an antigen. The findings from this paper have implications for our understanding of how vitamin D and folate may affect the risk of sensitization to other allergens. The final study extends the examination of vitamin D by studying a distantly related exposure, season of birth. Fall season of birth is a known risk factor for food allergy, and it is hypothesized that it may be mediated through decreased levels of vitamin D early in life. The third paper is an analysis of gene-by-environment interaction between common genetic variants and season of birth within a family-based genome wide study of food allergy, with the goal of understanding the mechanisms of this association and identifying the key environmental exposure that is related to season of birth. Together, these investigations aim to identify environmental risk factors which can be modified to prevent food allergy.

Definitions of Sensitization, Allergy and Related Terms

In general, allergy is defined as an abnormal immune response to a normally innocuous non-self antigen, and is divided into two broad categories: IgE-mediated and non-IgE mediated. ***IgE-mediated*** allergic reactions are characterized by their immediate onset and typical symptoms, including hives, swelling, respiratory and gastrointestinal responses, and hypotension; while ***non-IgE mediated*** allergic reactions have a more delayed course, and are typically limited to gastrointestinal or skin symptoms. This thesis primarily focuses on IgE-mediated allergy, although Paper #1 examines self-reported food allergy as a whole, a set of conditions encompassing both types of allergic reactions.

A fundamental requirement for IgE-mediated allergy is **sensitization**; the presence of IgE specific for an allergen. Not everyone sensitized to an allergen develops symptoms upon exposure; thus **clinical reactivity** is distinct from sensitization and requires a history of symptoms upon exposure to the allergen and the absence of clinical tolerance to the allergen, defined as exposure to the allergen with no symptoms.

Food allergy itself is defined as “an adverse health effect arising from a specific immune response that occurs reproducibly on exposure to a given food”². Although food allergy can develop to any food, the most common food allergies in the United States are allergies to cow’s milk, hen’s egg, peanut, tree nuts, shellfish, fish, soy, wheat and sesame^{3,4}.

Public Health Burden of Food Allergy and Other Allergic Diseases

Currently, there is no treatment for food allergy except for avoiding the offending allergen and prompt treatment of allergic reactions with epinephrine and antihistamines³. Because of anxiety related to the possibility of accidental exposures, and the social implications of restricted diets, families of children with food allergy report significantly impaired quality of life^{5,6}. The overall economic cost of food allergy in the U.S. was reported as nearly 25 million dollars per year⁷.

Food allergy typically develops in early childhood, and frequently resolves naturally during or before the school-aged years, but this varies by the food. Milk and egg allergy resolve more commonly, while peanut, tree nut and shellfish allergies less

likely to resolve ^{3,8-11}. However, a significant minority of milk and egg allergy persists into adulthood.

Unanswered Questions in Food Allergy

Prevalence/changes in prevalence/subgroups affected

The true prevalence of food allergy in the United States is unknown. There are several factors contributing to the lack of concrete understanding of food allergy prevalence including: uncertainty and differing definitions of food allergy in various studies of food allergy, secular changes in rates, and lack of systematic review of available data^{3,12}. In addition, underlying heterogeneity in prevalence rates by age, ethnicity and geographic area make estimation of nationwide rates difficult. Although two systematic reviews of food allergy prevalence have been published in the past 10 years^{13,14}, they both looked at *worldwide* rates of food allergy and combined data across multiple time points, despite the widespread understanding that large discrepancies in rates of allergic disease exist between countries, and changes have occurred over time. Individual studies using the same methodology over time have found increases in ambulatory care visits for food allergy, hospital admission, self-reported food allergy overall¹⁵, and self-reported peanut allergy in the U.S.¹⁶ and U.K.¹⁷, but the data have not been synthesized.

The effect of ethnicity on food allergy is poorly defined. In general, African Americans have higher total IgE than Caucasian and Hispanic populations, and they have

also been shown to have higher rates of high food specific IgE^{18,19}. However, some studies report lower rates of doctor diagnosed food allergy²⁰ and provision of self-administered emergency medication²¹. Thus, whether high specific IgE translates into more clinical disease is not clear.

Why Food Allergy Prevalence Might Be Increasing

There are varied hypotheses for why food allergy, along with other allergic diseases, may be increasing in prevalence in the developed world, including theories about the hygiene hypothesis, timing of allergen exposure, and the role of vitamins. The most widely accepted theory to explain this, at least in part, is the ***hygiene hypothesis***, which posits that relative lack of exposure to a wide range of pathogens in early life leads to lasting immune deviation toward allergic phenotypes²². Supporting data for this theory in general include strong epidemiologic data showing children growing up on European farms whose mothers drank unpasteurized milk have less allergy; weaker data linking cesarean section delivery with increased risk of allergy; and a wide variety of animal models showing specific variation in the microbial contents of the gastrointestinal tract affects the risk of allergy^{23,24}. However, thus far attempts to alter the composition of the gut flora have not led to substantial reductions in sensitization in most human studies²⁵, and it remains unclear how much of the allergy “epidemic” can be explained by the hygiene hypothesis. In addition, because we understand little about how the gut microbiome develops, we do not know whether other potential risk factors for allergy may act through this mechanism.

Other theories have to do with the **timing of introduction of foods** to infants, and center on a controversy about whether food sensitization proceeds through the gut or, instead, by way of skin contact with the allergen. Until the mid-2000s, it was widely assumed sensitization to food allergens occurred through the immature gastrointestinal tract. For this reason, and due to some mouse and limited epidemiological data, it was recommended infants delay introduction of potentially allergenic foods and pregnant and breast-feeding mothers consider avoiding certain allergenic foods as well²⁶. However, this strategy did not appear to decrease the prevalence of food allergy. An Israeli population where peanuts were introduced at a very young age had much lower rates of peanut allergy than a genetically similar population in the U.K. where peanuts were introduced later²⁷. Moreover, substantial animal data showed sensitization through irritated skin is much easier than through the gut²⁸⁻³⁴. Finally, the discovery that mutations in Filaggrin, a gene encoding a protein involved in the skin barrier, can be risk factors for food allergy supported the central role of the skin in sensitization process^{35,36}. Thus, a current hypothesis is that skin exposure to allergen can lead to sensitization, but this can be countered by earlier gastrointestinal exposure inducing tolerance. Currently this theory is being tested in some clinical trials^{28,37,38}.

Another theory involves dietary changes and changes in our outdoor exposure which play some role in the development of allergy. Here we focus on two vitamins:

Folate and **Vitamin D**.

Folate

Over the time frame in which allergic diseases have increased in the U.S., a parallel increase in vitamin supplementation has occurred. In the 1960s, it was first observed lower folate levels might be associated with increased risk of neural tube defects, a serious birth defect with a prevalence of at least 6 infants per 10,000 liveborns in the U.S. at its peak³⁹. By the 1980s, evidence began to accumulate that vitamin supplementation could reduce the incidence of neural tube defects, and coalesced around folic acid, the synthetic form of folate, as the important component of vitamin supplementation⁴⁰. In 1992, the US public health service began recommending folic acid supplementation to all pregnant women, and in 1998, the U.S. established mandatory fortification of certain grains, a measure associated with a doubling of serum folate levels in the overall U.S. population⁴¹ and a reduction in neural tube defects by 27%³⁹. However, folate has many effects beyond prevention of neural tube defects. In its metabolized form, folate is a potent methyl donor, and thus may induce epigenetic changes relevant to allergy.

In a mouse model, researchers have found pregnant mice supplemented with methyl donors had pups with more severe allergic airways disease, and this was associated with increased methylation of a specific transcription factor⁴². Following this finding, several prospective birth cohorts have found higher maternal or folate levels early in life are associated with higher risk of various allergic diseases in childhood⁴³⁻⁴⁵. However, sensitization was only measured in one study, and whether folate is associated with sensitization across the life span has not been assessed at all. Thus, the idea that folate is a partial contributor to the “allergy epidemic” has both biologic and

epidemiologic plausibility, but is not yet firmly established.

Vitamin D and Season of Birth

Vitamin D is another nutritional factor hypothesized to be related to allergic disease. Vitamin D has pleotropic effects on the immune system, including dampening of production of inflammatory cytokines and IgE by T and B Cells, respectively, as well as increasing T regulatory cells *in vitro*⁴⁶. Vitamin D has clear antimicrobial functions *in vitro* and *in vivo*, but its role in initiation of allergic diseases is controversial. A recent birth cohort found an association between higher vitamin D levels at birth and development of food allergy⁴⁷, and several other studies have similarly found either a positive association or no relationship⁴⁷⁻⁵⁰. However, other prospective cohorts have shown evidence of association between relative vitamin D deficiency and higher risk of development of allergic diseases^{51,52}.

Some of the epidemiologic data suggesting a role for vitamin D in risk of allergic diseases has come not from direct measurement of vitamin D levels but instead from observations related to variation in UV light exposure and rates of allergic diseases. This is because vitamin D is synthesized in the skin upon exposure to UV light. A variety of publications have linked decreased UV light to increased risk to food allergy, including variations by latitude in the rates of self-administered epinephrine prescriptions,⁵³ hypoallergenic formula use,⁵⁴ and acute anaphylactic reactions.^{55,56}

Another association suggesting some role for vitamin D in allergic diseases is the

longstanding observation that fall/winter birth is associated with higher risk of food allergy. This finding has been replicated in different populations and with different definitions of food allergy⁵⁷⁻⁶². Overall, the odds of food allergy are 30-90% higher among those born in the fall than in other seasons⁵⁷⁻⁶². We recently showed this again in our clinic population in Baltimore, and in NHANES III, a nationally representative sample⁵⁹. It has been hypothesized that this may be due to decreased vitamin D during a critical period of development of food allergy⁶³, although UV light may have other effects on the immune system. In the NHANES III population, the season of birth signal was only seen in White children, suggesting that vitamin D, which varies more strikingly by season in light skinned populations, may be implicated in this association⁵⁹. It is also possible this association may be driven by other seasonal factors, such as viruses and allergen exposures.

Genetic Variation and How it May Interact with Environmental Risk Factors

The heritability of food allergy is estimated at 15-82% for specific food allergies, depending on food allergy definition and the population studied.⁶⁴ However, there are only sparse data on specific genes responsible for this strong overall heritability. To date, several candidate genes, including HLA Class II, STAT6, FOXP3, TNF, SPINK5, IL-13, CD14, IL10, GSTP1 and Filaggrin have all been associated with food allergy, either alone, or in conjunction with eczema.^{36,65} With a few exceptions, these associations have generally not been replicated, and much of the heritability remains unexplained. This is a common problem for complex diseases, and may result from genetic risk factors

manifesting their effects only under specific environmental conditions. It has been suggested that such gene-environment-interactions (GEIs) could account for a substantial portion of the “missing heritability” for complex diseases in general,⁶⁶ and there are several examples suggesting the potential for strong GEIs in allergy. For example, CD14 and endotoxin show strong interaction in their effects on risk to asthma, total IgE and allergic sensitization.⁶⁷⁻⁷⁰ Other GEIs identified for food allergy include those found by Dr. Wang’s group between common markers in immunology genes and both breastfeeding and vitamin D.^{71,72}

How this thesis addresses these questions

This thesis seeks to contribute to our understanding of the epidemiology of childhood food allergy by addressing specific questions relating to its prevalence, the role of specific environmental factors in sensitization, and how environmental factors might interact with genetics to influence development of food allergy. The first paper, “Temporal Trends and Racial/Ethnic Disparity in Self-reported Pediatric Food Allergy in the US”, is a systematic review of the literature aimed at synthesizing data on prevalence of food allergy in the U.S., and its change over time, as well as differential trajectories in specific ethnic groups. The second paper, “Associations Between Serum Folate and Vitamin D Levels and Incident Mouse Sensitization in Adults”, is a nested case-control study within a prospective cohort of new workers at a large mouse laboratory. The goal of this paper is to examine whether vitamin D or folate levels are associated with subsequent development of incident mouse sensitization in the context

of new and/or intensified exposure to mouse allergens. Although this paper focuses on adults, and on a non-food allergen, it has general implications for the relationship between exposure to vitamin D and folate and the development of sensitization. This cohort provides a unique opportunity to examine sensitization as it occurs. The third paper, “Genome-wide Study of Interaction Between Season of Birth and Food Allergy Identifies a Region on Chromosome 3 as a Genetic Risk Factor for Peanut Allergy”, is a family based genome-wide by environment analysis to understand whether season of birth interacts with any common genetic variants to affect the risk of food allergy. Thus, it utilizes a very different study design to examine similar environmental risk factors and, ultimately, to address the fundamental question which runs through all of these investigations – ***why do some people develop allergic diseases while others do not?***

Summary of Methods to be Applied and Their Strengths and Limitations

Systematic Review and Meta-regression:

As outlined above, no systematic reviews of food allergy prevalence in the U.S. have been published. Paper #1 synthesizes the considerable data now available on food allergy prevalence. By using meta-regression, which examines study level factors as predictors of prevalence rates (such as the date of the survey, the type of questions asked and racial/ethnic features of participants) this analysis seeks to answer broad questions about the prevalence of food allergy in the U.S. Because it includes both published and unpublished data collected by the Centers for Disease Control, the potential for publication bias is limited. These data include reports and raw data from

CDC or FDA sponsored national surveys (i.e. NHANES I, NHANES II, NHANES III, NHANES 2005-10, NHIS, National Survey of Children's Health 2003 & 2007, Infant Feeding Practices Study), as well as several telephone surveys and various prospective cohorts.

As in any systematic review, the primary limitation of this analysis is the quality of the data available for synthesis. In this case, because no nationally representative studies in the U.S. have used the gold-standard for diagnosis of food allergy (i.e., food challenge) there is substantial potential for misclassification of disease status.

Nested case-control study

The nested case-control design is an efficient way to take advantage of the benefits of a cohort study – its prospective design, allowing for determination of temporal relationships between exposures and outcome, and its unbiased assessment of outcome – while minimizing resources used. This particular cohort is unique in that it allows for examination of developing sensitization to a new allergen. This is not generally possible otherwise, especially given the limitations of studying very young children in whom most sensitization develops. Other strengths of this cohort include a generally homogeneous population, allowing for isolation of particular environmental factors while minimizing confounding.

However, like all other observational models, confounding cannot be eliminated as a source of bias in cohort studies. Both folate and vitamin D levels are highly dependent on diet, and thus may be associated with many other factors including other

dietary factors and general health-related behaviors. Vitamin D is also highly affected by season and time spent outdoors. In this analysis, season is considered in the model, but it is difficult to model seasonal variation because the timing of the inciting event in sensitization is usually unknown.

Family-based genome-wide by environment association study

This cohort utilizes a family-based design to allow joint tests for linkage and association, which tests for deviation from expected Mendelian transmission of genetic markers. A significant advantage of this method compared to traditional case-control studies is its robustness to bias due to population substructure⁷³; this is particularly important for analyses involving environmental factors, where population level associations between genetic background and an environmental factor can lead to biased results from case-control studies. For this thesis, the R package TRIO is used to assess for gene-by-environment interactions within a genome-wide study. This program fits a conditional logistic regression model comparing observed transmission patterns within case-parent trios to expected transmission patterns, while testing for interaction with environmental factors. This genome-wide approach to looking for gene-by-environment interactions allows identification of novel pathways that may be involved in development of food allergy.

The most significant limitations of this approach are limitations on statistical power in a genome wide analysis considering gene-by-environment interactions, and that coverage of this genome-wide scan is limited to common polymorphic markers.

Because the underlying genetic architecture of food allergy is so poorly understood, it is unknown how much common versus rare variation might be important in influencing genetic risk to food allergy.

Populations Studied

In the first paper, we analyzed data from a broad cross-section of children in the U.S., using studies designed to be nationally representative. The second and third studies use much less diverse populations; the second is of adult employees of the Jackson Laboratories in Maine, and consists generally of White adults in generally good health. The third population is children living in the Chicago area with food allergy; this population is also mostly White. Season of birth effects on food allergy have mostly been described for White populations living at northern latitudes, and thus the geographical and ethnic composition of the Chicago cohort is ideal for the season of birth analysis. The homogeneity of these two populations may limit some types of confounding (such as those between vitamin D levels and skin color and population substructure for the genetic study), but also limits the generalizability of our findings to more diverse populations.

Public Health Significance of These Questions

Ultimately, these studies are all focused on understanding the problem of food allergy and identifying genetic and environmental factors which could be targeted for prevention. Each of these three papers contributes a key piece to our understanding of

this disease: The first paper, the systematic review and meta-analysis/meta-regression informs the critical question of whether food allergy is increasing, and if so, in which groups. This question is important because if food allergy is increasing, then identifying any environmental factor involved in this increase becomes crucial. Identifying certain high-risk subgroups similarly helps inform the search for causes of food allergy, and may also allow us to properly allocate resources, and plan for care of affected children. The second paper, the nested-case cohort study on the association between vitamin D and folate levels and new-onset mouse sensitization in adults, examines environmental factors which could also be prime targets for intervention. There is ongoing controversy about the optimal vitamin D levels for non-bone health, and this analysis could continue to contribute to that question. For folate, there is growing concern that fortification and supplementation strategies with folic acid to prevent neural tube defects may be contributing to increasing rates of allergy. While this analysis will not be a definitive, it can help inform this important public health debate. Finally, the third paper, the gene-by-environment analysis is more exploratory, but may assist in understanding how environmental factors interact to develop allergy. This may ultimately identify pathways which can be acted upon to prevent allergy.

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Paper One

PMID: 24428971

Temporal Trends and Racial/Ethnic Disparity in Self-reported Pediatric Food Allergy in the US

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Word Count: 3476

Number of Figures and Tables: 5

Key words: Food allergy, prevalence, systematic review, race, ethnicity, child.

Abbreviations:
CDC: Centers for Disease Control

CI: Confidence Interval

ES: Estimate

NHANES: National Health and Nutrition Examination Survey

NHIS: National Health Interview Survey

NMIHS: National Maternal and Infant Health Study

NSCH: National Survey of Children's Health

US: United States

Short Title: Temporal Trends and Ethnic Disparities in Food Allergy

Funding Source: The study described was made possible in part by Grant Numbers 1KL2RR025006-01(to CAK) from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH), and NIH Roadmap for Medical Research, and by Grant 1K23AI103187-01 (to CAK). Its contents are solely the responsibility of the authors and do not necessarily represent the official view of NCRR or NIH. Information on NCRR is available at <http://www.ncrr.nih.gov/>. Information on Re-engineering the Clinical Research Enterprise can be obtained from <http://nihroadmap.nih.gov/clinicalresearch/overview-translational.asp>.

Financial Disclosure: The authors have no financial disclosures relevant to this manuscript.

Conflict of Interest: Jessica Savage has received research support from the American Academy of Allergy, Asthma and Immunology and the Food Allergy and Anaphylaxis Network. Robert Wood discloses consulting with the Asthma and Allergy Foundation of America, royalties from Up-to-Date, and service on the Medical Advisory Board to the Food Allergy and Anaphylaxis Network. Elizabeth Matsui discloses receipt of a monetary award from ThermoFisher. Corinne Keet, Shannon Seopaul and Roger Peng have no pertinent disclosures.

Contributor's Statement:

Corinne A. Keet: Dr. Keet conceptualized and designed the study, performed data collection, carried out the analyses, drafted the initial manuscript, and approved the final manuscript as submitted.

Jessica H. Savage: Dr. Savage contributed to the design of the study, performed data collection and analysis, critically reviewed the manuscript and approved the final manuscript as submitted.

Shannon Seopaul: Ms. Seopaul performed data collection and analysis, critically reviewed the manuscript and approved the final manuscript as submitted.

Roger D. Peng: Dr. Peng contributed to the design of the study, advised on the analyses, created figures, critically reviewed the manuscript, and approved the final manuscript as submitted.

Robert A. Wood: Dr. Wood contributed to the design of the study, critically reviewed the manuscript and approved the final manuscript as submitted.

Elizabeth C. Matsui: Dr. Matsui contributed to the design of the study, performed data collection and analysis, contributed to the analysis of the study, critically reviewed the manuscript and approved the final manuscript as submitted.

Abstract:

Background: The prevalence of food allergy is thought to be increasing, but data regarding the US have not been systematically synthesized.

Objective: (1) To summarize the data on prevalence of food allergy in the US pediatric population, and to (2) estimate the effects of time, race/ethnicity and method of assessing food allergy on the estimated prevalence.

Methods: EMBASE, MEDLINE, bibliographies of identified reports, and data from publically available datasets were searched. Studies were limited to those in English with data from the general pediatric US population. Study synthesis was done by meta-analysis, and by meta-regression to estimate the effect of study and participant level covariates, using STATA SE/11. Meta-regression was limited to nationally-representative surveys conducted by the Centers for Disease Control.

Results: 10,090 publications were identified, from which twenty-seven different survey administrations representing 452,237 children were identified, covering the period from 1988 to 2011. Because of heterogeneity among surveys in the estimated food allergy prevalence, a summary estimate of food allergy prevalence was not possible. Meta-regression was performed using 20 of these surveys. Temporal trends were pronounced, with an estimated increased prevalence of self-reported food allergy of 1.2 percentage points/decade (95% CI: 0.7-1.6). The increase/decade varied by race/ethnicity: 2.1 among Non-Hispanic Blacks (95% CI: 1.5-2.7%), 1.2 among Hispanics (95% CI: 0.7-1.7%) and 1.0 among Non-Hispanic Whites (95% CI: 0.4-1.6%).

Conclusion: Self-report of food allergy among US children has sharply increased in the past two decades. The increase has been greatest among Non-Hispanic Black children, a disparity that needs to be investigated.

Introduction:

Food allergy is one of the most common chronic diseases of childhood, with widely varying estimates of prevalence worldwide and in the United States (US)¹⁻³. Several studies have suggested that food allergy has increased dramatically in prevalence over the past several decades in developed countries^{4,5}, and it is perceived that it has increased in the US, but data regarding trends in the US have not been systematically synthesized and evaluated⁶⁻⁸. In this systematic review and meta-analysis with meta-regression we aimed to (1) determine the prevalence of self-reported food allergy in children in the US, and (2) explore sources of variation in prevalence estimates, including case definition, changes over time, and racial/ethnic differences.

