HHS Public Access

Author manuscript

J Allergy Clin Immunol Pract. Author manuscript; available in PMC 2023 August 01.

Published in final edited form as:

J Allergy Clin Immunol Pract. 2022 August ; 10(8): 2117–2124.e4. doi:10.1016/j.jaip.2022.04.042.

Atopic dermatitis mediates the association between an *IL4RA* variant and food allergy in school-aged children

Tina M. Banzon, MD[1,2,*], **Michael S. Kelly, MD**[2,3,*], **Lisa M. Bartnikas, MD**[1,2], **William J. Sheehan, MD**[4,5], **Amparito Cunningham, MD, MPH**[1], **Hani Harb, PhD**[1,2], **Elena Crestani, MD, MS**[1,2], **Linda Valeri, MSc, PhD**[6], **Kimberly F. Greco, MPH**[7], **Talal A. Chatila, MD, MSc.**[1,2], **Wanda Phipatanakul, MD, MS**[1,2,7,#], **Peggy S. Lai, MD, MPH**[2,8,#]

[1]Division of Allergy and Immunology, Boston Children's Hospital, Boston, MA

[2]Harvard Medical School, Boston, MA

[3]Department of Internal Medicine, Massachusetts General Hospital, Boston, MA

[4]Division of Allergy and Immunology, Children's National Hospital, Washington DC

[5]George Washington University School of Medicine and Health Sciences, Washington DC

[6]Department of Biostatistics, Columbia University Mailman School of Public Health, New York, NY

[7]Institutional Centers for Clinical and Translational Research, Boston Children's Hospital, Boston, MA

[8]Division of Pulmonary and Critical Care, Massachusetts General Hospital, Boston, MA; Department of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, MA

Abstract

Background: Atopic dermatitis (AD) and food allergy (FA) may share genetic risk factors. It is unknown whether genetic factors directly cause FA or are mediated through AD, as the dual-allergen hypothesis suggests.

Objective: To test the hypothesis that AD mediates the relationship between an interleukin-4 receptor alpha chain gene (*IL4RA*) variant, the IL4Rα-R576 polymorphism, and FA.

Methods: 433 children with asthma enrolled in the School Inner-City Asthma Study underwent genotyping for the $IL4RA^{576}$ allele. Surveys were administered to determine FA, AD and associated allergic responses. Mediation analysis was performed adjusting for race and ethnicity, age, gender, and household income. Multivariate models were used to determine the association between genotype and FA severity.

Corresponding Author: Peggy S. Lai, MD, MPH, Bulfinch 148, 55 Fruit Street, Boston, MA 02114, PLAI@mgh.harvard.edu, (617) 726-2000.

*equal contribution and should be considered co-first authors, authorship order listed alphabetically

#equal contribution and should be considered co-last authors

Conflicts of Interest:

Dr. Phipatanakul reports consultancy fees from Genentech, Novartis, Regeneron, Sanofi, GSK, and Astra Zeneca for therapeutics related to Asthma, outside the submitted work. Drs. Banzon, Kelly, Bartnikas, Sheehan, Cunningham, Harb, Crestani, Chatila, Valeri, and Lai declare no conflicts of interest.

All tables are attached in a separate Tables file. All supplemental tables are located in the online repository file.

Results: AD was reported in 193 (45%) and FA in 80 children (19%). Each risk allele increased odds of AD 1.39-fold ([1.03 – 1.87], $P = 0.03$), and AD increased odds of FA 3.67-fold ([2.05 – 6.57], $P < 0.01$). There was an indirect effect of genotype, mediated by AD, predicting FA; each risk allele increased the odds of FA by 1.13 (OR [95% CI]: $Q/R = 1.13$ [1.02 – 1.24], $R/R = 1.28$ [1.04 – 1.51]; $P = <0.01$). Each risk allele increased the odds of severe FA symptoms 2.68-fold ([1.26 – 5.71], $P = 0.01$).

Conclusion: In a cohort of asthmatic children, AD is part of the causal pathway between an *IL4RA* variant and FA. This variant is associated with increased risk of severe FA reactions. Addressing AD in children with an *IL4RA* polymorphism may modulate the risk of FA.

Keywords

Food allergy (FA); atopic dermatitis (AD); *IL4RA* ; genetics; asthma; food anaphylaxis; epicutaneous sensitization; mediation analysis

Introduction:

Atopic dermatitis (AD), a chronic inflammatory skin disease characterized by intense pruritus, eczematous lesions and a relapsing disease course, is the first manifestation of the allergic march. Beginning with AD in the first few months of life, the allergic march continues with IgE-mediated food allergies (FA) presenting between 1 and 3 years of age and asthma appearing in early childhood (1, 2). Early-onset AD has been linked with subsequent diagnosis of FA and asthma (3–5). The temporal relationship with AD preceding FA has raised questions about whether they are the result of a common risk factor or if development of AD itself increases the likelihood of FA (6).

The dual-allergen exposure hypothesis proposes that allergic sensitization to food can occur through the skin, whereas early consumption of food proteins induces oral tolerance, protecting against development of food allergies (7). Several studies have described an increased risk of peanut sensitization and clinically confirmed peanut allergy due to epicutaneous exposure to peanut through an impaired skin barrier (8–10). The dual allergen hypothesis posits that whether a child will develop allergy or tolerance hinges on the timing and balance of cutaneous versus oral exposure. Low-dose cutaneous exposure to environmental foods, such as on tabletops, dust and hands, is thought to penetrate the skin barrier, where it is taken up by Langerhan's cells and leads to T_H2 responses and IgE production by B cells (11, 12). Alternatively, it is proposed that tolerance is induced via early high-dose oral consumption, whereby T_H1 and regulatory T-cell responses are believed to occur in gut-associated lymphoid tissue (11, 12). However, only a fraction of children with AD goes on to develop FA, raising questions of alternative explanations including shared genetic risk factors or environmental triggers (6, 13).

AD, asthma and FA may share genetic risk factors; however, it is unknown whether genetic risk alleles associated with FA are mediated through concomitant AD diagnosis. A human interleukin-4 receptor alpha chain gene variant, which results in a glutamine to arginine substitution at amino acid residue 576 (IL4Rα-R576 polymorphism), has been identified as a possible locus of genetic predisposition for atopic disease (14, 15). IL4Rα-R576

results in a gain of function mutation at the IL-4 receptor causing increased IL-4 pathway signaling (14). This IL4Rα-R576 polymorphism has been associated with asthma diagnosis and severity (16, 17). An anti-IL-4 monoclonal antibody, dupilumab, has been shown to be effective in treating allergic diseases, including asthma and AD (18, 19). However, less is known about the relationship between IL4Rα-R576, AD, and FA.

We hypothesized that the human interleukin-4 receptor alpha chain gene (*IL4RA*) variant $IL4RA^{R576}$ would be associated with increased risk of FA through AD as a mediator and associated with severe FA reactions.

Methods:

Study Population and Design

The School Inner-city Asthma Study (SICAS-1) and School Inner-city Asthma Intervention Study (SICAS-2) were conducted between 2008 and 2013 in children (ages 5 to 17 years) with persistent asthma attending inner-city schools in a city in the northeast United States (Online Repository) (20, 21). Full details of the methodology of selection of this cohort are published elsewhere (20, 21). Inclusion criteria included physician diagnosis of asthma with either controller medication use or symptoms of exacerbation in the last year. Exclusion criteria included lung disease other than asthma and cardiovascular disease. All children from the SICAS cohorts who had genotyping for $IL4RA^{576}$ performed were included in this study.