Systematic reviews of self-reported food allergy prevalence have previously been published, but had several limitations that we address in the current study^{1,2}. Earlier studies have focused on broad geographic areas, did not account for differences in wording of questions, and did not account for date of survey administration in their estimates, all of which likely contributed to wide prevalence estimates. Further, we incorporate the Centers for Disease Control (CDC) sponsored surveys of the US population into our analyses, which include a wealth of data available on hundreds of thousands of children. While the gold standard for diagnosis of food allergy is the double blind, placebo controlled oral food challenge, this method has yet to be employed in epidemiologic studies in the US, and questionnaire-based data from high

quality surveys is the best we have available. Thus, here we examine the full range of publically available data on self-reported food allergy in US children.

Methods:

Search Methods:

We conducted a systematic literature search (up to February 2012) of MEDLINE and EMBASE for reports that included descriptions of the prevalence of food allergy in the United States. Search terms included prevalence and its synonyms combined with food allergy and its synonyms, including the combination of specific foods with allergy terms. In addition, we searched the reference list of all identified relevant publications and relevant reviews, and examined publically available databases by searching the Centers for Disease Control (CDC) websites^{9,10} (See **Supplementary Methods** for detailed description of search strategy). The search was updated on November 15th 2012, with one new relevant reference identified from reviewing titles/abstracts from 1,276 items found¹¹; however, the data that were reported in this reference had already been incorporated into the meta-analysis. In addition, two new survey estimates were identified from the CDC (of NHIS 2011 and National Health and Nutrition Examination Survey [NHANES 2009-10]¹²). The review protocol was not registered.

Eligibility Criteria:

Our first goal was to estimate the prevalence of self-reported food allergy in the general pediatric US population. Thus, studies that did not sample from the general

population, were not based in the US, did not report on individual level data, did not include estimates for children or did not include food allergy as an outcome were excluded. Included studies reported on the prevalence of food allergy overall and/or specific food allergies. All references were in English. There was no time restriction. Literature eligibility was assessed in duplicate (C.K. reviewed all of the references, and J.S., S.S. and E.M. jointly reviewed a duplicate search); discrepancies were resolved by consensus. References were first screened for eligibility by scan of titles and abstracts, followed by a full-text review. Our second goal was to assess the contribution of study and individual level covariates to variation in estimates of prevalence; specifically to assess temporal changes in food allergy prevalence, ethnic differences and changes over time, and the impact of questionnaire design on estimates of prevalence. Because only the surveys administered by the CDC were considered to be of high enough quality in terms of reporting response rate and accounting for non-response in their estimates, surveys included in meta-regression were limited to those conducted by the CDC (see details in results).

Data collection process:

The following information was extracted from each reference: report characteristics (article name, authors, publication year, journal), study characteristics (study name, year(s) of study, number of participants, method for selection of participants, age of participants), participation rate when available, and diagnostic method for food allergy (self-report of diagnosis or symptoms in the past year, self-report of ever diagnosis or

symptoms and/or self-report of food allergy with no time-period specified, laboratory testing [skin test or specific IgE], combination of laboratory testing and symptoms, or oral food challenge), prevalence and confidence intervals of food allergy in general and specific foods (when available), and ethnicity-specific prevalences when available. Ethnicity was defined as Non-Hispanic White (referred to as White here), Non-Hispanic Black (referred to as Black), Hispanic and other. When confidence intervals or standard errors were not published, binomial standard errors and confidence intervals were calculated from the published data, or, if the underlying data were publically available, from the data directly using appropriate survey methodology. See Supplementary Methods for details about methods of analysis for publically available data. Clarification about details of study design and results were obtained directly from the study authors by personal communication for two reports^{13,14}.

Statistical methods:

All analyses were conducted with STATA SE/11 (College Station, TX) or R (Vienna, Austria). The analysis of summary statistics, including heterogeneity, by meta-analysis was conducted with the package METAN in STATA using a random effects model. Heterogeneity was quantified using the I-squared measure¹⁵; this measure estimates the proportion of between study variation due to heterogeneity. Meta-regression is a method that explores sources of heterogeneity in meta-analysis associated with study-level covariates¹⁶. Temporal trends and differences in prevalence by method of determining food allergy (i.e., differences in questionnaire design) were analyzed by

meta-regression by creating a multivariate model that included both time and category of questionnaire using the package METAREG in STATA. In addition, analyses of ethnic differences over time were also done using meta-regression, adjusting for differences in questionnaire design. For this question, both stratified analyses by ethnicity, and interactions between ethnicity and time were explored. When studies spanned multiple years of data collection, the midpoint year was used for analysis. Only one of the surveys (two administrations) included in the meta-regression reported data on specific food allergies, and thus ethnic differences and changes over time were not analyzed for specific food allergies.

Results:

Study Selection and Characteristics:

10,090 references were identified from the database searches. After the title/abstract screening, full text screening and searches of CDC websites for unpublished surveys, a total of eight surveys were found that met the inclusion criteria^{11-14,17-31}, including one that was administered annually for 15 years (the National Health Interview Survey^{23,24}), one that was administered periodically and included estimates of self-reported food allergy on four separate occasions (the National Health and Nutrition Examination Survey^{12,22,25,26}), one that was administered twice (the National Survey of Children's Health²⁷) and one that was administered three times (the Sicherer et al. peanut and tree nut survey^{13,18,20}); each separate survey date is hereafter analyzed separately. Only one survey used a methodology other than self-report; the NHANES 2005-6 survey, which

measured IgE to milk, egg, peanut and shrimp²². Because data are lacking on population-level positive and negative predictive values for specific IgE, it is not currently possible to extrapolate the overall prevalence of food allergy from IgE data³², and this survey was thus not used in other analyses. All surveys were conducted cross-sectionally. These surveys included a total of 452,237 subjects and covered the period of time from 1988-2011. Search results are summarized in **Figure 1** and **Table 1**.

Seven surveys reported on the prevalence of self-reported food allergy overall in the United States^{11-14,17,20-31}, providing 23 point estimates. There were several ways of identifying food allergy by self-report. Surveys generally fell into one of two categories with respect to type of question used to ascertain self-reported food allergy: (1) questions pertaining to ever having had diagnosis or symptoms of food allergy and/or those that did not specify a time-frame^{12-14,17,20,25,26}, and (2) questions pertaining to current symptoms or diagnosis of food allergy^{11,23-30}. Studies were further split into those that were limited to the infant/toddler period^{17,31} and those that were not.

For specific food allergies, self-reported data were available for peanut, tree nut and shellfish allergy. For peanut, data were available from 4 surveys and 7 survey administrations^{12-14,18,20,22,25,31}. For tree nuts, 3 surveys and 6 survey administrations^{12-14,18,20,25} and for shellfish 3 surveys and 4 survey administrations^{12,14,19,22,25}. (See

Supplementary Figures 1, 2 and 3).

Results of individual studies and synthesis of results for the systematic review:

Figure 2 shows the point estimates and confidence intervals for the included surveys in the systematic review, stratified by method of identifying food allergy and presented by year of study. There was substantial heterogeneity between these estimates ($I^2=97.4\%$, $p<0.001$), and so we are unable to present a summary estimate.

Assessment of quality:

Two surveys that reported national prevalence were deemed to be of insufficient quality to be included in the meta-regression: the Infant Feeding Practices Survey II (IFPS II) used a consumer panel for sampling and was thus inadequately representative of the U.S. population³¹, and the Gupta survey did not report response rate and was an internet survey¹⁴, and thus had high potential for selection bias^{22,32}. Details of response rates and design for each study are included in **Table 1**. All remaining surveys were conducted by the CDC. This left 5 surveys and 20 survey administrations covering 395,220 children for meta-regression of prevalence.

Results of Meta-regression:

Method of identifying food allergy as a source of heterogeneity

Even when restricted to surveys conducted by the CDC, there was too much heterogeneity to calculate a summary measure of food allergy prevalence ($I^2=91\%$, $p<0.001$). We then investigated the sources of this heterogeneity, and identified the question type and year of survey as significant sources of heterogeneity in the estimates. Specifically, 51% of between study variability was explained by method of

identifying food allergy alone, while 88% of the variability was explained by the combination of method and year of survey administration. For example, compared to estimates of prevalence of self-reported current food allergy, the prevalence of self-reported history of food allergy ever was considerably higher, even after adjusting for year of study (difference: 2.5 percentage points between current and ever/time undefined food allergy, 95% CI: 1.5-3.4%, $p < 0.001$ for all children).

Changes over time

The estimated prevalence of self-reported food allergy among children in the US increased significantly over the period from 1988-2011. After adjusting for method of defining food allergy, the self-reported prevalence of food allergy among children was estimated to have increased by 1.2 percentage points per decade during this time (95% CI: 0.7-1.6, see **Figure 3**). For example, within the NHIS survey, estimates of current food allergy increased from 3.4% (95% CI: 3.0-3.8%) in 2000 to 4.6% (95% CI: 4.2-5.1%) in 2010.

Relationship between race/ethnicity and food allergy prevalence

The rate of increase in estimated food allergy prevalence varied significantly by race/ethnicity (see **Figure 4**); the estimated increase in food allergy prevalence per decade among Black children was 2.1 percentage points (95% CI: 1.5-2.7%) compared to 1.2 percentage points among Hispanics (95% CI: 0.7-1.7%) and 1.0 percentage points (95% CI: 0.4-1.6%) among Whites ($p = 0.01$ for comparison of trends between Blacks and Whites, and $p = 0.04$ for comparison between Blacks and Hispanics). As an example of

this trend, Blacks tended to report less food allergy than Whites until approximately the late 2000s, when Blacks began to report food allergy more frequently in nearly all surveys.

Sensitivity Analyses:

To assess the effect of excluding surveys that did not meet the quality criteria, we also did analyses that included the three survey administrations that had been excluded in the primary analyses^{14,22,31} (the IFPS II survey, the Gupta survey and NHANES 2005-6). For the NHANES 2005-6 survey, definitions of allergy according to Liu et al. were used. The overall estimate of change over time did not change appreciably: instead of an estimate of 1.2 percentage point increase/decade, the estimate was 1.3 percentage points/decade (95% CI: 0.5-2.2). Between ethnicity differences were more marked: in this analysis Blacks had a 3.5 percentage point increase per decade (95% CI: 2.4-4.6%), compared to 1.4 percentage points (95% CI: 0.9-1.9%) among Hispanics (95% CI: 0.9-1.9) and 1.1 percentage points (95% CI: 0.4-1.9%) among Whites ($p=0.002$ for comparison of trends between Blacks and Hispanics, and $p<0.001$ for comparison between Blacks and Whites).

Because the questionnaire structure for NHANES III was distinctive from the other surveys in that it asked about history of reactions to foods but did not use the words “food allergy”, we also did a sensitivity analysis excluding NHANES III from the meta-regression. These analyses yielded similar results as the main analyses: in this analysis, change over time was 1.4 percentage points/decade (95% CI: 0.9-1.8), the rate

of increase among Blacks was 2.2 percentage points (95% CI: 1.4-2.9%) compared to 1.3 percentage points among Hispanics (95% CI: 0.8-1.8%) and 1.2 percentage points (95% CI: 0.7-1.8%) among Whites (p=0.03 for comparison between Blacks and Hispanics and p=0.01 for comparison of trends between Blacks and Whites).

Discussion

In this study, we identified twenty-seven surveys that asked about food allergy in the United States. Due to substantial heterogeneity in survey design, we were unable to arrive at a point estimate for current prevalence of self-reported food allergy in US children, although it appears that the prevalence of self-reported food allergy is between three and six percent. However, when we examined temporal trends by race/ethnicity using the 20 surveys administered by the CDC, we did find clear evidence of increasing rates of self-reported food allergy among children, with significant racial/ethnic disparities in the rate of increase. The combined data suggest that the rate of self-reported prevalence of food allergy has increased by more than 1 percentage point per decade. Interestingly, the data show that the rate of increase of self-reported food allergy was twice as high among Blacks as among Whites.

The very few systematic reviews of self-reported food allergy prevalence previously conducted had important limitations that we were able to address here^{1-3,33}. First, previous studies analyzed data from a wide geographical range, although it is well known that substantial differences in the rate of allergy exist between regions of the world³⁴. By restricting our analyses to the US, we are able to more meaningfully assess

trends over time and differences between sub-populations. Second, previous systematic reviews did not account for differences in wording of questions, a factor that contributed considerably to heterogeneity here, and prevented us from presenting a summary measure of self-reported prevalence. Third, they did not account for date of survey administration in their estimates, another large source of heterogeneity, and here we clearly demonstrate significant changes over time in prevalence. Finally, previous systematic reviews did not include the CDC and FDA sponsored surveys of the US population, although there is a wealth of data available in these surveys. Here we examined the full range of publically available data and because we took a comprehensive approach to identifying sources of data, both published and unpublished, our analysis is also unlikely to be influenced by publication bias. Our results showing increases in self-reported food allergy prevalence over time are consistent with more geographically localized studies of peanut allergy in the US³⁵, and with longitudinal data on peanut allergy in the United Kingdom³⁶ and Canada³⁷, although in the United Kingdom, increases over the 2000s were not seen³⁶. Together, these data point to an alarming increase in this disease, one that could cause more and more societal impact if current trends continue. Moreover, although much attention is given to racial/ethnic disparities in asthma, relatively little research has been focused on racial/ethnic disparities in food allergy, despite the rapid increase in self-report of this disease among Blacks.

Because the data are all based on self-report, we cannot distinguish between true increases in food allergy prevalence, increased recognition of true food allergy,

and/or greater awareness resulting in over diagnosis of food allergy, a phenomenon that is clearly documented³⁸. None of the studies included oral food challenges, the gold standard for diagnosis of food allergy³⁹. Previous analyses have shown that self-report of food allergy overestimates the true rate of clinical disease⁴⁰⁻⁴³. Nonetheless, although food challenges are the gold standard for diagnosis of food allergy, they are not feasible in large epidemiologic studies for both practical and potential ethical reasons, and so questionnaires are the best data that we currently have and are useful for examining changes over time.

Here we show that the nature of what was being estimated (current allergy vs. history of allergy/no timeframe defined) was a substantial source of heterogeneity in estimates of food allergy prevalence. We did not have enough data to examine the effects of further variation in specific wording on prevalence estimates. Questions varied substantially, from wording designed to identify the symptoms consistent with an IgE mediated food allergy (such as done by Sicherer, Gupta and in NHANES III), to much more general questions, such as those asking for doctor diagnosis of “food or GI/digestive allergy” (such as asked in NHIS and NCHS) to simply “do you have any food allergies?” (NHANES 2007-8 and NHANES 2009-10). In general, the data point to the need for clarity about what we are estimating when we report prevalence, and to the need for the development of validated and easily administered methods that can accurately estimate clinical disease.

Although it is known that there are large disparities in asthma prevalence and severity by race/ethnicity, the role of race/ethnicity as a risk factor for food allergy has not been well established. Here we show that while the current prevalence of self-reported food allergy may be higher among Blacks than Whites or Hispanics, this has not always been the case. We demonstrate a more rapid increase in the rate of self-reported food allergy among Blacks over the past several decades than among Whites or Hispanics. Racial/ethnic disparities have also been seen in the few studies done that looked at specific food allergies^{13,20}.

In general, Blacks have higher total IgE levels than White and Hispanic Americans and genetic risk factors have been implicated in these differences. A higher proportion African Ancestry has been associated with higher risk of sensitization to foods among young children, even among those self-identified as Black⁴⁴⁻⁴⁷. Consistent with this observation, the estimated differences in food allergy between Blacks and Whites are substantially higher for IgE than for self-report. Specifically, in NHANES 2005-6, based on IgE, food allergy prevalence was 8.4% among Black children, compared to 2.5% among Whites and 4.2% among Hispanics [data not shown]²². At the same time, in NHIS, self-reported food allergy prevalence was 3.9-4.6% among Blacks, 4.3-4.8% among Whites and 3.1-2.5% among Hispanics²⁹. Thus, despite this apparent genetic predisposition towards higher total and specific IgE levels, before the mid-2000s Blacks reported food allergy at rates equal to or lower than Whites. This suggests that either there are biologic differences in how specific IgE and clinical symptoms relate between racial/ethnic groups, or that recognition of food allergy has lagged among Blacks.

However, because we do not have food-specific IgE data from multiple time points, we cannot distinguish between lagging recognition of food allergy among Blacks and a real increase in the rate of allergic disease, perhaps as a result of heightened susceptibility to some changing environmental factor⁴⁸. Because of the limitations of meta-regression, which is a study-level analysis, we are not able to assess how much of the apparent racial/ethnic disparities are due to socio-economic factors. Both Whites and Hispanics also showed an increase in food allergy prevalence, although at a slower rate than Blacks, suggesting that whatever is driving this increase is not isolated to Blacks. Further investigation should be focused on substantiating the disparity in rate of increase of food allergy in Blacks, and in examining whether increases can be explained by other changes, such as access to care, dietary changes or other environmental factors. These investigations will not only identify at risk populations, but also help elucidate the mechanisms of food allergy.

After accounting for differences in survey questionnaire design in this comprehensive synthesis of the available data on self-reported food allergy in US children, we find a marked increase of self-reported food allergy of approximately 1 percentage point per decade that shows no signs of abating. Whether the apparent rise in prevalence of food allergy is real not only has important implications for our understanding of the pathophysiology of food allergy, but also for whether we should be allocating resources preferentially to prevention and treatment or to improving the accuracy of diagnosis. In addition, Blacks have had a disproportionate increase in self-reported food allergy, highlighting an underappreciated and emerging health disparity.

From this wealth of survey data, it is clear that that more parents believe that their children have food allergies than ever, and that disparities are growing. Whether this represents a true biological increase, and if so, why, are urgent questions to answer.

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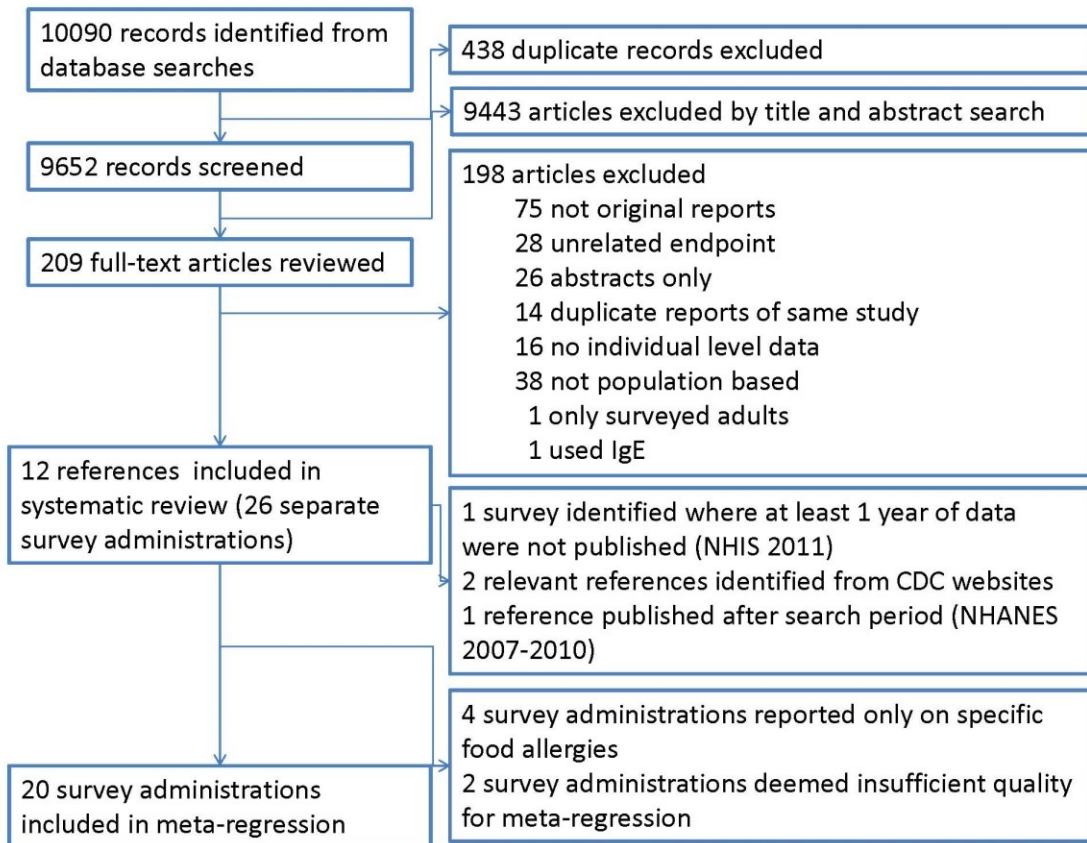


Figure 1: Search results. Abbreviations: NHIS: National Health Interview Survey, NHANES: National Health and Nutrition Examination Survey. CDC: Centers for Disease Control

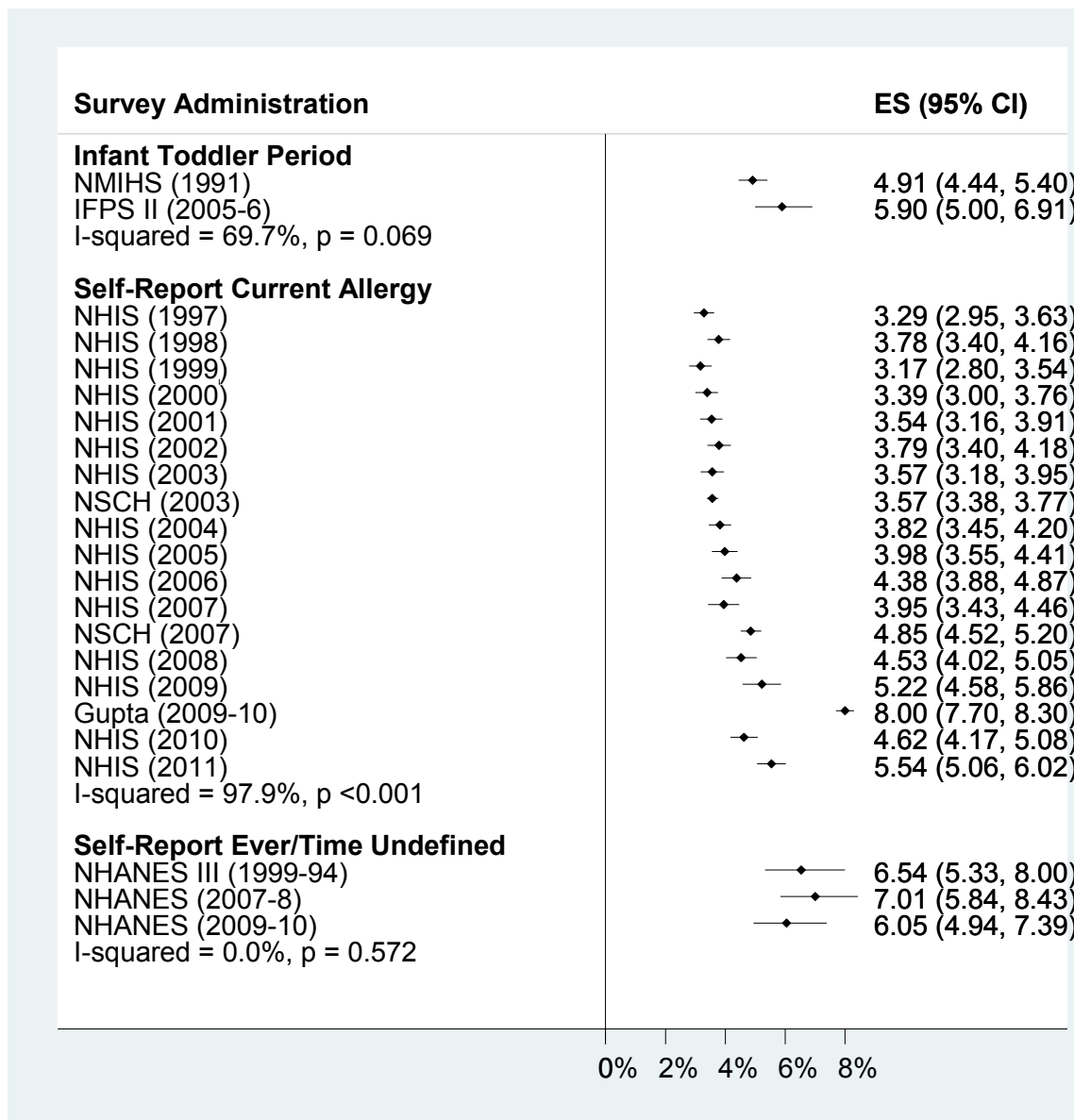


Figure 2: Forest plot of food allergy prevalence in children in the US, by category of food allergy definition, ordered by year of survey; surveys done repeatedly are included separately by year of survey. Abbreviations: ES: Estimate, NMIHS: National Maternal and Infant Health Study, NHANES: National Health and Nutrition Examination Survey, NHIS: National Health Interview Survey, NSCH: National Survey of Children’s Health, IFPS II: Infant Feeding Practices Study II.

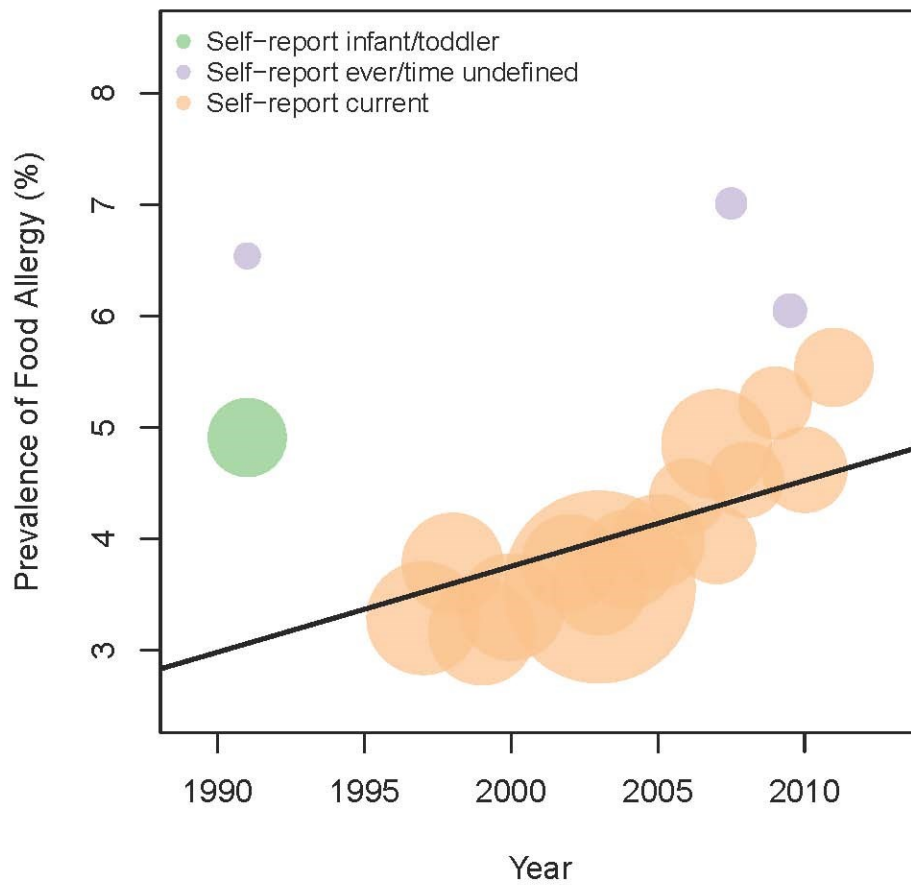


Figure 3: Prevalence of food allergy over time. Individual dots reflect individual survey dates. The size of the dots is proportionate to the number of subjects surveyed.

Figure 4A: Whites

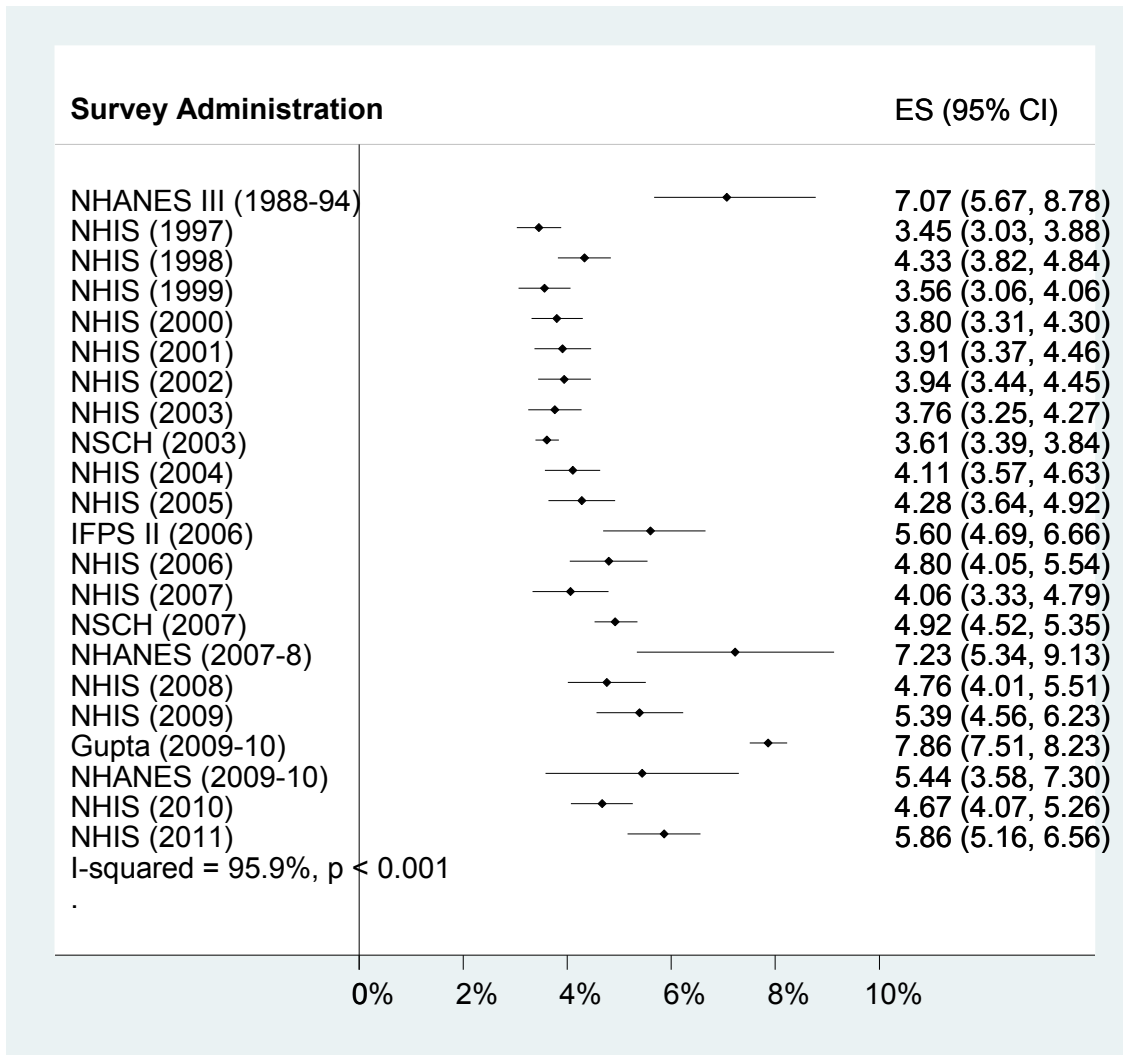


Figure 4B: Blacks

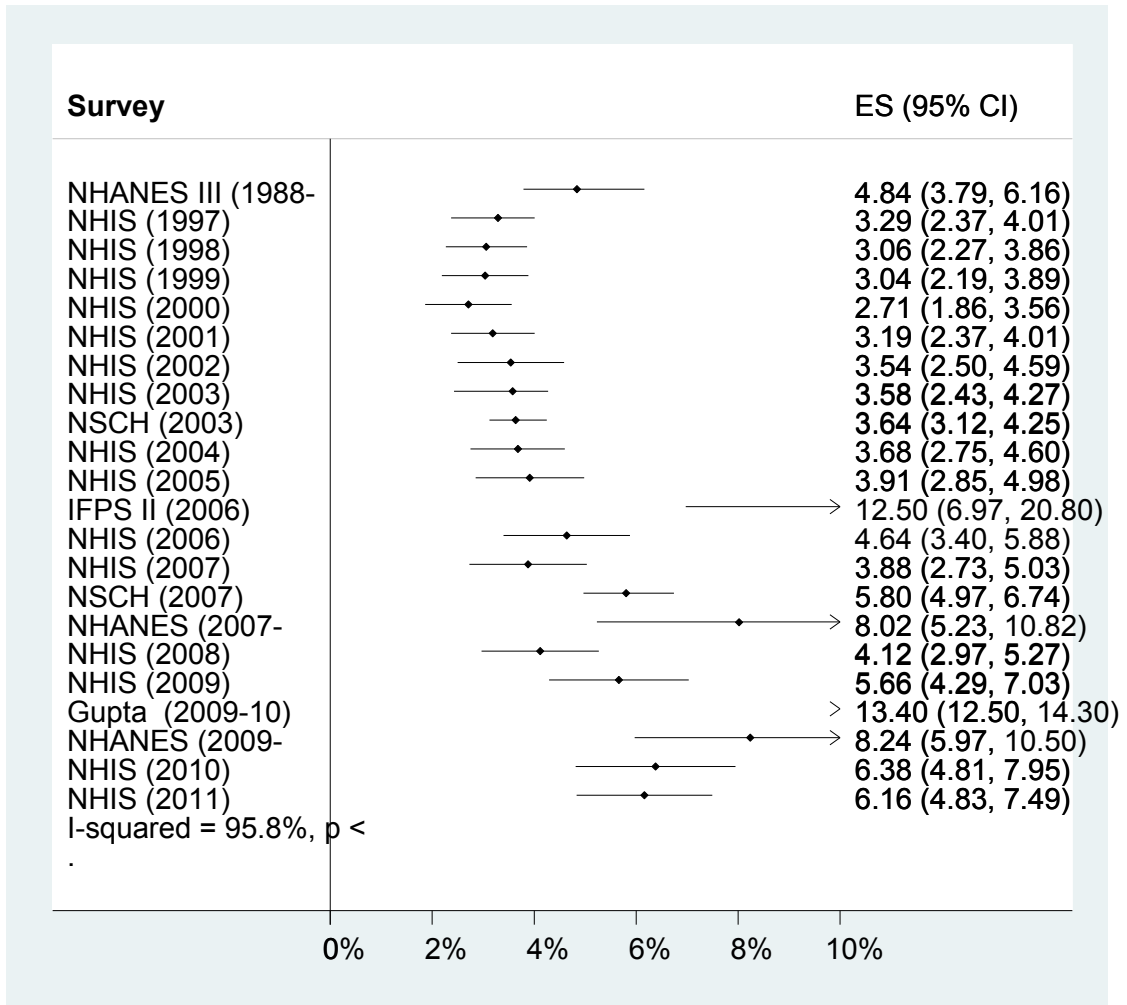


Figure 4C: Hispanics

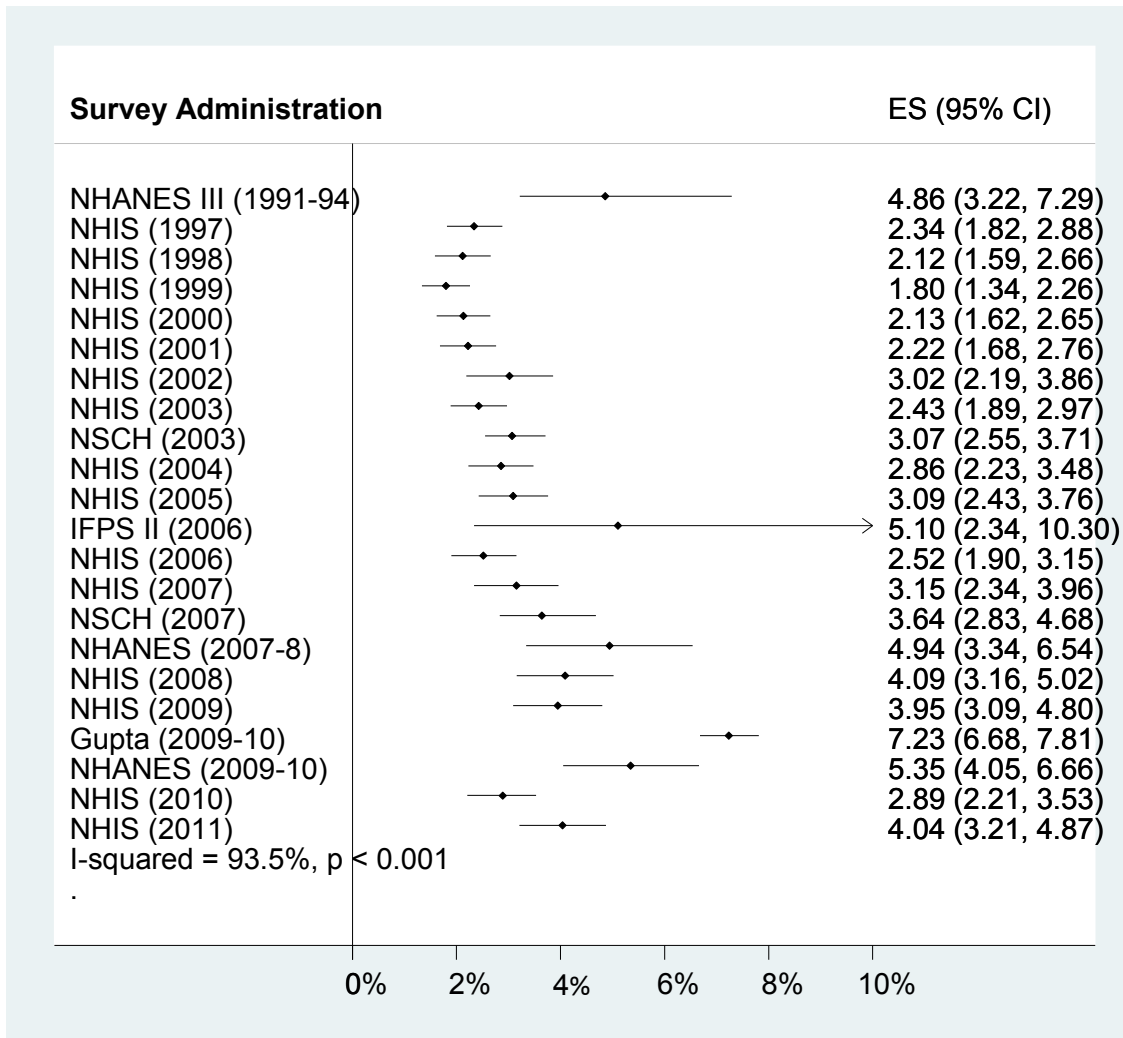


Figure 4: Forest plot of self-report of current food allergy by ethnicity, ordered by time; surveys done repeatedly are included separately by year of survey. A: Non-Hispanic Whites. B: Non-Hispanic Blacks. C: Hispanics. Abbreviations: ES: Estimate, NHANES: National Health and Nutrition Examination Survey, NHIS: National Health Interview Survey, NSCH: National Survey of Children’s Health, IFPS II: Infant Feeding Practices Study II.

Study Name	Year	N	Setting	Diagnostic Criteria	Primary Data Used?	Age Range	Data on overall prevalence available	Participation Rate	Used in meta-regression
National Maternal and Infant Health Survey ¹⁷	1991	8285	Phone and Mail	Self-report of ever food allergy. Infant/toddler.		3	X	90%	X
National Health and Nutrition Examination Survey III ^{25,26}	1988-1994	6600	In-person	Self-report of ever reaction to a food.	X	<18	X	86%	X
Sicherer Peanut/Tree nut ¹⁸	1997	2998	Phone	Self-report ever of peanut/tree nut allergy.		<18		67%	
Sicherer Shellfish ¹⁹	2002	3814	Phone	Self-report ever of shellfish allergy.		<18		67%	
Sicherer Peanut/Tree nut ²⁰	2002	3127	Phone	Self-report ever of peanut/tree nut allergy.		<18		52%	
National Survey of Children's Health 2003 ^{21,28}	2003	102166	Phone	Self-report of food allergy in past 12 months.	X	<18	X	55%	X

Table 1.

Study Name	Year	N	Setting	Diagnostic Criteria	Primary Data Used?	Age Range	Data on overall prevalence available	Participation Rate	Used in meta-regression
Infant Feeding Practices Study II ³¹	2005-6	2441	Mail	Self-report ever. Infant/toddler.		<1	X	76%	
Sicherer Peanut/Tree nut ¹³	2008	2915	Phone	Self-report ever of peanut/tree nut allergy.		<18		42%	
National Health Interview Survey 1997-2011 ^{23,24,29,30}	1997-2011	179,774	In-person	Self-report of food allergy in past 12 months.	X	<18	X	~80%	X
National Survey of Children's Health 2007 ^{24,27}	2007	91393	Phone	Self-report of food allergy in past 12 months.	X	<18	X	47%	X
National Health and Nutrition Examination Surveys 2007-8, 2009-10 ^{12,25*}	2007-2010	7002	In-person	Self-report of food allergy – no time frame defined	X	<18	X	>85%	X
Gupta ¹⁴	2009-2010	38480	Internet	Self-report of food allergy – current.		<18	X	Not published	

Table 1 Continued

Paper Two

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PMID: 24290285

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16 Associations between serum folate and vitamin D levels and incident mouse
17 sensitization in adults

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49 Sources of support: This research was funded in part by NIAID/NIH grant number
50 1K23AI103187 (to C.A.K) and NIAID/NIH grant number R01AI081845 (to E.C.M.).

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52 Word count: 2992

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54 Capsule summary:

55 In an analysis of mouse workers, higher baseline serum folate, but not vitamin D, levels
56 were associated with new mouse sensitization, indicating that folate may be a risk factor
57 for allergic diseases across the life-span.

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Abstract:

Rationale: Although both folic acid intake and vitamin D levels are hypothesized to be contributors to increased incidence of allergic diseases, prospective studies of these relationships have not been done in adults.

Objectives: To determine whether serum folate or vitamin D levels are associated with incident mouse sensitization among new workers at a mouse facility.

Methods: Subjects started employment at the Jackson Laboratory between June 2004 and July 2007. Skin testing to mouse and other allergens, and collection of questionnaire data, was performed at baseline and every 6 months. Serum folate and vitamin D levels were assessed on baseline samples stored at -80°C. Folate was categorized into tertiles (2.5-10.5 ng/ml, 10.5-16.2ng/ml and 16.2-78.4ng/ml). Vitamin D was categorized as <20 ng/ml, 20-29 ng/ml or ≥30 ng/ml. This was a nested case/control study in which 5 controls were matched to each case on baseline atopy and type of employment.

Multivariate analyses controlled for age, sex, education, smoking, season, personal mouse exposure, serum folate and vitamin D levels.

Measurements and Main Results: 35 cases and 47 controls were included. The odds of incident mouse sensitization were higher in the intermediate and highest tertiles of serum folate, compared to the lowest tertile of serum folate (OR: 10.5 [95% CI: 1.8-61.5], p=0.009, and OR: 5.6 [95% CI: 1.8-31.3], p=0.049, respectively in the multivariate model). Serum vitamin D was not associated with incident mouse sensitization.

Conclusions: These findings support a role for higher serum folate levels in increased risk of incident allergic disease, even during adulthood.

Word Count: 250

Key Messages:

- Higher serum folate levels may be associated with increased risk of new allergic sensitization among adults.
- No evidence was found that low vitamin D levels increase the risk of new allergic sensitization among adults.

Key Words: Folate, folic acid, vitamin D, allergy, mouse allergy, sensitization.

Abbreviations:

CI: Confidence interval.

Introduction:

Among the hypothesized causes of the recent increase in allergic diseases are changes in micronutrients among the U.S. population. Two leading contenders for this hypothesis are folate and vitamin D. Specifically, increased folic acid intake, due to supplementation and fortification of foods, and decreased vitamin D production, due to more time indoors, have both been linked to increased risk of allergic sensitization(1-11). However, data supporting either hypothesis are mixed. Studies have generally either been cross-sectional or birth cohorts, and, for folate, have shown different associations with sensitization in studies addressing prenatal and early life exposure(1-3) than in those studying older children or adults(12, 13). It has been hypothesized that the increased risk of allergic outcomes with higher prenatal folate seen in some birth cohorts is due to epigenetic effects, and that this mechanism is relevant at critical developmental times only(5, 14). To our knowledge, there are no published studies that specifically examine whether either folate or vitamin D levels are associated prospectively with new allergen sensitization in adults.

The Jackson Laboratory cohort offers a unique opportunity to explore predictors of de novo sensitization in adults. This longitudinal cohort is composed of new employees at the Bar Harbor, Maine, Laboratory, a mouse research and production facility that sells over 2 million mice per year. Enrolled subjects are assessed at baseline and every six months for mouse sensitization, and detailed information, including direct

measurement of personal mouse allergen exposure, are collected periodically. Using this cohort, we sought to determine whether baseline serum folate or vitamin D levels were associated with incident mouse sensitization.

Methods:

Description of cohort:

As has been previously described(15), this cohort was drawn from subjects who started non-temporary, full-time employment at The Jackson Laboratory between July 2004 and December 2007. Eligibility criteria included age of at least 18 years, provision of written informed consent, and, for this analysis, absence of mouse sensitivity by skin prick test at baseline. This study was approved by Institutional Review Boards at the Johns Hopkins Medical Institution and the Jackson Laboratory.

Assessment of outcomes and confounders:

Briefly, skin prick testing was performed at baseline and every 6 months using the Multi-Test II device (Lincoln Diagnostics, Decatur, Ill). A positive result was defined as an orthogonal wheal size of at least 3 mm more than that elicited by the negative control.

At the baseline visit the following allergens were tested using two Multi-testers and atopy was defined as a positive result to at least one: mouse, rat, cat, dog,

Dermatophagoides pteronyssinus, *Dermatophagoides farinae*, pine, birch, oak, orchard grass, *Alternaria* species, *Aspergillus* species, *Penicillium* species, and ragweed. At

follow-up visits the following allergens were tested using one Multi-tester: mouse, rat, cat, dog, dust-mite mix, and pine.

Baseline demographics were captured by a questionnaire at the first visit administered by the study staff. A follow-up questionnaire was administered at subsequent visits and captured interval allergic and occupational history. Subjects were followed for up to 36 months.

The methods for assessing personal mouse allergen exposure have been described in detail elsewhere(15). Briefly, personal air samples were collected during 2 full 8-hour shifts within one week period every 6 months using Buck VSS-12 personal sampling pumps (A.P. Buck, Inc, Orlando, FL). Mus m 1, the major mouse allergen, was quantified using sandwich ELISA. Personal exposure was defined as the mean of each participant's repeated Mus m 1 measurements and was categorized into tertiles of exposure to capture the non-linear relationships with mouse sensitization that were previously reported(15).

Serum folate and vitamin D levels were measured on sera from the baseline visit that were stored at -80°C. Serum folate was quantified by a paramagnetic particle, chemiluminescent immunoassay (Access Immunoassay Systems, Beckman Coulter, Brea, CA). Serum folate was categorized into tertiles (2.5-10.5 ng/ml, 10.5-16.2 ng/ml and 16.2-78.4 ng/ml). Vitamin D was quantified by LIAISON 25 OH Vitamin D assay from DiaSorin, Inc. (Stillwater, MN). The assay is a direct competitive chemiluminescent

immunoassay (CIA) for the quantitative determination of total 25 OH vitamin D in serum. Based on prior literature, vitamin D levels were categorized as <20 ng/ml, 20-29ng/ml, and \geq 30 ng/ml, which some consider insufficient, potentially insufficient and optimal, respectively, although there is considerable controversy about these labels, particularly for consideration of conditions apart from bone health(16). This resulted in groups of 26, 28 and 28 subjects respectively.

Selection of cases and controls:

Cases and controls were selected using a nested case control method. Cases were defined as subjects with incident sensitization to mouse on skin prick testing. Each case subject was individually matched to five control subjects using incidence density sampling according to baseline atopic status and whether their employment at The Jackson Laboratory included mouse handling. With incidence-density sampling, each subject's eligibility to be a control was assessed at the same follow-up time as each incident case; thus, each subject could be a control for more than one case, and cases who became mouse sensitized at later follow-up dates could serve as controls for cases presenting earlier(17, 18). With this method, time to development of sensitization is incorporated into the outcome. Most importantly, incidence density sampling is more robust to bias introduced by loss to follow-up than other methods.

Statistical analysis:

Analysis of predictors of case and control status was done using conditional logistic regression except for the demographic characteristics shown in Table 1, which were

analyzed by chi square statistics for dichotomous variables and Wilcoxon rank sum statistics for continuous variables and which categorized subjects by whether they ever became cases or remained controls. Power calculations, assuming a standard deviation of 8 ng/ml in each serum micronutrient level and 35 cases and 35 matched controls, found 80% power to detect a difference of at least 3.9 ng/ml in serum vitamin D or folate levels between cases and controls. Potential confounders included in the analysis were baseline age, sex, education, smoking status, and season, average personal Mus m 1 exposure, and either baseline serum vitamin D or folate levels, for analyses of serum folate and vitamin D levels, respectively. When used as a confounder, serum folate was divided into tertiles, and serum vitamin D was categorized as described above. Education was classified as either completing less than a college degree versus completing at least a college degree. Smoking history was classified as never, former or current. Confounders were selected based on prior plausibility; only season was significantly associated with sensitization in the multiple regression model. Analyses were limited to subjects with data on personal Mus m 1 exposure, which excluded 6 control subjects. All analyses were performed using STATA SE/11 (College Station, TX).

Results:

Baseline Characteristics:

The cohort from which this nested study was drawn consisted of 260 subjects. Overall, the cohort was predominantly female (57%), Caucasian (91%), and had positions that involved handling mice (66%). Similar to the U.S. population as a whole(19), 10%

reported current asthma at baseline. For this analysis, 35 cases and 47 controls were selected. As can be seen in Table 1, cases and controls were similar except in smoking history; subjects who had incident mouse sensitization were much more likely to be never smokers than controls ($p=0.02$). Both cases and controls were overwhelmingly Caucasian, and were generally highly educated. (Table 1)

Predictors of serum folate levels:

No demographic characteristics were statistically significant predictors of serum folate levels except smoking status; folate levels were higher among never smokers (median folate 14.0 ng/ml) compared to former (median folate 10.6 ng/ml) and current (median folate 11.0 ng/ml) smokers ($p=0.046$). Sex, education, race/ethnicity, season and vitamin D levels were not predictors of serum folate levels (>0.35 for all). (Table 2) Similarly, age was not a predictor of serum folate ($p=0.85$).

Association between serum folate levels and incident mouse sensitization:

The odds of incident mouse sensitization was highest in the second tertile of serum folate in unadjusted models (OR: 5.4 [95% CI: 1.9-15.2], $p=0.001$ when compared to the lowest tertile of serum folate). This association persisted when analyses were adjusted for age, sex, education, smoking status, mouse allergen exposure, season and vitamin D group (OR: 10.5 [95% CI: 1.8-61.5], $p=0.009$). The highest tertile of serum folate was also associated with a higher odds of incident mouse sensitization than the lowest tertile, although the odds were not as high as in the second tertile of serum folate (unadjusted analyses: OR: 2.4 [95% CI: 0.9-6.9], $p=0.09$, fully adjusted model: OR: 5.6 [95% CI: 1.8-

31.3], $p=0.049$, compared to lowest tertile). When folate was treated in a continuous manner, there was not a statistically significant relationship with incident mouse sensitization, although there was a positive trend (OR: 2.0 [95% CI: 0.7-5.3], $p=0.17$ in fully adjusted model) (Table 3).

Predictors of serum vitamin D levels:

Serum vitamin D levels were higher among female subjects than male subjects (medians: 30 and 20 ng/ml, respectively; $p=0.0006$) and in the summer and fall (medians: 28 and 29 ng/ml, respectively) compared to winter and spring (medians: 20 and 17 ng/ml, respectively; $p=0.003$). Vitamin D levels tended to be higher among Caucasians (median 25 ng/ml) compared to non-Caucasians (medians: 25 and 18 ng/ml, respectively; $p=0.09$). Smoking history, tertile of serum folate and education did not predict serum vitamin D level ($p=0.31$, 0.76 and 0.75, respectively). (Table 2) Similarly, age was not a predictor of serum vitamin D ($p=0.17$).

Association between serum vitamin D levels and incident sensitization:

Category of vitamin D level was not associated with incident mouse association in either unadjusted or adjusted models, although there was a trend towards increased odds of sensitization in the intermediate category of serum vitamin D (unadjusted model: OR: 2.3 [95% CI: 0.8-6.1], $p=0.09$, fully adjusted model: OR: 2.6 [95% CI: 0.7-10.1], $p=0.17$, compared to lowest category of vitamin D). However, this trend was not evident for the highest category of vitamin D (unadjusted model: OR: 1.7 [95% CI: 0.6-4.7], $p=0.27$, fully adjusted model: OR: 1.6 [95% CI: 0.4-6.5], $p=0.55$, compared to lowest category of

vitamin D). In addition, there was no linear relationship between vitamin D and incident sensitization (OR: 3.2 [95% CI: 0.7-14.1], $p=0.13$ in fully adjusted model).

Discussion:

In this prospective cohort of new employees at a mouse facility, we found that serum folate levels were associated with incident sensitization to mouse. Specifically, we found that those in the middle tertile of serum folate had a 10-fold higher odds of sensitization compared to those in the lowest tertile, while those in the highest tertile had a 6-fold higher odds of sensitization compared to the lowest tertile. Our findings that higher levels of serum folate are associated with higher risk of incident sensitization are consistent with a small but growing literature showing higher risk of allergic outcomes with higher folate levels. Intriguing evidence from animal models suggests that folate's role as a methyl-donor may mediate its association with allergic disease through epigenetic changes, but this mechanism has previously been hypothesized to be relevant only during prenatal and early life. Our findings suggest that folate may continue to have an effect on allergic outcomes throughout the lifespan. In contrast, although we hypothesized that lower vitamin D levels would be associated with higher risk of incident sensitization, we did not find a consistent relationship in this cohort; in fact, there was a trend towards an association between higher levels of vitamin D and increased odds of sensitization.

In the U.S., mandatory fortification of certain grain products with folic acid for the prevention of neural tube defects began in 1998, and was associated with a more than

doubling in mean serum folate levels in the general U.S. population(20). Over the same period, recommendations for prenatal and preconception supplementation with folic acid were also strengthened(21). Because these interventions occurred simultaneously with increases in certain allergic diseases, the role of folate in development of atopy has received recent attention. Several birth cohorts have examined the association between prenatal or early life folate levels and atopic outcomes. Most relevantly, Okupa et al. found that early life serum folate levels were associated with higher risk of incident allergic sensitization(1). Similarly, Kiefte-de Jong et al.(2) and Haberg et al.(3), found that higher maternal folate levels were associated with higher risk of eczema and asthma, respectively, in children, although sensitization was not assessed in either cohort. Others studies examining folic acid intake but not serum folate levels have found mixed results with regard to allergic sensitization, perhaps because of the biases associated with relying on maternal report of folic acid intake (4, 22-24).

To our knowledge, prospective studies of the relationship between serum folate levels and atopic outcomes outside of early childhood have not been done. Two cross-sectional studies in general populations showed that higher serum folate levels were associated with lower risk of asthma diagnosis and exacerbation, but were mixed in their findings of allergic sensitization (12, 13). In contrast, among asthmatics, Lin et al. found that moderate levels of folate were associated with higher exhaled nitric oxide and IgE, but did not find an association with asthma symptoms(14). Serum folate may have a relationship with asthma symptoms and diagnosis that is distinct from that with incident sensitization, as many allergic and non-allergic factors play a role in asthma

pathology. Our prospective data, adjusted for important confounders, suggests that higher folate levels may increase the risk for new allergic sensitization well into adulthood. Thus, although cross-sectional data initially suggested a protective role for folate in allergic sensitization, prospective data, including this study, now suggest the opposite. Previous cross-sectional data may have been inaccurate because it did not adequately measure folate at the critical time for development of sensitization, or because of unmeasured confounding.

Epigenetic modifications have been proposed as the pathway by which higher folate may increase the risk of allergic disease. As a methyl-donor, folic acid could contribute to increased methylation of genetic areas relevant to allergy. Work by Hollingsworth et al. supports this mechanism. In seminal mouse models, they supplemented pregnant mice with a diet high in methyl donors, including folic acid, and found increased severity of allergic airways disease in their offspring, a change that was inherited trans-generationally. Moreover, they tied this outcome to excessive methylation of the Runt-related transcription factor 3, a known regulator of allergic airways disease(5). Whether this mechanism mediates the effects of folate on allergic diseases in humans has not yet been explored.

Folate has a myriad of other biological functions that could also be relevant to sensitization. Recently it was discovered that folate metabolites activate mucosa-associated invariant T cells(25), a cell type that could potentially be involved in the early steps of sensitization. Other functions include key roles in DNA turnover, which may also

be important in allergic diseases. For example, it is possible that low folate levels interfere with Th2 skewing by impairing T cell differentiation rather than by epigenetic mechanisms (14). In our results, there was not a clear linear dose response curve for the association between folate and sensitization; in fact it appeared that the highest risk of sensitization occurred at intermediate levels of serum folate. This could be because of a threshold effect related to methylation, or could be due to other effects at higher levels of folate. It has been speculated that other anti-inflammatory properties of folate may become more important at higher levels, potentially explaining the relatively smaller effect of higher folate levels compared to intermediate folate levels on incident sensitization seen here(14). Further research needs to be done to clarify the mechanisms by which folate may affect the development of allergy.

Although several studies have shown cross-sectionally that children and adults with various atopic conditions have lower vitamin D levels(6, 7, 11, 26, 27), prospective studies of vitamin D in association with allergic sensitization have shown much more mixed results, with some showing higher risk of sensitization with lower vitamin D(8, 9), and others showing no association or the opposite relationship(10, 28-30). Our data do not support a role for vitamin D deficiency or insufficiency in new-onset allergic sensitization in adults, and in fact show a trend towards higher risk of sensitization with higher levels of vitamin D. Because vitamin D has clear anti-microbial properties, it is possible that previous relationships seen with respiratory outcomes are primarily due to this property of vitamin D, and not its effects on the allergic diathesis(31). However, one complication of our current analysis, and studies analyzing vitamin D in general, is that

serum vitamin D levels are highly dependent on season, and because we do not know the key time-point at which vitamin D might influence development of sensitization, it is possible that seasonal variations obscure a real relationship between vitamin D and sensitization. Although we did adjust for season in our analyses, both residual confounding and the time-varying nature of this variable could have biased our results. In contrast, seasonal changes are not likely to be a problem for analyses of associations with folate, as this micronutrient is not influenced by sun exposure or other seasonal factors.

The strengths of this study are that it took advantage of the natural experiment that is new employment at The Jackson Laboratory to prospectively determine the effects of folate and vitamin D on incident sensitization in subjects with new or intensified exposure to allergen, a question that has not been studied in adults. In addition, subjects and controls were matched on the most important predictors of incident sensitization, atopic status and mouse handling status, and important confounders, including smoking status and season, were adjusted for in the analyses. However, it is possible that we missed other important confounders of the relationship between these micronutrients and incident sensitization. Another important caveat is that we measured incident sensitization but did not have a large enough sample to assess allergic symptoms here. However, allergic sensitization is a first step in developing allergic symptoms and is a reasonable surrogate for increased risk of symptomatic allergy. Despite these caveats, this cohort offers a rare opportunity to study incident sensitization in a relatively homogenous group of adults.

In summary, this study extends previous findings that have tied higher folate levels to higher risk of allergic outcomes in children to adults, but did not find evidence that higher vitamin D levels protect against sensitization. Although it would be quite premature to recommend reduction in folic acid supplementation based on these results, especially given the very compelling reasons for folic acid supplementation in pregnant women, this finding does add to a small but growing literature that supports folic acid fortification and supplementation as a possible cause of recent increases in incidence of allergic diseases. More research aimed at understanding the mechanisms of this association may lend insight into whether folic acid supplementation might be responsible, in part, for the relatively recent increase in allergic disease. Additional prospective studies extending this study's findings to other allergic diseases are also needed, and will help inform the design of clinical trials aimed at determining if there is an optimal folate level for allergic disease prevention.

Acknowledgements:

The authors acknowledge Lori Sokoll, PhD and Barbara Detrick, PhD for analysis of the folate and vitamin D levels and Shannon Seopaul for facilitating sample analysis.

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Figure 1. Associations between serum folate level and odds of incident mouse sensitization. Tertile of serum folate: 1 (2.47-10.45 ng/ml, reference), 2 (10.53-16.16 ng/ml), 3 (16.22-78.44 ng/ml). Odds ratios are compared to tertile 1, and are adjusted for age, sex, education, smoking status, mouse allergen level, season and vitamin D levels. CI: Confidence interval.

Table 1. Characteristics of cases and controls.

Characteristic	Cases (n=35) n (%)	Controls (n=47) n (%)	p value
Age, median (IQR)	29 (22-35)	32 (24 – 40)	0.15
Female sex	19 (54%)	25 (53%)	0.89
Caucasian race	32 (89%)	43 (86%)	0.69
Education (at least college graduate)	23 (66%)	25 (53%)	0.18
Smoking History			0.02
Never	25 (71%)	20 (43%)	
Former	3 (9%)	13 (28%)	
Current	7 (20%)	14 (30%)	
Tertile of Personal Mus m 1*			0.59
1 (0.01 – 0.45 ng/m ³)	9 (26%)	15 (32%)	
2 (0.45 – 6.51 ng/m ³)	16 (46%)	16 (34%)	
3 (6.58 – 629.39 ng/m ³)	10 (29%)	16 (34%)	
Sensitized to cat or dog	11 (31%)	12 (26%)	0.62
Previously worked with mice	9 (26%)	13 (28%)	0.84
Handle mice†	19 (54%)	29 (62%)	0.65
Atopic†	27 (77%)	36 (76%)	0.95
Folate level (ng/ml), median (IQR)	15.1 (9.5-20.6)	14.1 (6.7-78.4)	0.03
Vitamin D level (ng/ml), median (IQR)	27 (20-36)	23 (14-33)	0.09
*Personal Mus m 1 exposure was defined as the mean of all of the personal Mus m 1 exposure assessments for each subject.			
†These variables were matched on for selection of controls.			

Table 2. Predictors of serum folate and vitamin D levels.

Predictors of serum folate levels		
	Median folate level (IQR), ng/ml	p value
Smoking history		
Never	14.0 (11.1 – 21.4)	0.046
Former	10.6 (8.1 - 16.2)	
Current	11.0 (8.5 – 15.8)	
Sex		
Female	14.1 (9.1 – 19.4)	0.52
Male	12.2 (10.3 – 15.2)	
Education		
Less than college graduate	12.1 (8.7-16.2)	0.25
College graduate or higher	14.1 (9.7-18.9)	
Race/ethnicity		
Caucasian	12.8 (9.6 – 17.3)	0.88
Non-Caucasian	12.4 (6.9 – 27.6)	
Age		
<30 y	12.8 (10.3-16.2)	0.75
≥30 y	13.0 (9.1-19.4)	
Season		
Winter	12.9 (9.6 – 19.4)	0.88
Spring	15.2 (8.3 – 21.4)	
Summer	12.2 (8.8 – 17.0)	
Fall	13.0 (9.3 – 17.3)	
Vitamin D category		
<20 ng/ml	11.9 (7.9 – 15.8)	0.38
≥20–29 ng/ml	13.0 (10.3 – 23.0)	
≥30 ng/ml	13.4 (9.6 – 19.0)	
Predictors of serum vitamin D level		
	Median vitamin D level (IQR)	p value
Smoking history		
Never	23.5 (17 – 33)	0.31
Former	22.5 (15 – 32.5)	
Current	26.5 (23 – 36)	
Sex		
Female	30 (22 – 36)	0.0006
Male	20 (16 – 26)	
Education		
Less than college graduate	25 (20– 33)	0.75
College graduate or higher	24 (17 – 36)	
Race/ethnicity		0.09

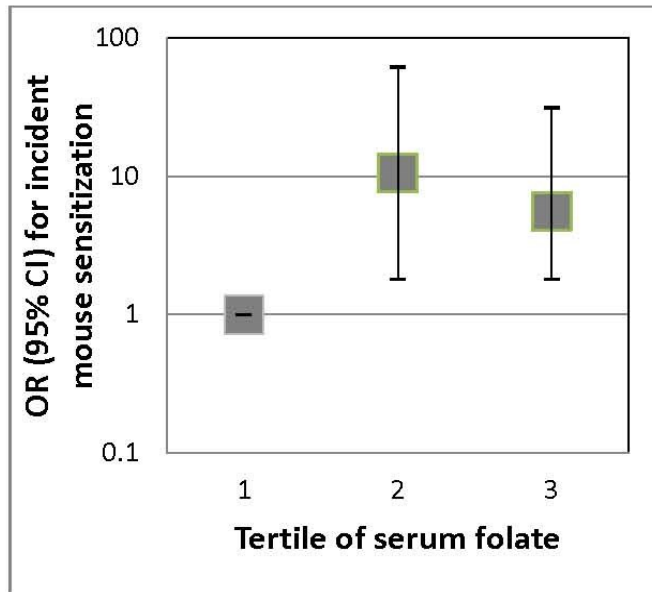
Caucasian	25 (18 – 33)	
Non-Caucasian	18 (14 – 31)	
Age		
<30	27 (17-35)	0.33
≥ 30	23 (18-31)	
Season		
Winter	20 (16 – 26)	0.003
Spring	17 (14 – 24)	
Summer	27.5 (22 – 37)	
Fall	28.5 (18 – 33)	
Folate tertile		
1 (2.5 - 10.5 ng/ml)	25 (17 – 33)	0.76
2 (10.5 – 16.2 ng/ml)	24 (17 – 32)	
3 (16.2 – 78.4 ng/ml)	24 (21 – 32)	

Table 3. Associations between incident sensitization and serum folate levels.

	Unadjusted Model	Adjusted Model 1*	Adjusted Model 2†
	OR (95% CI) p value		
Folate (natural log)	1.6 (0.9-3.1) p=0.13	2.3 (0.9-5.8) p=0.09	2.0 (0.7-5.3) p=0.17
Folate tertiles			
Tertile 1 (2.47-10.45 ng/ml)	REF	REF	REF
Tertile 2 (10.53-16.16 ng/ml)	5.4 (1.9-15.2) p=0.001	10.3 (1.9-55.7) p=0.007	10.5 (1.8-61.5) p=0.009
Tertile 3 (16.22-78.44 ng/ml)	2.4 (0.9-6.9) p=0.09	6.5 (1.3-32.9) p=0.02	5.6 (1.8-31.3) p=0.049
*adjusted for age, sex, education, smoking status, season, mouse allergen level †adjusted for age, sex, education, smoking status, season, mouse allergen level, and vitamin D category Statistically significant findings are bolded			

Table 4. Associations between incident sensitization and serum vitamin D levels.

	Unadjusted	Adjusted Model 1*	Adjusted Model 2†
	OR (95% CI) p value		
Vitamin D (natural log)	2.4 (1.0-5.9) p=0.06	3.9 (0.9-17.0) p=0.07	3.2 (0.7-14.1) p=0.13
Vitamin D categories			
Category 1 (<20 ng/ml)	REF	REF	REF
Category 2 (20-29 ng/ml)	2.3 (0.8-6.1) p=0.09	3.4 (1.0-11.7) p=0.054	2.6 (0.7-10.1) p=0.17
Category 3 (≥30 ng/ml)	1.7 (0.6-4.7) p=0.27	1.65 (0.4-6.4) p=0.47	1.6 (0.4-6.5) p=0.55
*adjusted for age, sex, education, smoking status, season, mouse allergen level †adjusted for age, sex, education, smoking status, season, mouse allergen level, and folate tertile Statistically significant findings are bolded			



Paper Three

Title: Genome-wide study of interaction between season of birth and food allergy identifies a region on chromosome 3 as a genetic risk factor for peanut allergy

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Sources of support: This research was funded in part by NIAID/NIH grant number
1K23AI103187 (to C.A.K).

Abstract:

Rationale: Fall season of birth has been identified as a possible risk factor for food allergy, but underlying biological mechanisms remain unknown. We hypothesized at least one common single nucleotide polymorphism (SNP) would show evidence of gene-environment interaction (GxEI) with season of birth and risk of food allergy.

Methods: A total of 589 case-parent trios were included in a genome wide association study of food allergy using the Illumina HumanOmni1-Quad BeadChip. Analysis of GxEI between season of birth and individual SNPs was done using the package TRIO in R. The 1 degree of freedom test comparing the additive genetic model with and without GxEI is reported here. Season of birth was defined as fall (September, October and November) versus all other months combined. Food allergy in general, and peanut, milk and egg allergy in particular, were defined as history of an immediate onset of typical allergy symptoms when exposed to the respective food with confirmatory positive specific IgE or skin testing.

Results: No SNPs achieved genome-wide significance in tests for GXEIs for milk, egg or food allergy combined. For peanut allergy, however, one SNP in an intron of *PBRM1* on chr.3p21 yielded genome-wide significance in this test for GxEI (rs2590838, $p=4.8 \times 10^{-8}$).

Forty-six SNPs in this region approached genome-wide significance ($10^{-7} < p < 10^{-5}$).

PBRM1 is necessary for signaling by nuclear hormone receptors, including those activated by vitamin D, but has not been previously associated with any allergic diseases.

Conclusions: Genetic markers near *PBRM1* on chr.3p21 appear to interact significantly with fall birth to increase risk of peanut allergy in children. More research will be needed to identify the functional significance of this statistical association.

Introduction

Food allergy (FA) has a strong heritability, estimated at 15-82% for specific FAs, depending on its definition and the population studied.¹ However, there are sparse data on specific genes that could underlie this apparently strong heritability. To date, several candidate genes, including HLA Class II, STAT6, FOXP3, TNF, SPINK5, IL-13, CD14, IL10, GSTP1 and filaggrin have shown evidence of association with FA, either alone, or in conjunction with eczema.^{2,3} With a few exceptions, these associations have generally not been replicated, and little of the estimated heritability can be accounted for. This is a common problem for complex diseases, and may result from the fact that some genetic risk factors manifest only under specific environmental conditions. It has been suggested that gene-by-environment interactions could account for a substantial portion of “missing heritability” for complex diseases in general⁴, and there are several examples suggesting some potential for these interactions in allergy. For example, CD14 and endotoxin show strong interaction in their effects on asthma, total IgE and allergic sensitization.⁵⁻⁸ For food allergy, interactions between breastfeeding, vitamin D levels and genetic variants have been found^{9,10}.

Fall birth may be one environmental factor where considering GEIs could help explain the heritability of FA and the environmental factors which affect it. The so-called ‘horoscope effect’, whereby fall birth increases the risk of food sensitization and allergy, has long been seen for FA¹¹⁻¹⁷. Overall, the odds of food allergy is estimated to increase by 30-90% if a child is born in the fall months¹⁷. However, what this seasonal factor is

and how it might interact with genetic risk for food allergy remains unknown. Identifying genetic variants that interact with season of birth to modify the risk of FA could help elucidate the mechanisms through which this disease develops.

In this study, we hypothesized season of birth would interact with genetic background to influence the development of food allergy. Using data collected for a genome-wide family based association study of food allergy, we performed a genome-wide environmental interaction study to find variants that may interact with season of birth.

Methods:

Description of the Chicago Food Allergy Study:

Samples were drawn from the Chicago Food Allergy Study, which has been described in detail previously^{18,19}. In the overall cohort, families of children with and without food allergy living in the Chicago area were recruited for completion of a questionnaire, medical record review, allergy skin prick testing, measurement of food specific IgE and collection of DNA. These families had a variety of structures, but for the analyses described here, subjects were limited to the index child with food allergy (milk, egg, peanut allergy or any food allergy) and his or her two parents. If more than one child in the family had a food allergy, only one child was selected for each analysis. The study protocol was approved by the Institutional Review Boards of the Children's Memorial Hospital and the Johns Hopkins Bloomberg School of Public Health.

Definition of food allergy:

Food allergy was defined as a convincing history of reaction to the food in question, with confirmatory positive food specific IgE and/or skin prick test. Skin prick testing was done using the Multi-Test II to egg white, cow milk, peanut, soy, wheat, walnut, fish mix, shellfish mix and sesame seed with histamine and saline as positive and negative controls, respectively. Skin prick tests were considered valid if the mean wheal diameter was ≥ 3 mm for histamine, <3 mm for saline, with a difference between the two of ≥ 3 mm. Food specific IgE (to egg white, cow's milk, peanut, soy, wheat, walnut, cod fish, shrimp and sesame seed) was quantified by the Clinical Immunology Laboratory of Children's Memorial Hospital using the Phadia ImmunoCAP (Phadia AB, Uppsala, Sweden).

Genotyping and Quality Control:

Genotyping was done using the Illumina HumanOmni1-Quad BeadChip, according to specifications listed in the Illumina protocol²⁰. Quality control steps for this study are described in detail elsewhere [manuscript submitted]. SNPs were removed for the following reasons: (1) 45,100 markers were monomorphic in our sample, (2) 14,948 SNPs had $\geq 5\%$ missing genotype calls, (3) 595 markers had >2 discordant calls in study duplicates, (4) 1,784 SNPs showed departure from HWE ($p < 10^{-6}$) when tested in either males, females or both from samples from parents or non-affected siblings (when parents were not available), and (5) 2,145 SNPs had Mendelian errors in 10 or more families. Only autosomes were considered for this analysis. In total, 929,536 autosomal

SNPs were available for downstream analysis. Fifty-three subjects were removed from analysis: 2 subjects were misloaded on the chip, 1 subject had <95% genotyping call rate, 12 subjects had gender inconsistency, 32 had inconsistency between reported relatedness and estimated identity by descent, and 6 subjects had >5000 SNPs with Mendelian errors. Reproducibility rate in the raw data was above 99.99% among 101 duplicated subjects.

Statistical analyses:

Analysis of GxEI between season of birth and individual SNPs was done using the package TRIO in R, which rapidly fits a conditional logistic regression model comparing observed transmission patterns to expected transmission patterns while testing for interaction²¹. The 1 degree of freedom test comparing the additive model with and without GxEI is reported. Season of birth was defined as fall (September, October and November) versus all other months combined. Sensitivity analysis of significant results was done using subjects of European ancestry only as defined by self-report and confirmed by principal components analysis.

Functional information on identified SNPs was annotated using SCAN²².

Vitamin D response elements:

Because vitamin D is a hypothesized mechanism of the association between fall birth and food allergy, for regions identified by the GXEI analysis, we then checked for the presence of vitamin D response elements (VDRE) near significant SNPs. Using published

data from Ramagopalan et al.²³, we searched for VDRE identified in that study within 150kb of our significant or suggestive SNPs.

Results:

Characteristics of the subjects:

A total of 589 unique families were included in this analysis, including 653 subjects with any food allergy and their two biological parents (Table 1). Of these, 307 cases were allergic to peanuts, 270 were allergic to milk and 207 were allergic to eggs. Overlap between these groups is shown in Supplementary Table 1. As can be seen from Table 1, families were nearly all of European heritage, and subjects were predominantly male. Over 90% had a history of eczema and asthma, and nearly 80% had allergic rhinitis. Thirty-one percent of affected children were born in the fall, compared to 26% of their parents (OR: 1.28, 95% CI: 1.07-1.58, $p=0.02$). In total, 929,536 autosomal SNPs were genotyped on 1,831 individuals.

Gene-by-environment analysis:

Peanut Allergy

In genome-wide analyses, a region on chromosome 3p21 showed interaction between fall season of birth and food allergy at a genome wide significant level ($p=4.8 \times 10^{-8}$, Figure 1). Q-Q plot showed no evidence of inflation in the test statistic, and the genomic control parameter (λ) was 1.00043 (Supplementary Figure 1). In this region, the minor allele, C, for the most significant SNP, rs2590838, found in the intronic region

of the PBRM1 gene, was significantly less likely to be transmitted among subjects born in the fall (GXE OR: 0.25, 95% CI 0.14-0.42, $p=4.8 \times 10^{-8}$), and in fact showed an inverse relationship with PA among children born in the fall compared to non-fall (Table 2). Forty-six other SNPs in high linkage disequilibrium (LD) with this top SNP also showed evidence approaching genome-wide significance ($5 \times 10^{-8} < p < 10^{-5}$). These SNPs spanned a region of 500k base pairs (from 52262508 to 52931958) and are located within or 2k base pairs near 12 different genes (Figure 2).

Egg, Milk and all Food Allergy

No SNPs reached genome wide significance in test of interaction between fall birth and egg, milk considered separately or all food allergy groups combined (Supplementary Figures 2-4). For all food allergy, one SNP in the same region as the top hits for peanut allergy gave suggestive significance (rs3617, $p=6.58 \times 10^{-6}$). An additional 10 SNPs on chromosomes 3, 4, 7, 8, 10 and 12 yielded $p < 10^{-5}$ (Table 3). For egg allergy, five SNPs on chromosomes 6, 12, 15, and 20 yielded $p < 10^{-5}$. For milk allergy, three SNPs on chromosomes 1, 5 and 7 had $p < 10^{-5}$.

As can be seen from Table 4, SNPs identified in the analysis of peanut allergy only were also marginally significantly associated with the interaction between season and egg allergy ($p=0.03$ for the top SNP), and were not significant in tests involving milk allergy. For the analysis of any food allergy, although no SNP identified in the peanut allergy analysis reached genome wide significance, all the SNPs all had $p < 0.0005$.

Sensitivity Analyses for Peanut Allergy:

When analyses were limited to the 259 families with an index subject of European ancestry by principal components analysis or self-identification, the results were largely the same. In this analysis, the top peanut SNP, rs2590838, had a $p=4.52 \times 10^{-7}$ (data not shown).

Assessment of VDREs in this region:

None of the VDREs identified by Ramagopalan et al. were in the region around SNP rs2590838²³. The closest VDREs were 489,568 bp and 617,208 bp, respectively, from the index SNP (rs2590838) and did not fall within the region defined by the top 47 SNPs (data not shown).

Discussion:

In this family based analysis, we identified a region on chromosome 3p21 showing evidence of linkage and association in a model that considered SNP effects interacting with season of birth. To our knowledge, this is the first example of a gene-by-environment interaction for FA identified from a genome-wide analysis.

Potent gene-environment interactions have been described for allergic diseases in general; for example, a strong interaction between variants in CD14 (part of the endotoxin receptor complex) and endotoxin exposure initially obscured the role of both the genetic variants and the environmental factors in asthma^{5,24}. More relevantly for this analysis, in a recent GWAS by environment study, Du et al. identified variants in CRTAM (class I MHC-restricted T cell-associated molecule) that interacted with vitamin

D levels to influence risk of asthma²⁵, and further showed not only that a VDRE was located close to the identified SNP, but also that vitamin D induced gene expression differed based on variants at that SNP. Although there are data suggesting gene-environment interactions for FA^{9,10,26}, the genetic basis of food allergy and how it may interact with the environment has been poorly studied. In part, this is due to the relative rarity of FA, making prospective study of environmental exposures difficult, and to the difficulty of phenotyping FA, which contributes to limited sample sizes. One of the strengths of this study is its large cohort of well phenotyped cases and their families. Our environmental exposure, season of birth, is unique in that it is easily ascertained years after the relevant exposure, is not likely subject to confounding and is common. Another strength of this study facilitating identify significant GxEI was the family-based design which ensures identified variants are both in linkage and association with FA, while being robust to population stratification.

However, there are several limitations which must be acknowledged. Even using a common exposure like fall birth, our sample had limited power to detect genome-wide GxE interactions, and we may have missed other associations. As outlined in detail below, the functional implications of the identified associations are not clear at this time and future fine mapping and functional studies will be necessary to delineate the role of genetic variation in this region in influencing risk to food allergy.

Season of birth was chosen as a candidate environmental factor in this study because it has been repeatedly associated with FA in a variety of populations, but the

underlying mechanism through which fall birth increases risk of FA remains unknown. It has been suggested that low early life vitamin D levels due to limited sunlight during a critical period for development of allergy may be responsible. Further, it has been theorized that vitamin D acts by (1) reducing skin barrier defects¹⁷ or (2) affecting gut microbiome constituents²⁷. The top SNP in this analysis, rs2590838, lies in an intronic region of the gene PBRM1. This gene encodes a protein also known as BAF180 which is an integral part of complexes involved in ligand-dependent activation by nuclear hormone receptors (such as vitamin D)²⁸. This protein is also a critical regulator of p53, and is thus involved in apoptosis, a pathway involved in inducing tolerance²⁹. In addition, it regulates IL-10 transcription, a key cytokine involved in tolerance³⁰. Loss of function of PBRM1 is clearly associated with renal cell carcinoma³¹⁻³⁸, but this gene has not yet been associated with any allergic diseases. Because PBRM1 has been shown to be required for proper functioning of the vitamin D receptor, one could hypothesize that functional variation in PBRM1 levels could lead to differential responsiveness to seasonally based vitamin D fluctuation, therefore interacting with season of birth to affect the risk of FA.

However, the top SNPs identified in this study are in high linkage disequilibrium over a fairly large area of the genome, making identification of a single causative gene difficult. Two of the top 10 SNPs in this region are missense variants in other genes (rs2289247 in GNL3, and rs3617 in ITIH3), and both are predicted to affect exonic splicing²². GNL3, also known as nucleostemin, plays a role in prevention of DNA damage³⁹, is thought to interact with p53 and is important for stem cell proliferation; it

is also widely expressed in epithelial tissues⁴⁰. ITIH3 is a plasma serine protease inhibitor that is part of a large family of inter-alpha-trypsin inhibitors involved in epithelial repair and inflammatory processes. For both of these genes, a role in the skin's response to UV light could be theorized. Thus, at least three genes in the identified region could have biologically plausible involvement in food allergy. In contrast to the gene-by-vitamin D interaction and asthma in the CRTAM gene previously described, this region did not have any known VDREs, suggesting differential response to vitamin D on the transcriptional level is not the basis of this interaction. Although this region has not been associated with allergic diseases previously, and was not in the parent GWAS study, the top SNPs identified in this analysis have been associated at genome-wide significant or suggestive levels with adiponectin levels, bipolar disorder and osteoarthritis⁴¹⁻⁴³. It is not clear what the overlap between these conditions and FA might be. Future work will be needed to explain the association demonstrated here.

Our data add to the growing body of literature showing different allergic diseases have both allergic specific and shared genetic risk factors⁴⁴. We found a significant association only among cases with peanut allergy, and did not find any evidence of association with this region among trios ascertained through a case with egg or milk allergy. This could be due to different critical periods for development of the allergies, or to different mechanisms of allergy.

In summary, we identified a genome-wide significant interaction between SNPs in and around the PBRM1 gene, fall season of birth and food allergy. More work will need to be done to understand the functional implications of this work.

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Table 1. Demographic features of included subjects.

	Peanut Allergy	Milk Allergy	Egg Allergy	All Food Allergy[#]
Number of trios	307	270	207	589
Characteristics of Index Child				
Fall birth (n, %)	96 (31%)	90 (33%)	66 (32%)	177 (30%)
European Maternal Heritage (n, %)	260 (85%)*	238 (88%)	182 (88%)	507 (87%) ⁺⁺
Sex (n, % male)	205 (67%)	178 (66%)	139 (67%)	384 (65%)
Age, mean (SD), years	5.4 (3.6)	4.9 (3.4)	5.5 (3.4)	5.7 (3.6)
Associated allergic conditions				
Eczema	215 (93%)**	201 (96%) [†]	162 (96%) [‡]	419 (95%) ^{‡‡}

[#]All food allergy is not the sum of peanut, milk and egg allergy because some subjects had multiple allergies, some families had multiply affected siblings with different food allergies, and some subjects had other food allergies.

Information was not present on all subjects: denominator was *305, **230, †210, ‡168, ††586, and ‡‡ 440.

Table 2: Top 10 peanut allergy SNPs.

SNP	Minor Allele	Minor Allele Frequency	GXE OR	95% CI LL	95% CI UL	GXE 1 df p value	OR in non-Fall Birth	95% CI LL	95% CI UL	Gene-only p value
rs2590838	C	0.496	0.245	0.145	0.416	4.76E-08	1.581	1.207	2.072	8.79E-04
rs1108842	C	0.502	3.997	2.359	6.774	7.53E-08	0.635	0.485	0.831	9.26E-04
rs3796353	A	0.427	3.635	2.169	6.092	4.37E-07	0.578	0.437	0.764	1.15E-04
rs3617	T	0.464	3.678	2.165	6.250	5.38E-07	0.680	0.518	0.892	5.46E-03
rs13071584	C	0.425	3.567	2.122	5.996	7.16E-07	0.609	0.462	0.803	4.34E-04
rs13079063	G	0.430	3.591	2.129	6.055	7.44E-07	0.595	0.450	0.788	2.89E-04
rs1866268	T	0.429	3.535	2.110	5.925	7.71E-07	0.594	0.450	0.785	2.45E-04
rs2289247	T	0.429	3.535	2.110	5.925	7.71E-07	0.594	0.450	0.785	2.45E-04
rs3733041	C	0.429	3.535	2.110	5.925	7.71E-07	0.594	0.450	0.785	2.45E-04
rs2300149	A	0.388	3.645	2.146	6.192	8.26E-07	0.558	0.418	0.745	7.37E-05

Table 2. Continued.

SNP	OR in Fall Birth	95% CI LL	95% CI UL	GXE 2 df p value	# trios Non-fall	# trios Fall	Gene	Function	Position
rs2590838	0.388	0.247	0.610	2.95E-07	162	72	[PBRM1]	Intronic	52622086
rs1108842	2.538	1.612	3.996	4.47E-07	163	71	[GNL3]	Synonymous	52720080
rs3796353	2.100	1.360	3.244	1.12E-06	159	70	[PBRM1]	Intronic	52533230
rs3617	2.500	1.586	3.940	3.48E-06	163	72	[ITIH3]	Missense Q->K	52773805
rs13071584	2.172	1.399	3.373	2.72E-06	158	70	[NEK4]	Intronic	52804487
rs13079063	2.138	1.376	3.323	2.46E-06	157	69	[NEK4]	Intronic	52744460
rs1866268	2.100	1.360	3.244	2.40E-06	158	70	(near PBRM1 and GNL3)	Intergenic	52659398
rs2289247	2.100	1.360	3.244	2.40E-06	158	70	[GNL3]	Missense	52727257
rs3733041	2.100	1.360	3.244	2.40E-06	158	70	[GLT8D1]	Intronic	52731598
rs2300149	2.034	1.304	3.173	1.50E-06	151	68	[ITIH1]	Intronic	52822921

Table 3: Top SNPs for other food allergies, and for peanut allergy not on Chromosome 3

SNP	Minor Allele	Minor Allele Frequency	GXE OR	GXE 1 df p value	Chr	Position	Gene	Function	left_gene	right_gene
All food Allergy										
rs4897645	A	0.400	2.43	2.29E-06	8	135011554	NA	NA	LOC100129104	ZFAT
rs12775752	T	0.105	4.24	3.43E-06	10	57763200	NA	NA	LOC389970	ZWINT
rs1547382	T	0.108	0.26	3.68E-06	4	99712978	TSPAN5	Intron	RAP1GDS1	BTF3L3
rs1558475	C	0.113	0.27	4.35E-06	7	7883885	NA	NA	RPA3	LOC100132073
rs7618915	A	0.343	2.41	5.07E-06	3	52254634	PPM1M	Near-gene-5	TWF2	PPM1M
rs4935649	C	0.112	3.94	5.30E-06	10	57909670	NA	NA	ZWINT	LOC100128586
rs7825229	A	0.224	0.36	5.60E-06	8	330067	NA	NA	FAM87A	FBXO25
rs1602837	C	0.469	2.31	5.92E-06	12	58760705	NA	NA	SLC16A7	PGBD3P1
rs3617	T	0.464	2.32	6.58E-06	3	52808845	ITIH3	Missense	ITIH1	ITIH4
rs4876124	C	0.223	0.36	6.65E-06	8	1646312	NA	NA	DLGAP2	LOC100130321
rs3852064	T	0.344	2.33	9.50E-06	3	52324452	DNAH1	Near-gene-5	GLYCTK	DNAH1
Egg Allergy										
rs331603	G	0.309	4.59	3.23E-06	20	55052353	NA	NA	PTMAP6	LOC728902
rs13329098	C	0.119	0.09	7.91E-06	15	57541378	FAM81A	intron[NM_152450.2]	LOC100130999	GCNT3
rs9264882	T	0.111	0.03	8.15E-06	6	31379855	NA	NA	HLA-C	HLA-B
rs637307	T	0.234	5.88	8.54E-06	12	116624242	KSR2	intron[NM_173598.4]	NOS1	RFC5
rs17302162	G	0.163	7.29	9.65E-06	20	53565401	NA	NA	RPL12P4	CBLN4
Milk Allergy										
rs1569661	C	0.290	4.54	1.82E-06	1	37625921	NA	NA	GRIK3	LOC728431
rs644358	C	0.126	6.50	2.34E-06	5	17160465	NA	NA	MYO10	FLJ34047
rs12699131	A	0.486	3.24	8.51E-06	7	71389252	CALN1	Intronic	LOC728453	TYW1B

Peanut allergy not on Chromosome 3										
rs17646244	C	0.368	0.30	2.40E-06	15	24312809	NA	NA	LOC100128714	GABRB3
rs7498199	C	0.409	0.30	2.66E-06	16	79806896	PKD1L2	intron	GCSH	BCMO1
rs7186093	A	0.410	0.30	2.87E-06	16	79807238	PKD1L2	intron	GCSH	BCMO1
rs7191351	T	0.410	0.31	3.25E-06	16	79807455	PKD1L2	missense	GCSH	BCMO1
rs697578	T	0.429	3.28	4.79E-06	4	140726406	NA	NA	SETD7	MGST2
rs7088326	G	0.483	3.35	4.99E-06	10	90718761	NA	NA	ACTA2	FAS
rs11550670	T	0.184	0.22	8.93E-06	14	24351408	STXBP6	utr-3	GZMB	LOC649445
rs10271156	A	0.280	0.27	9.45E-06	7	22479405	NA	NA	RAPGEF5	MGC87042
rs10109080	G	0.454	3.19	9.70E-06	8	135012300	NA	NA	LOC100129104	ZFAT

Table 4: Comparison of Peanut, Egg, Milk and All Food Allergy for Top SNPs identified by Peanut GXEI Analysis

SNP	Peanut			All Food Allergy			Egg allergy			Milk Allergy		
	GXE beta (OR)	GXE 1 df p value	# trios	GXE beta (OR)	GXE 1 df p value	# trios	GXE beta (OR)	GXE 1 df p value	# trios	GXE beta (OR)	GXE 1 df p value	# trios
rs2590838	0.247	4.76E-08	234	0.472	3.72E-05	450	0.522	3.08E-02	162	0.06	8.26E-01	208
rs1108842	4.015	7.53E-08	234	2.117	3.70E-05	452	1.954	2.42E-02	163	0.00	9.97E-01	210
rs3796353	3.633	4.37E-07	229	1.935	2.54E-04	450	1.859	4.09E-02	157	0.04	8.92E-01	207
rs3617	3.669	5.38E-07	235	2.316	6.58E-06	444	2.117	1.60E-02	157	0.09	7.33E-01	206
rs13071584	3.561	7.16E-07	228	1.974	1.78E-04	445	1.804	5.46E-02	155	0.06	8.25E-01	203
rs13079063	3.597	7.44E-07	226	1.878	4.87E-04	447	1.768	6.10E-02	156	0.03	9.24E-01	205
rs1866268	3.525	7.71E-07	228	1.896	3.87E-04	450	1.804	5.06E-02	157	0.02	9.26E-01	207
rs2289247	3.525	7.71E-07	228	1.896	3.44E-04	449	1.804	5.06E-02	157	0.03	8.93E-01	206
rs3733041	3.525	7.71E-07	228	1.896	3.87E-04	450	1.804	5.06E-02	157	0.02	9.26E-01	207
rs2300149	3.633	8.26E-07	219	1.994	2.15E-04	433	1.507	1.87E-01	151	0.10	7.18E-01	195

Figure Legends:

Figure 1. Manhattan plot of interaction P values for peanut allergy.

Figure 2. Regional association plot for rs2590838 showing chromosome position (National Center for Technology Information build 36.3) and recombination rate (hg18 build). Rs2590838 is shown as a diamond and other colors represent r^2 values for SNPs to the sentinel SNP (HapMap CEU phase II).

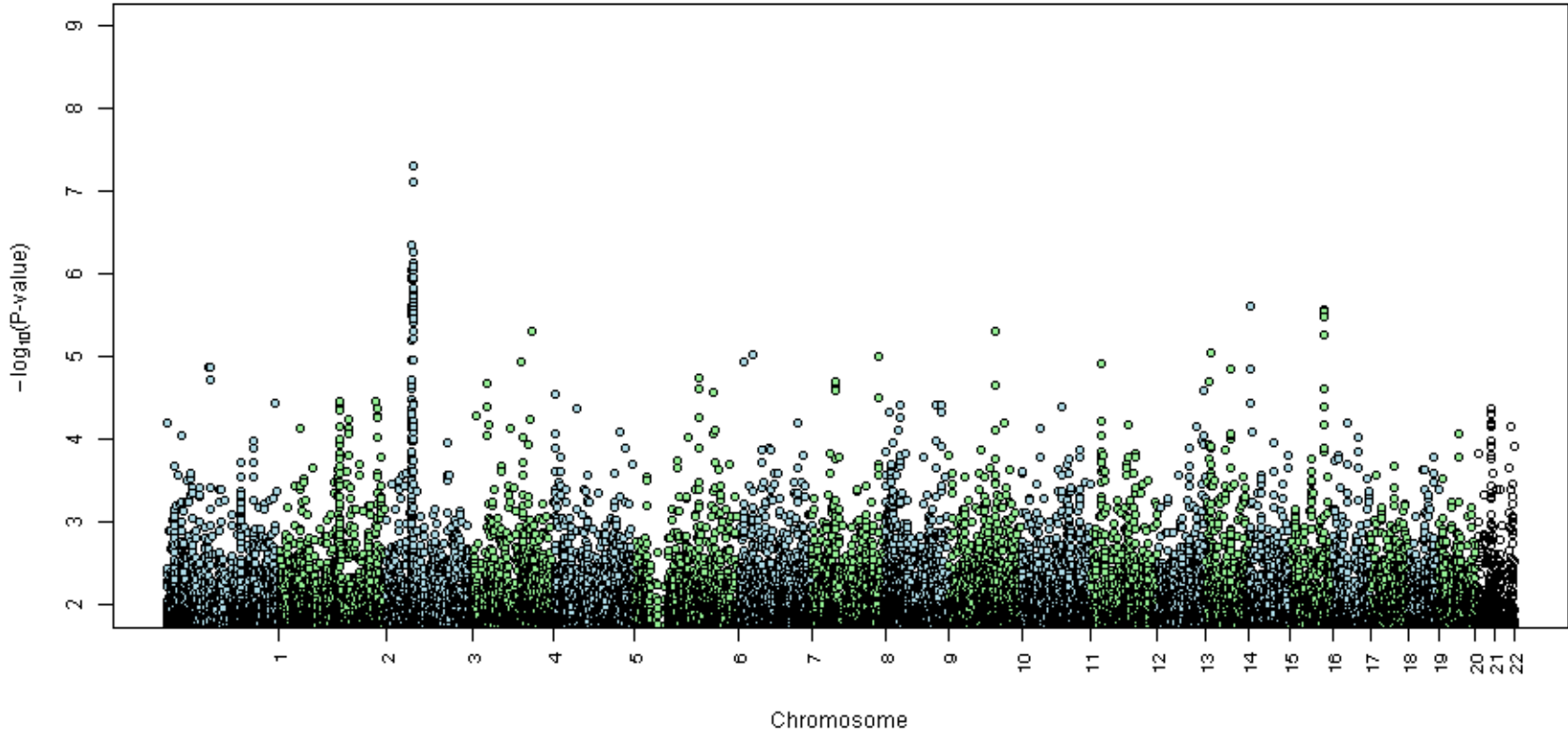
Supplementary Figure 1: QQ plot for GXEI for Peanut Allergy

Supplementary Figure 2: Manhattan Plot of GXEI for Egg Allergy

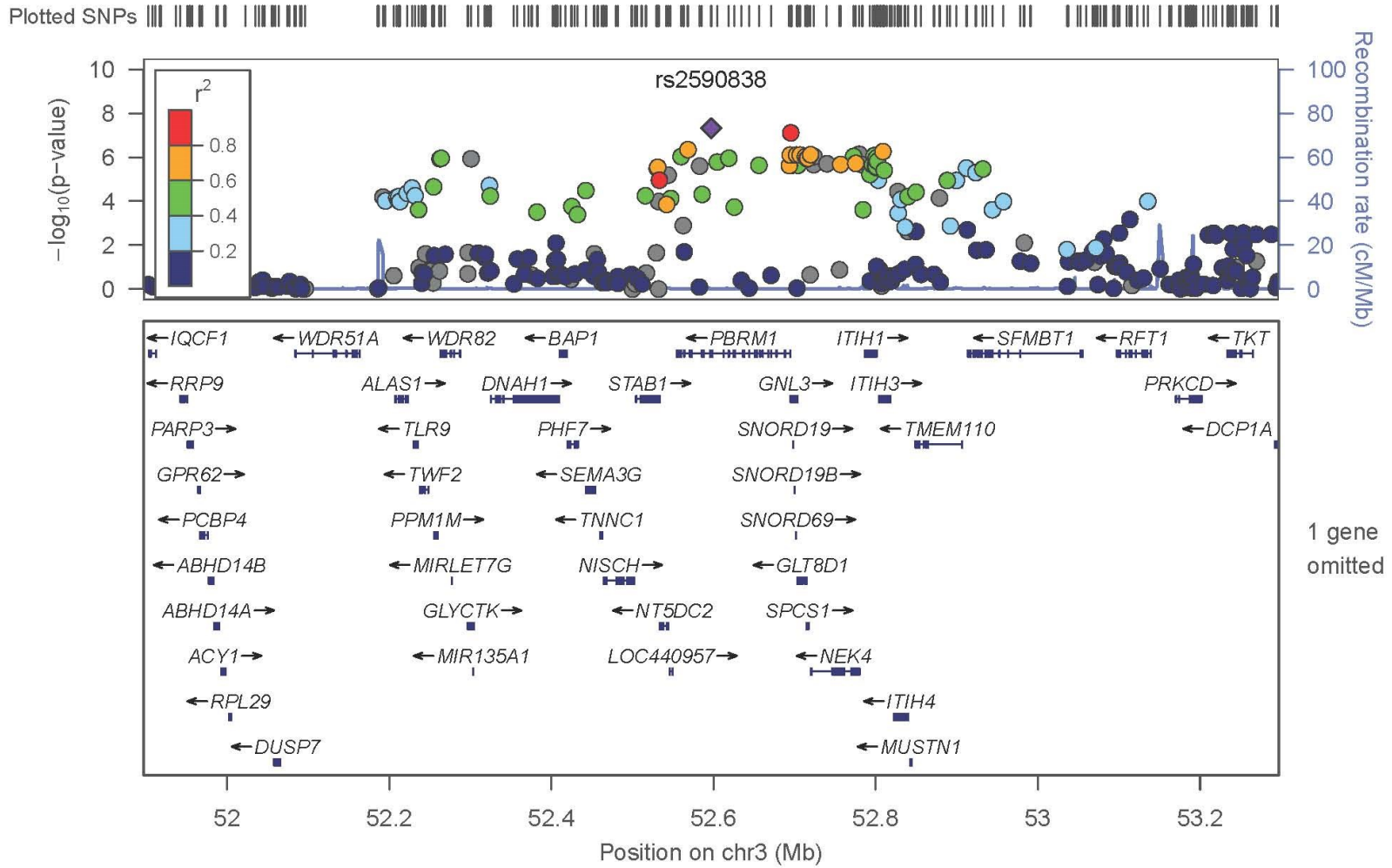
Supplementary Figure 3: Manhattan Plot of GXEI for Milk Allergy

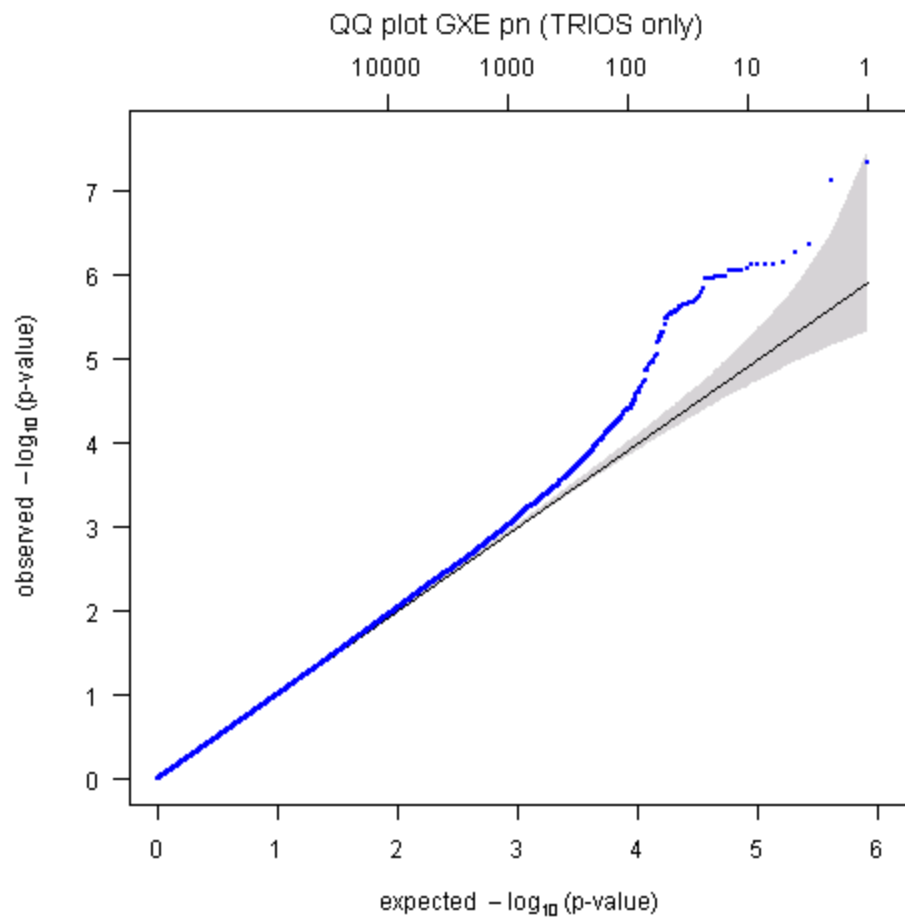
Supplementary Figure 4: Manhattan Plot of GXEI for Food Allergy Overall

Season of Birth by Gene interaction for PN allergy



Peanut Allergy





Concluding Section

Summary of Results and Implications:

In the three papers that make up this thesis, we examined several aspects of the epidemiology of allergic disease, attempting to answer the following questions: (1) what is the prevalence of food allergy in the U.S. and has it increased in recent years?; (2) could variation in certain micronutrients, namely vitamin D and folate, be associated with development of allergy, with implications for temporal trends in allergy?; and (3) are there genetic variants which are associated with differential sensitivity to the pro-food allergic effects of season of birth, potentially helping us understand the mechanism of this risk factor?

In the first paper(1), a meta-analysis and systematic review of food allergy prevalence in the U.S., we found that prevalence of self-reported food allergy increased by an estimated 1.2 percentage points per decade, and that this increase was highest among non-Hispanic Blacks, in whom the estimated increase was 2.1 percentage points per decade. The overall increase in food allergy prevalence is in keeping with several other studies of food allergy in the U.S. and other countries, but the differential increase in non-Hispanic blacks is a novel observation. The fact that trends in food allergy have differed by race/ethnicity suggests that environmental factors may have differential effects on different groups or that different groups may have differential exposure to a changing environment. Several possible theories present themselves as explanations for differential increase between groups, including differential genetic susceptibility to

changing microbial patterns, different dietary exposures, including micronutrients as examined in the second and third papers, and other different environmental exposures, including pollution, antibiotics and household chemicals. As outlined below, more work is needed to determine whether the observed changes in food allergy represent true increases in allergy and to determine the environmental factors responsible for the disparity if it is real.

The second paper(2), a nested case-control study of the association between baseline vitamin D and folate levels and incident mouse sensitization among mouse workers showed that baseline folate levels, but not vitamin D, were associated with development of mouse sensitization, with middle and higher tertiles of folate associated with higher risk of sensitization. This result is in keeping with a small number of prospective studies in younger children showing that higher folate may be a risk factor for the development of allergic disease(3-5), but is the first study to prospectively look at folate and allergy in adults. Folate is an interesting potential risk factor for allergy because there are demonstrated increases in average folate levels among Americans in the past two decades(6)and because there are animal data supporting a role for folate in the development of allergy(7). Thus, this work contributes to the data suggesting that changes in folic acid intake may play a role in the epidemic of allergic disease. If this hypothesis is true, research should be directed at determining how to balance the benefits of folic acid supplementation and fortification against its potential harms.

The third paper, a genome-wide association by environment interaction study, was a search for genetic variants associated with fall season of birth and food allergy. In this family based study, we found that a region on chr.3p21 yielded genome-wide significance in the gene-by-environment analysis. The top SNP in this area is in an intron for the gene PBRM1, which codes for a protein also known as BAF-180. BAF-180 has numerous functions, including as a critical part of complexes required for activation of the vitamin D hormone(8). Thus, it is plausible that this, or another variant linked to it, could affect risk for food allergy differently depending on the level of vitamin D available. Because season is a major determinant of vitamin D levels, the effects of a variant that modified the vitamin D pathway could have different effects in different seasons. The literature regarding the role of vitamin D in the development of allergic disease is now quite mixed. In our own work, in paper #2, we did not find a relationship between vitamin D levels and incident mouse sensitization. Work published after these studies were complete suggests that the way we measure vitamin D may be flawed in that it may be bioavailable vitamin D rather than total vitamin D that is most relevant for human disease(9). Thus it is possible that, despite our negative findings in paper #2, vitamin D may be involved in the development of allergic disease. It is also possible that it may play a different role at different times in the lifespan. As outlined below, more work is needed to verify our genetic findings and determine their functional significance, and to determine whether vitamin D is indeed involved in the pathway by which fall season of birth increases the risk of food allergy.

Limitations and Future Directions:

Each of these papers has significant limitations that suggest the need for more research on these subjects to fully answer the questions posed.

For the first paper, the data are limited by the fact that they are drawn from self-report, a method well known to exaggerate the prevalence of food allergy. It is possible that the increases seen represent changing diagnosis or perception of food allergy rather than a true underlying increase in the disease. Future planned study to determine whether perception or real disease has increased includes measurement of specific IgE to foods from different time points in nationally representative samples in order to compare rates of sensitization over time. This and other work will also be needed to determine why food allergy may be increasing more quickly among Non-Hispanic blacks than other groups. If sensitization has in fact increased in non-Hispanic blacks more than other groups, exploration of potential genetic and environmental causes for this increase could shed light more broadly on the mechanisms of food allergy.

For the second paper, the fact that these data are observational makes it possible that the observed associations are due to some unknown underlying confounder. Although we adjusted for known confounders, it is possible that some other factor, such as the composition of the gut microbiome, jointly affects folate levels and risk of sensitization. Future studies will need to replicate this finding in other populations and examine more closely for potential confounders. If the epidemiologic data confirm these findings, interventional trials of prevention would have to balance

the beneficial effects of increased folate, such as reduction in neural tube defects, with potential risks for allergic disease.

For the third paper, the genome-wide-by-environment findings need to be replicated. If replicated, more work is needed to identify the causal variant associated with this interaction and to determine the functional path by which it may interact with season to affect the risk of food allergy.

Synthesis and conclusions:

Together, this work finds that food allergy is increasing in prevalence, suggesting an environmental cause, suggests that folic acid supplementation/fortification may be one factor which could be a potential mediator of this rise, and that environmental factors, such as season of birth, may interact with genetic background to affect the risk of food allergy. Together, this work does not suggest a unified theory for the development of food allergy, but instead indicates several paths that future research should explore. These include more attention to the potential role of folic acid supplementation in development of allergic disease across the lifespan, replication and more analysis of variants that may interact with season of birth to affect the risk of food allergy, and further delimitation of epidemiologic trends in the prevalence of food allergy.

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Appendix 1

Supplementary materials for Paper #1

Supplementary Materials

1. Supplementary Methods
2. Supplementary References

Supplementary Methods

Search Strategy:

Tier 1 (prevalence)

AND

Tier 2 (food allergy)

A: Food allergy itself OR

B: allergy term AND food term

PUBMED strategy: 5935 results

("epidemiology"[Subheading] OR "epidemiology"[All Fields] OR "incidence"[All Fields]
OR "incidence"[MeSH Terms] OR "prevalence"[All Fields] OR "prevalence"[MeSH Terms]
OR "epidemiology"[MeSH Terms] OR "morbidity"[All Fields] OR "morbidity"[MeSH
Terms]

OR "data collection"[MeSH Terms] OR ("data"[All Fields] AND "collection"[All Fields]) OR
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OR "Questionnaires"[All Fields] OR "Cross-Sectional Studies"[MeSH Terms] OR "federal
government"[MeSH Terms] OR ("federal"[All Fields] AND "government"[All Fields]) OR
"federal government"[All Fields] OR "national"[All Fields])

AND

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Fields]) OR "food hypersensitivity"[All Fields] OR ("food"[All Fields] AND "allergy"[All
Fields]) OR "food allergy"[All Fields])

OR

((("Allergens/immunology"[MeSH Terms] OR "skin tests"[MeSH Terms] OR ("skin"[All Fields] AND "tests"[All Fields]) OR "skin tests"[All Fields] OR "immunoglobulin e"[MeSH Terms] OR "immunoglobulin e"[All Fields] OR "challenge"[All Fields] OR provocation[All Fields] OR "allergy and immunology"[MeSH Terms] OR ("allergy"[All Fields] AND "immunology"[All Fields]) OR "allergy and immunology"[All Fields])

AND

("food"[MeSH Terms] OR "food"[All Fields] OR "milk"[All Fields] OR "milk"[MeSH Terms] OR "egg"[All Fields] OR "ovalbumin"[MeSH Terms] OR "ovalbumin"[All Fields] OR shrimp[All Fields] OR shrimp/crab/fish/sea[All Fields] OR shrimp/fish[All Fields] OR shrimp/prawn[All Fields] OR crustacean[All Fields] OR crustacean/shellfish[All Fields] OR crustacean's[All Fields] OR crustaceana[All Fields] OR crustaceanartemia[All Fields] OR crustaceans[All Fields] OR crustaceans/crayfish[All Fields] OR crustaceans'[All Fields] OR crustaceansaquatic[All Fields] OR "shellfish"[MeSH Terms] OR "shellfish"[All Fields] OR "arachis hypogaea"[MeSH Terms] OR ("arachis"[All Fields] AND "hypogaea"[All Fields]) OR "arachis hypogaea"[All Fields] OR "peanut"[All Fields] OR soy[All Fields] OR "soybeans"[MeSH Terms] OR "soybeans"[All Fields] OR "soybean"[All Fields] OR "fishes"[MeSH Terms] OR "fishes"[All Fields] OR "fish"[All Fields] OR "nuts"[MeSH Terms] OR "nuts"[All Fields] OR ("tree"[All Fields] AND "nut"[All Fields]) OR "tree nut"[All Fields] OR "dairy products"[MeSH Terms] OR ("dairy"[All Fields] AND "products"[All Fields]) OR "dairy products"[All Fields] OR ("dairy"[All Fields] AND "product"[All Fields]) OR "dairy product"[All Fields]) OR "wheat"[All Fields] OR "wheat"[MeSH Terms]

NOT ("animals"[MeSH Terms] NOT ("humans"[MeSH Terms] AND "animals"[MeSH Terms])) AND English[lang]

EMBASE strategy: 5757 results

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AND

[('food allergy'/exp OR 'food allergy' OR 'food hypersensitivity'/exp OR 'food hypersensitivity')

OR

(('allergens'/exp OR allergens OR 'immunology'/exp OR immunology OR ('skin'/exp OR skin AND test) OR 'immunoglobulin e antibody'/exp OR 'immunoglobulin e antibody' OR 'provocation test'/exp OR 'provocation test' OR 'challenge' OR 'allergy and immunology'/exp OR 'allergy and immunology')

AND

'food'/exp OR food OR 'milk'/exp OR milk OR dairy OR 'dairy product'/exp OR 'dairy product' OR 'egg'/exp OR egg OR 'ovalbumin'/exp OR ovalbumin OR 'shellfish'/exp OR shellfish OR 'shrimp'/exp OR shrimp OR 'crab'/exp OR crab OR 'crustacean'/exp OR crustacean OR 'peanut'/exp OR peanut OR 'arachis hypogaea' OR 'soybean'/exp OR soybean OR 'soy' OR 'fish'/exp OR 'fish' OR 'wheat' OR 'wheat'/exp OR 'tree'/exp OR tree AND ('nut'/exp OR nut))]

Methods for analysis of publically available data

NHANES III^{1,2}, 2007-8 and 2009-10³ The National Health and Nutrition Examination Survey (NHANES) is survey of the non-institutionalized civilian population of the United States, and is conducted periodically by the National Center for Health Statistics (NCHS), a part of the Centers for Disease Control (CDC). Since 2000, the survey has been conducted continuously in 2 year blocks. For NHANES, interviews are initially conducted in subject's homes, followed by examination in mobile centers (vans). In NHANES III, young children, older people, African Americans and Mexican Americans were oversampled. In NHANES 2005-6, 2007-8 and 2009-10, low-income people, adolescents, older people, African Americans and Mexican Americans were oversampled. For these analyses, subjects younger than 18 years of age were included. Separate surveys were of independent subjects.

Data included that had not previously been published:

For NHANES III, the prevalence of food allergy in this age group had been previously reported², but not the standard error of this measurement or ethnicity specific prevalences.

Measure of food allergy:

For NHANES III, self-report of ever history of food allergy was by a positive response to the following question: "Within an hour after eating something, have you ever had a severe reaction, such as itching all over, trouble breathing, flushing, hives, or swelling of the face or hands or feet?"

For NHANES 2007-8 and 2009-10, self-report of food allergy was by a positive response to the following question: “Do you have any food allergies?”

Ethnicity: In NHANES III, categories of self-reported race/ethnicity were Non-Hispanic White, Non-Hispanic Black, Mexican American and Other.

Statistical Methods: To account for the oversampling, complex sampling method and non-response, weights and survey strata provided with the surveys were used for all analyses. For NHANES III, overall and ethnic-specific weighted rates were calculated using simple tabulation. All analyses were done in STATA SE/11 (College Station, TX).

National Survey of Children’s Health 2003⁴ and 2007^{5,6}: The National Survey of Children’s Health is a survey conducted periodically by the NCHS at the CDC. The survey is conducted through the State and Local Area Integrated Telephone Survey (SLAITS). The surveys use the sampling frame of the National Immunization Survey, which is a random-digit-dialed telephone survey using computer assisted telephone interviews. Each state survey was designed to include at least 1700 completed interviews. The 2003 and 2007 interviews were very similar except that some questions were revised. Separate interviews were of independent subjects.

Data included that had not previously been published: For NSCH 2003, the overall estimated prevalence of food allergy among children has been published⁷, but not the standard error or ethnicity specific rates. For NSCH 2007, no data had been published at the time of our search. After our search, one reference reported the overall estimated prevalence of food allergy⁶, but did not report the standard error or ethnicity specific rates.

Measure of food allergy: Food allergy was by self report using the following question:

“During the past 12 months, have you been told by a doctor or other health care provider that [the subject] had any kind of food or digestive allergy?”

Ethnicity: For these analyses, race/ethnicity categories were generated by back-coding of self-reported categories of race and ethnicity, and were categorized into Non-Hispanic White, Non-Hispanic Black, Hispanic, Multi-racial and Other.

Statistical Methods: Sampling weights included in the surveys were used in all analyses. Overall and ethnic specific prevalences were calculated by simple tabulation. All analyses were done using STATA SE/11 (College Station, TX).

National Health Interview Survey 1997-2011⁸: The National Health Interview Survey is an annual survey conducted by the NCHS of the CDC. Starting in 1997, a revised questionnaire included a question related to food allergy. The target population is the civilian non-institutionalized population of the U.S. It has a multi-stage design with oversampling of African American, Hispanic and Asian persons. It is an in-person household interview. Separate interviews were of independent subjects. Data were obtained from the University of Minnesota⁹.

Data included that had not previously been published: Data on overall and ethnicity specific prevalence of food allergy for selected years of the survey were previously published^{10,11}, but no data on the 2011 survey was previously published.

Measure of food allergy: In these surveys, food allergy prevalence was estimated from the following question: “During the past 12 months, has [the subject] had any kind of food or digestive allergy?”

Ethnicity: For these analyses, race/ethnicity categories were generated by back-coding of self-reported categories of race and ethnicity, and were categorized into Non-Hispanic White, Non-Hispanic Black, Hispanic, Multi-racial and Other.

Statistical Methods: Sampling weights, pseudostrata and sampling units included in the surveys were used in all analyses. Overall and ethnic specific prevalences were calculated by simple tabulation. All analyses were done using STATA SE/11 (College Station, TX).

eReferences:

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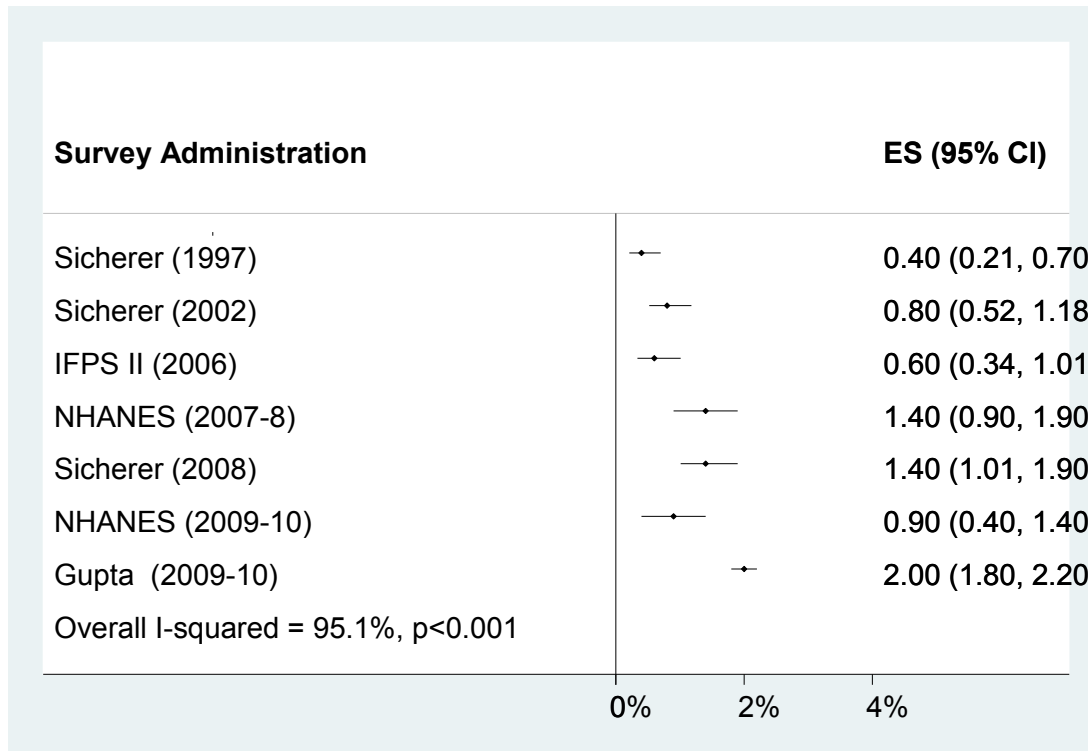
Supplementary Figure Legends

Supplementary Figure 1: Forest plot of peanut allergy prevalence in children in the US ordered by year of study. Abbreviations: ES: Estimate, NHANES: National Health and Nutrition Examination Survey, IFPS II: Infant Feeding Practices Study II.

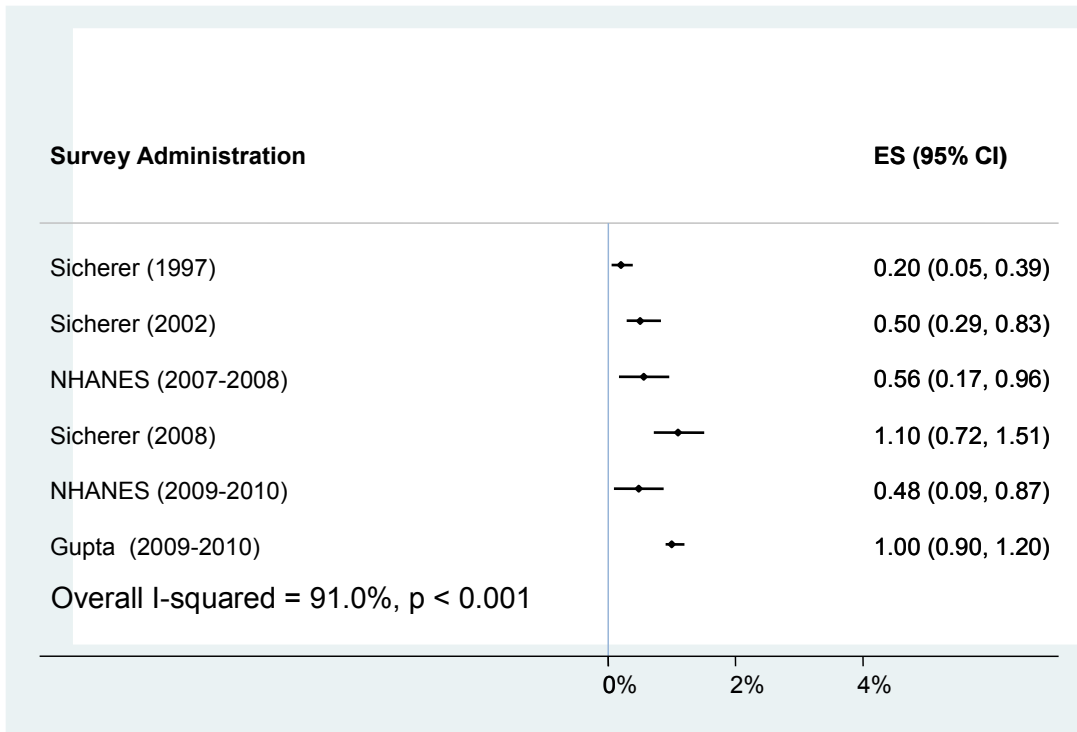
Supplementary Figure 2: Forest plot of tree nut allergy prevalence in children in the US ordered by year of study. Abbreviations: ES: Estimate, NHANES: National Health and Nutrition Examination Survey, IFPS II: Infant Feeding Practices Study II.

Supplementary Figure 3: Forest plot of shellfish allergy prevalence in children in the US ordered by year of study. Abbreviations: ES: Estimate, NHANES: National Health and Nutrition Examination Survey, IFPS II: Infant Feeding Practices Study II.

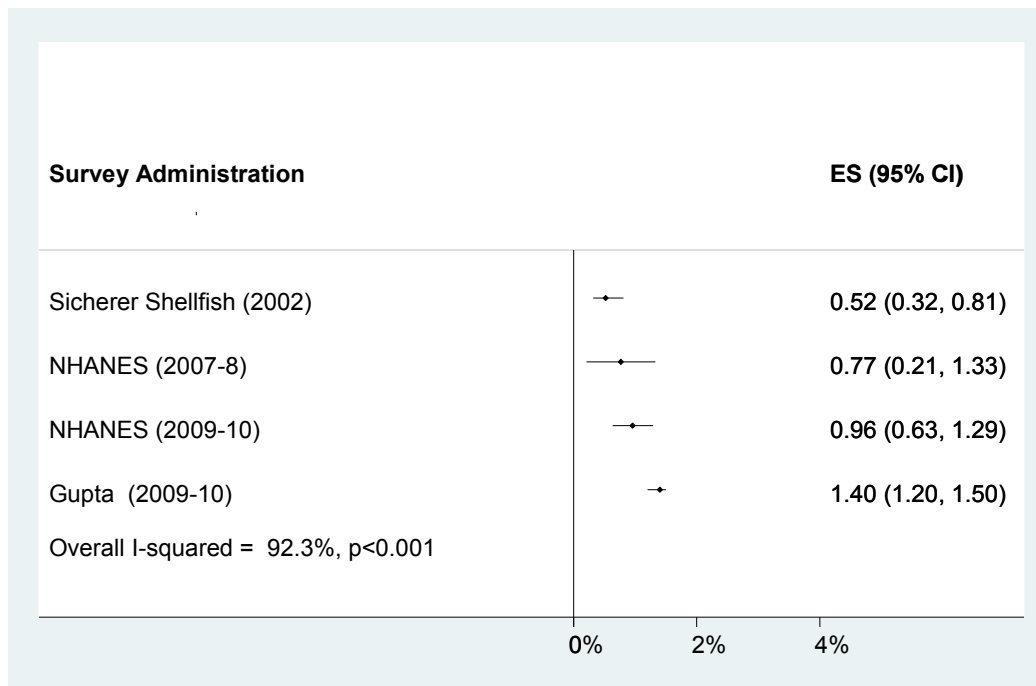
Supplemental Figure 1.



Supplemental Figure 2



Supplemental Figure 3



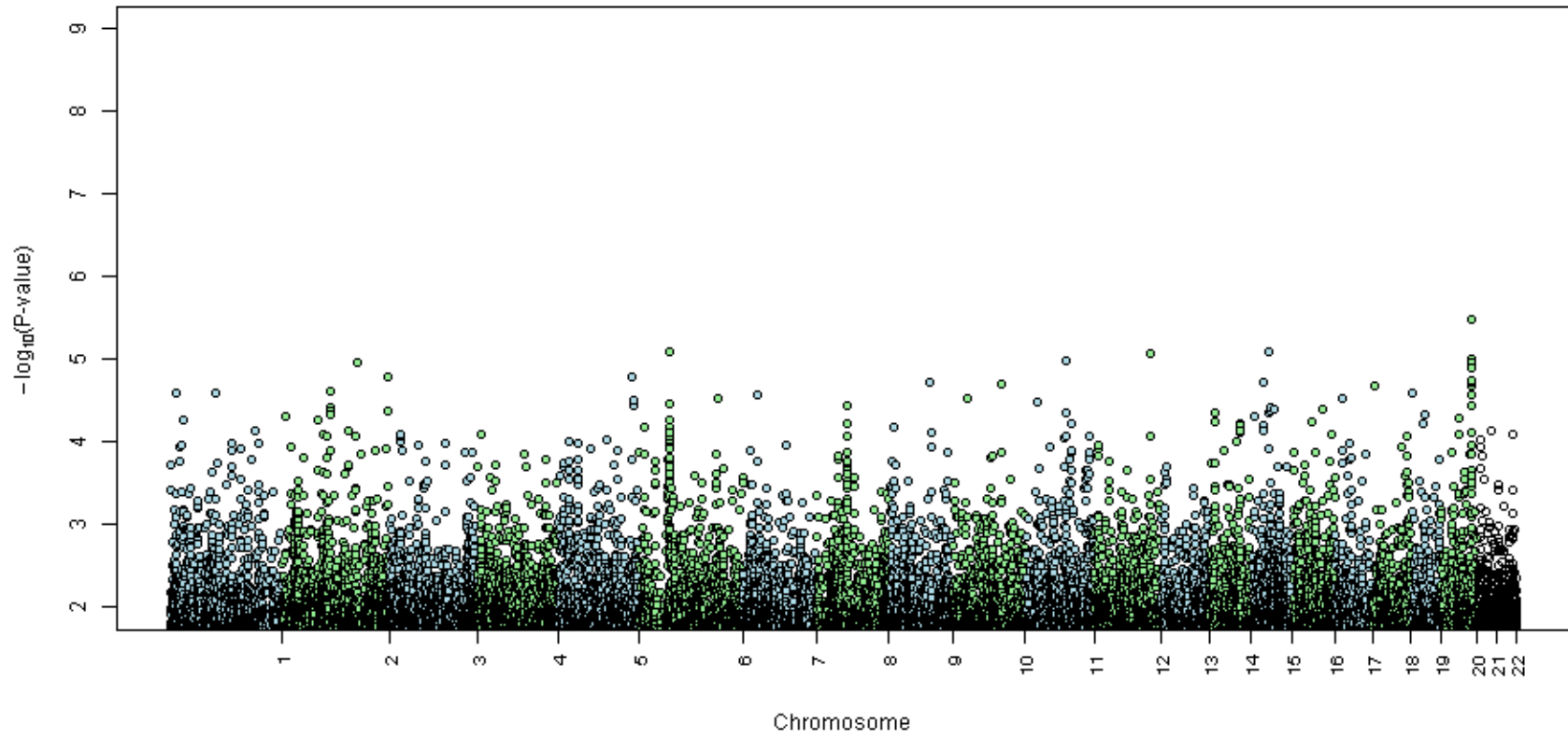
Appendix 2

Supplementary Materials for Paper #3

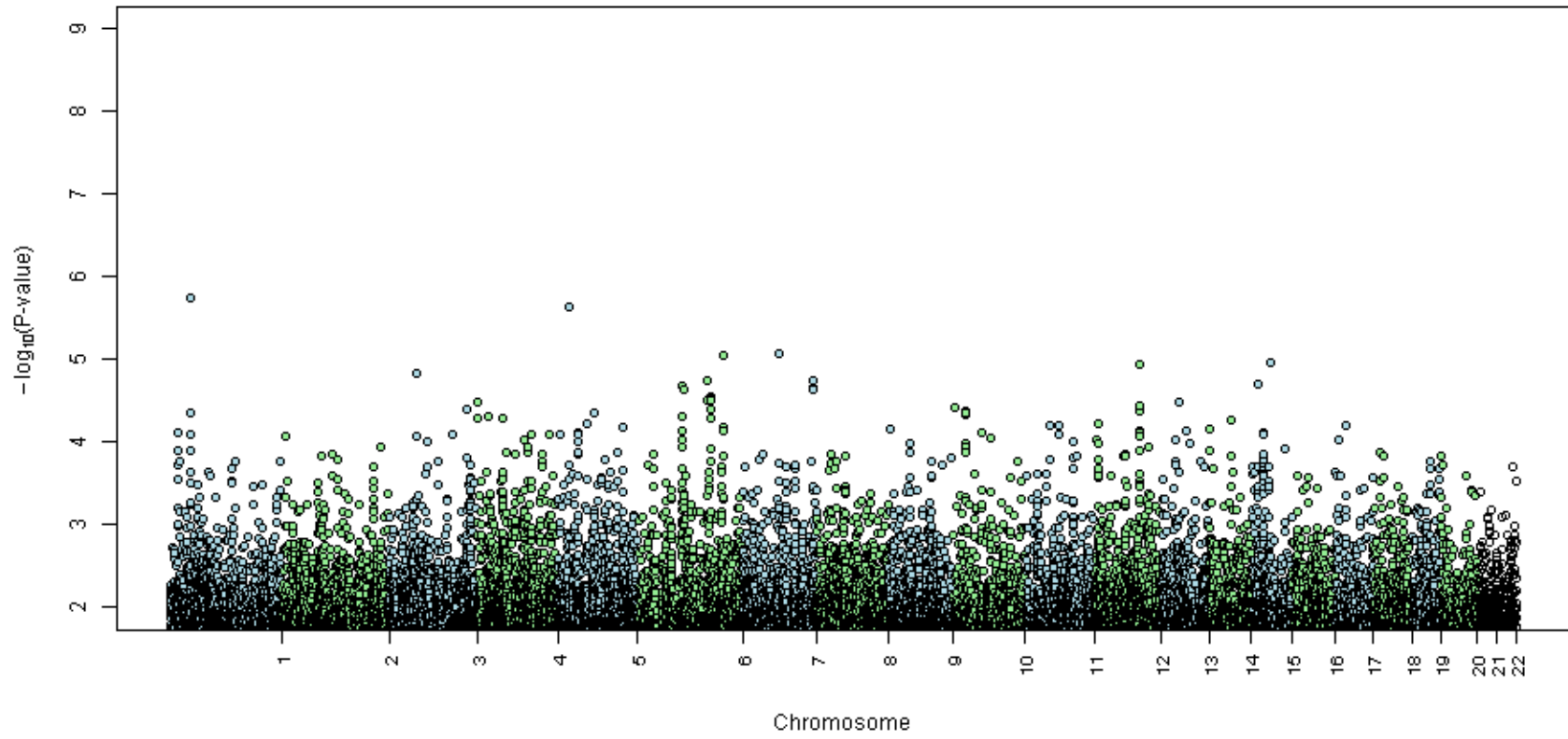
Supplementary Table 1

	Food Allergy	Peanut Allergy	Milk Allergy	Egg Allergy
	589	307	270	207
Peanut Allergy	283		58	69
Milk Allergy	241	58		105
Egg Allergy	173	69	105	
No Peanut, Milk or Egg Allergy	72	-	-	-

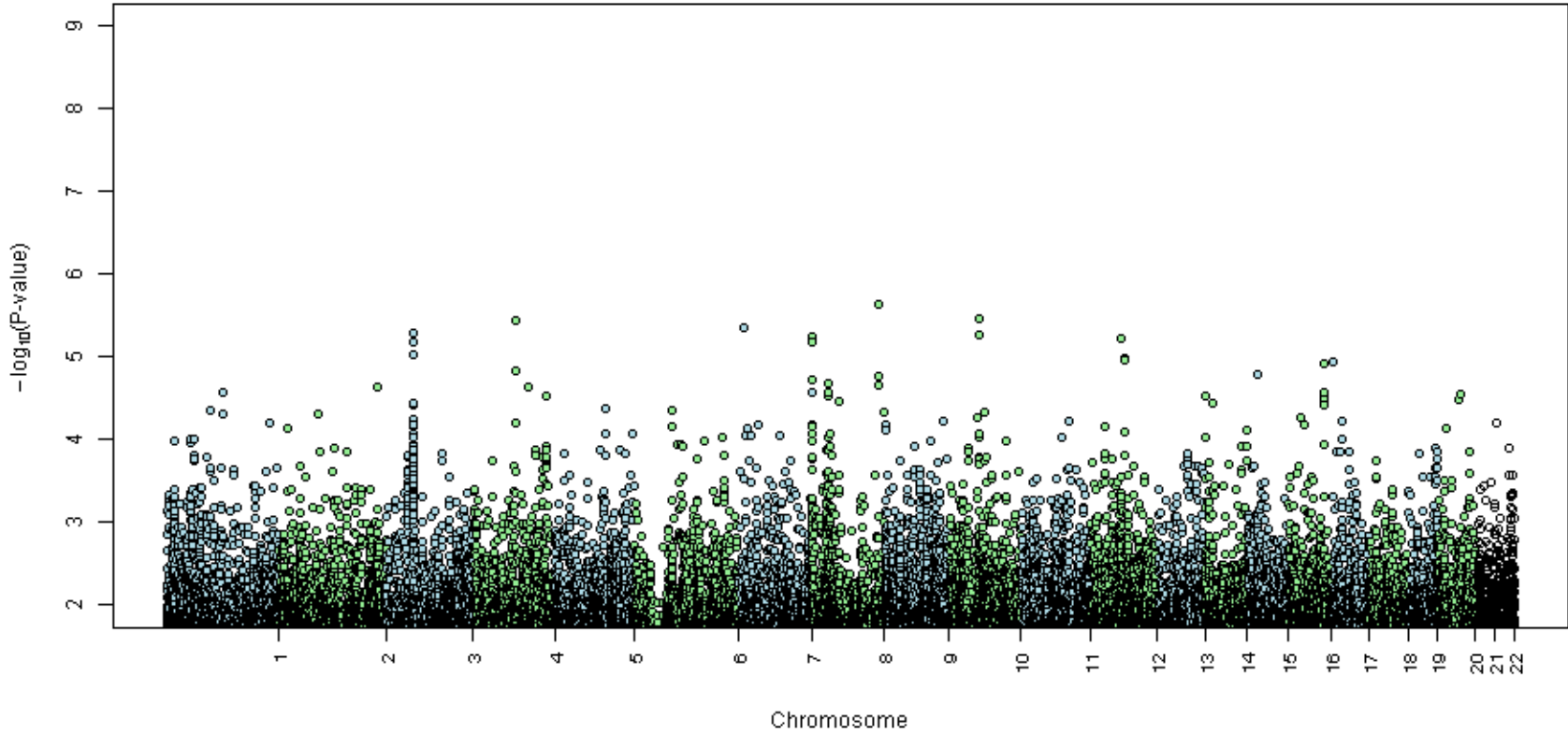
Season of Birth by Gene Interaction for Egg Allergy



Season of Birth by Gene Interaction for Milk Allergy



Season of Birth by Gene interaction for FA allergy



CURRICULUM VITAE

Corinne Keet

3/2014

DEMOGRAPHIC AND PERSONAL INFORMATION

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Department of Pediatrics
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PhD Candidate
Epidemiology
Johns Hopkins Bloomberg School of Public Health

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Birth Date: June 1, 1976

Birth Place: San Francisco, California

Education and Training (in chronological order):

Education

Year	Degree/Certificate	Institution	Discipline
1998	B.A.	University of California, Berkeley,	Chemistry
2001	M.S.	University of California, Berkeley,	Health Sciences
2004	M.D.	University of California, San Francisco	
2010-	PhD Candidate,	Johns Hopkins Bloomberg School of Public	Health, Epidemiology

Post-doctoral Training

2004 - 2005 Internship in Pediatrics Johns Hopkins Hospital

2005 - 2007 Residency in Pediatrics Johns Hopkins Hospital
2007 - 2010 Fellowship Allergy/Immunology Johns
Hopkins University

Professional Experience

Dates	Positions	Institutions
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RESEARCH ACTIVITIES

Publications: Peer-reviewed Original Science Research

1. Hamrick SE, Gremmels DB, **Keet CA**, Leonard CH, Connell JK, Hawgood S, Piecuch RE. Neurodevelopmental outcome of infants supported with extracorporeal membrane oxygenation after cardiac surgery. *Pediatrics*. 2003 Jun;111(6 Pt 1):e671-5.
2. **Keet CA**, Matsui EC, Dhillon G, Lenehan P, Paterakis M, Wood RA. The Natural History of Wheat Allergy. *Annals of Allergy Asthma Immunol*. 2009 May;102(5): 410-415.
3. **Keet CA**, McCormack MC, Peng RD, Matsui EC. Age- and Atopy- Dependent Effects of Age- and Atopy-Dependent Effects of Vitamin D on Wheeze and Asthma. *Journal of Allergy and Clinical Immunology*. 2011 Aug;128(2):414-16.
4. Neuman-Sunshine DL, Eckman JA, **Keet CA**, Matsui EC, Peng RD, Lenehan PJ, Wood RA. "The natural history of persistent peanut allergy", *Annals of Allergy Asthma Immunol*. 2012 May;108(5):326-331
5. **Keet CA**, Wood RA, Masui EC. Personal and parental nativity as risk factors for food sensitization. *Journal of Allergy and Clinical Immunology*. 2012 Jan;129(1):169-175.
6. **Keet CA**, Frischmeyer-Guerrerio PA, Thyagarajan A, Schroeder JT, Hamilton RG, Boden S, Steele P, Driggers S, Burks AW, Wood RA. The Safety and Efficacy of Sublingual and Oral Immunotherapy for Milk Allergy. *Journal of Allergy and Clinical Immunology*. 2012 Feb; 129(2):448-55,455.e1-5.
7. **Keet CA**, Matsui EC, Savage JH, Neuman-Sunshine D, Skripak J, Peng RD, Wood, RA. "Potential mechanisms for the association between fall birth and food allergy", *Allergy*. 2012 Jun;67(6):775-82
8. Savage J, Matsui EC, Wood RA, **Keet CA**. Urinary levels of triclosan and parabens are associated with aeroallergen and food sensitization. *Journal of Allergy and Clinical Immunology*. 2012 Aug;130(2):453-460.e7

9. **Keet CA**, Wood RA, Matsui, EC. Limitations of reliance on specific IgE for epidemiologic surveillance of food allergy. *Journal of Allergy and Clinical Immunology*. 2012 Nov; 130(5): 1207-1209
10. **Keet CA**, Johnson K, Savage JH, Hamilton RG, Wood RA. Evaluation of Ara h2 IgE thresholds in the diagnosis of peanut allergy in a clinical population. *JACI: In Practice*. 2013, Jan 1(1): 101-103
11. **Keet CA**, Seopaul S, Knorr S, Narisety S, Skripak J, Wood R. Long-Term Follow-up of Oral Immunotherapy for Cow's Milk Allergy. *Journal of Allergy and Clinical Immunology*, 2013 Sep;132(3):737-739
12. Holbrook T, **Keet CA**, Guerrerio P, Wood R. Use of Ondansetron in the Treatment of Food Protein Induced Enterocolitis Syndrome (FPIES) Reactions. 2013 Nov; 132(5): 1219-20. *Journal of Allergy and Clinical Immunology*
13. McGowan E, **Keet CA**. Prevalence of self-reported food allergy in the National Health and Nutrition Examination Survey (NHANES) 2007-2010. 2013 Nov; 132(5):1216-1219.e5 *Journal of Allergy and Clinical Immunology*.
14. Schroeder, JT, Bieneman AP, Chinester KL, **Keet, CA**; Hamilton, RG; MacGlashan DW Jr., Wood, R, Guerrerio, P; Spontaneous Basophil Responses in Food Allergic Children are Transferable by Plasma and are IgE-dependent. 2013 Dec;132(6):1428-31. *Journal of Allergy and Clinical Immunology*.
15. **Keet CA**; Shreffler WG; Peng RD; Matsui W; Matsui EC; Associations between serum folate and vitamin D levels and incident mouse sensitization in adults. 2013 Nov 27. [Epub ahead of print] *Journal of Allergy and Clinical Immunology*
16. **Keet CA**; Savage JH; Seopaul S; Peng RD; Wood RA; Matsui EC. Temporal Trends and Racial/Ethnic Disparity in Self-reported Pediatric Food Allergy in the US. 2014 Jan 7. [Epub ahead of print.] *Annals of Allergy, Asthma and Immunology*
17. Savage JH; Matsui EC; McCormack M, Wood RA; **Keet CA**. The association between asthma and allergic disease and mortality – a 30 year follow-up study". In press at *Journal of Allergy and Clinical Immunology*.

Extramural Funding (current, pending, previous)

Current:

1K23AI103187: (Keet) 08/15/2012-07/31/2017 9
calendar
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
\$633,050 (direct)
Genetic and Environmental Determinants of Food Allergy
This grant supports the career development of the candidate in a project focused on the genetic determinants of food allergy.

1R21HD: 073557 (Keet) 06/10/2013-05/31/2015 1.2 calendar (no salary)
EUNICE KENNEDY SHRIVER \$282,702 (direct)
NATIONAL INSTITUTE OF CHILD HEALTH & HUMAN DEVELOPMENT
Development of a Dissolving Film for Allergen Immunotherapy in Children
We have developed a film for use in the treatment of peanut allergy. In this grant, we propose to extend this method to other important allergens for children and explore methods to optimize its use in children.

1R21AI107085-01 (Keet) 07/13-03/15 1.2 calendar (no salary)
NIAID \$282,702 (direct)
Temporal Trend in Food Sensitization in U.S. Children
This proposal is to measure specific IgE to selected foods in the NHANES III survey (1988-1994) in order to compare rates of sensitization with the NHANE 2005-6 survey to determine whether sensitization is increasing in parallel with self-report of food allergy.

Gerber Family Foundation (Yung) 10/13-10/16 0.2 calendar
\$300,00 (direct)
Early Risk Factors for Gastrointestinal Mucosal Food Allergies
This proposal is to establish a birth cohort to examine early life risk factors for food allergy.

Previous:
5KL2RR025006 (Ford) 6/1/10 – 5/31/12 9.6 calendar
Institute for Clinical and Translational Research KL2
NIH/NCRR
This CTSA grant supports clinical and translational research throughout Johns Hopkins. It includes support for education and training of new translational investigators, facilities in which clinical research can take place and infrastructure support of patient recruitment, bioinformatics, biostatistics and translational core centers.
Role: KL2 Scholar

EDUCATIONAL ACTIVITIES

Educational Publications

Invited Review Articles

1. **Keet CA**, Wood RA. Food allergy and anaphylaxis. *Immunol Allergy Clin North Am*. 2007 May; 27(2):193-212, vi.
2. **Keet CA** and Wood RA. Risk Factors for Peanut Allergy. *Journal of Allergy and Clinical Immunology*. 2009 Aug;124(2):387
3. **Keet C**. Recognition and Diagnosis of Food-Related Anaphylaxis. *Pediatric Clin North Am*. 2011 Apr;58(2):377-88.
4. Narisety SD, **Keet CA**. Sublingual vs. Oral Immunotherapy for Food Allergy: Identifying the Right Approach. *Drugs*. 2012 Oct 22; 72(15):1977-89

Editorials

1. **Keet CA** and Wood RA. Baby – and toddler -- steps toward immunotherapy for food allergy. *Clin Exp Allergy*. 2011 Sep; 41(9):1175-6

Case Reports

1. **Keet CA**, Fox CK, Margeta M, Marco E, Shane AL, Dearmond SJ, Strober JB, Miller SP. Infant botulism, type F, presenting at 54 hours of life. *Pediatr Neurol*. 2005 Mar;32(3):193-6.
2. Ezzell JE, **Keet C**, McDonald W, Banerjee A. Medulloblastoma simulating acute myeloid leukemia: case report with a review of “myeloid antigen” expression in nonhematopoietic tissues and tumors. *J Pediatr Hematol Oncol*. 2006 Nov;28(11):703-10.

Letters, correspondence

1. **Keet CA** and Wood RA. Risk Factors for Peanut Allergy. *Journal of Allergy and Clinical Immunology*. 2009 Aug;124(2):387

Book Chapters, Monographs

1. **Keet CA**, Wood RA. New Aspects of Peanut and Tree Nut Allergy. In: Pawankar R, Holgate S, and Rosenwasser LJ, eds. Allergy Frontiers. From Epigenetics to Future Perspectives. Springer Japan KK, Tokyo, Japan 2009.
2. **Keet CA**, Wood, RA. Overview of Mucosal Immunology and the Development of Oral Tolerance. In: James JM, Burks W, Eigenmann P, eds. Food Allergy. Saunders, September 2011.
3. **Keet CA**, Wang J. Acute Reactions and Anaphylaxis to Foods. In: Sicherer S et al. Food Allergy: Practical Diagnosis and Management. CRC Press. In press.

Teaching

Classroom instruction (dates, course title, role, location)

2012, 2013 Graduate Immunology Medical Student Lecture Series,
Guest Small Group Leader, Johns Hopkins

2012 Graduate Student Instructor, Principles of Epidemiology,
Summer Session, Johns Hopkins Bloomberg School of Public Health

CME instruction (dates, course title, role, location)

2007, 2009, 2011, 2012, 2013. Pediatric Trends, Speaker, Johns
Hopkins

2009, 2010. Allergy Grand Rounds, Speaker, Johns
Hopkins

2010 Pediatric Grand Rounds, Johns Hopkins

2013- Clinical Research Methods for Post-doctoral
Fellows in Allergy/Immunology and Pulmonology, Co-director of course, Johns Hopkins

Mentorship

1. Jessica Savage. Current position: Instructor at Brigham and Women's Hospital
Item #8 in Peer Reviewed Original Research Articles above. (Editor's choice featured
article), AAAAI/FARE Howard J. Gittis 3rd/4th Year Fellowship/Junior Faculty
Research Award. Item #17 in Peer Reviewed Original Research Articles above.
Featured poster, Best of HEDQ Interest Section, 2013 AAAAI Meeting

2. Emily McGowan. Current position: Fellow in Adult Allergy and Clinical Immunology, Johns Hopkins. Projects: Item #13 in Peer Reviewed Original Research Articles above (featured on AAAAI website).
3. Karen Robbins. Current Position: Fellow in Pediatric Allergy and Immunology, Johns Hopkins. Robbins, K, Wood, RA, **Keet, CA**. Growth Of Children Aged 2-17 With Cow's Milk, Peanut, and Egg Allergy In NHANES. Accepted for oral presentation at the 2014 AAAAI meeting, San Diego, CA.
4. Shannon Seopaul. MPH Student, Johns Hopkins Bloomberg School of Public Health. Capstone Advisor. Seopaul S., Keet C. Infant Feeding Practices Changed Following Publication of the 2000 American Academy of Pediatrics Guidelines. *Featured Poster, Best of Allied Health*. 2013 AAAAI Meeting, San Antonio, Texas.

Preliminary Oral Exam Committee Membership

10/30/13 Huan He, PhD Student, Population, Family and Child Health, Johns Hopkins Bloomberg School of Public Health

CLINICAL ACTIVITIES

Certification

9/30/2012 Maryland License Number: D0071006

2007 Diplomat, Pediatrics
American Board of Pediatrics

2010 Diplomat, Allergy and Immunology
American Board of Allergy and Immunology

Clinical (Service) Responsibilities (dates, specialty, role, time commitment)

2011-current. Pediatric Allergy Clinic. 10%

ORGANIZATIONAL ACTIVITIES

Editorial Activities (dates, role)

Contributor, Best Articles in Pediatric Allergy and Immunology, Pediatrics 2008-2013

Journal Peer Review Activities

Reviewer for:

- ? Annals of Asthma, Allergy, and Immunology (2009-present)
- ? Journal of Allergy and Clinical Immunology (2011-present)
- ? Journal of Immunologic Methods (2011-present)
- ? International Archives of Allergy and Immunology (2011-present)
- ? Pulmonary Pharmacology and Therapeutics (2011-present)
- ? American Journal of Clinical Dermatology (2012-present)
- ? JACI: In Practice (2012-present)
- ? Allergy (2012-present)
- ? Clinical and Experimental Allergy (2012-present)
- ? PLOSone (2013-present)
- ? AAAAI Annual Meeting Abstracts (2013)
- ? Acta Paediatrica (2013-)
- ? Thorax (2014-)

Professional Societies (date, membership, committees, role)

2004 - American Academy of Pediatrics

2011 - Fellow, American Academy of Pediatrics

2007 - American Academy of Asthma, Allergy and Immunology, Member, Subcommittee on Food Allergy

RECOGNITION

Awards, Honors (date, title, description, sponsor)

- | | |
|------|---|
| 1997 | Phi Beta Kappa |
| 1998 | High Honors and Distinction in General Scholarship |
| 2000 | University Fellowship for Graduate Study in Health and Medical Sciences |
| 2003 | American Medical Students Association Health Policy Fellowship |
| 2004 | Study Abroad Fellowship |
| 2004 | Alpha Omega Alpha |
| 2004 | Sadie Berkov Award (awarded to top three female graduates of U.C.S.F.) |
| 2010 | American Academy of Pediatrics (AAP) Section on Allergy and Immunology (SOAI) Outstanding Abstract Award - Fellow |
| 2010 | American Academy of Allergy, Asthma and Immunology (AAAAI) FADDA Section Outstanding Abstract Award |

2011 American Academy of Pediatrics (AAP) Section on Allergy and Immunology (SOAI) Outstanding Abstract Award – Junior Faculty
2013 American Academy of Pediatrics (AAP) Section on Allergy and Immunology (SOAI) Outstanding Abstract Award – Junior Faculty

Invited Talks, Panels (date, title, venue, sponsor)
Johns Hopkins:

1. “Epidemiology of Food Allergy”, March 2013, Johns Hopkins Pulmonary/Allergic Diseases Conference

National:

1. “New Therapies for Food Allergy”, 2011 Annual Meeting American Academy of Allergy, Asthma and Immunology, San Francisco, California
2. “The PI’s Perspective on the FDA Audit”, 2012 Annual Meeting American Academy of Allergy, Asthma and Immunology, Orlando, Florida
3. “Food Allergy: How Much Do We Really Know?” 2013 NIH STEP Program, NIH, Bethesda, Maryland
4. “Is the Prevalence of Food Allergy Increasing, and, If So, Why?” 2013 Swineford Conference, Charlottesville, Virginia
5. “Born Under a Bad Sign; Gene-by-Environment Interactions in Food Allergy” 2013 Stanford University, Palo Alto, California
6. “Update on Food Allergy” 2013, Annual Meeting, American Academy of Pediatrics, Orlando, Florida
7. “Are antibacterial products causing food allergy?” 2014, Colorado Allergy and Asthma Society, Denver, Colorado
8. “Possible Environmental Causes of the Food Allergy Epidemic” 2014, Denver Allergy/Immunology Rounds, National Jewish Hospital, Denver, Colorado