Outcome Measures

Genotyping of the $IL4RA^{Q576}$ and $IL4RA^{R576}$ alleles was performed using the amplification resistance mutation screen PCR method on DNA extracted from either whole blood (Gentra Puregene Blood Kit; Qiagen) or saliva (prepIT L2P; DNA Genotek). In the SICAS studies, parental surveys were administered at 3, 6, 9, and 12 months after enrollment (20). Physician-diagnosed AD was reported by parents. Physician-diagnosed FA and FA symptoms occurring within one hour of food ingestion were reported by parents. IgE-mediated FA defined in our work was consistent with other studies (22), and included gastrointestinal (abdominal pain, vomiting, mouth/throat itching), respiratory (dyspnea, wheezing, throat tightness, cough), cardiovascular (syncope, hypotension), and cutaneous (urticaria, pruritis, edema) symptoms which occurred within one hour of ingestion. FA diagnosis was independently assessed based on history and reported symptoms by two study physicians (TMB and LMB). If ever there was disagreement between TMB and LMB on FA diagnosis, a blinded third study physician certified by the American Board of Allergy and Immunology (EC) adjudicated. Participants were not considered to have FA if reported symptoms after food ingestion were inconsistent with IgE-mediated FA. Severe FA symptoms were classified according to a grading system developed by Sampson, and defined as the presence of any of the following symptoms (Grade 3 anaphylaxis or above per Sampson anaphylaxis severity scoring system): throat tightness, difficulty breathing, coughing, wheezing, drop in blood pressure or change in neurologic status (passing out) (23).

Statistical Analysis

Characteristics of the study participants were compared among the three genotype groups (*Q/Q, Q/R*, and *R/R*) using analysis of variance (ANOVA) for continuous variables and Pearson's chi-squared statistic for categorical variables. In statistical models, the IL4Rα-R576 polymorphism was primarily modeled as a continuous variable using the number of *R* alleles (0, 1, or 2), consistent with a gain of function mutation. Thus, all logistic regression output in which number of risk alleles was used as a predictor is presented as odds ratio per *R* allele. As a sensitivity analysis, genotype was also modeled as a categorical variable by genotype (*Q/Q* versus *Q/R* versus *R/R*) or as a categorical variable with groupings based on presence or absence of a risk allele (*Q/Q* versus *Q/R* and *R/R*). Unless otherwise stated, *Q/Q* was used as the reference group. All multivariable regression models adjusted for possible confounding from age, sex, annual household income (above or below $25,000), and race and ethnicity (White, non-Hispanic versus any other combination of races and ethnicities).

Logistic regression was used for models identifying the association between risk alleles and AD, risk alleles and FA, and AD and FA. All models adjusted for potential confounding by age, sex, income, and race and ethnicity as above. To investigate potential interaction and mediation effects of AD on the relationship between genotype and FA and test the hypothesis that AD is part of the causal pathway between risk alleles and FA, we performed a mediation analysis including a four-way decomposition of the total effect using the CMAverse package in R (24, 25). The purpose of the mediation analysis was to test the hypothesis that the effect of the risk genotypes on food allergy is mediated by atopic dermatitis. While traditional mediation analyses are predicated on a significant total effect (i.e. association between the $IL4RA^{576}$ risk genotypes and food allergies) (26), in the presence of an *a priori* hypothesized indirect effect (dual allergen hypothesis), there is precedent for pursuing a mediation analysis (27). The presence of an indirect effect, but not total effect, may be seen when the direct and indirect effects are in different directions. Under some models, the indirect effect may have greater power than the total effect, thus using the total effect as a gatekeeper for deciding whether or not to continue with a mediation analysis may not always be the correct choice (27, 28). For these reasons, *a priori* we decided to proceed with a mediation analysis regardless of the total effect. Confidence intervals for the indirect effects were based on 1,000 bootstrapped replications, and multiple imputation of 10 iterations were used for missing income data (25% of study participants declined to answer questions on household income). A sensitivity analysis for unmeasured confounding in the mediation analysis was performed by estimating the E-value (29). Logistic regression, using genotype as the predictor, was used to predict FA symptoms and specific food allergens in the subset of patients with FA. All statistical analysis was performed in R (R version 4.0.4; R Foundation for Statistical Computing, Vienna, Austria). All tests were two-tailed with alpha set at 0.05.

Results

A total of 433 children from the SICAS cohorts consented to $IL4RA^{576}$ genotyping and were included in the study (Table 1). Sex, race, ethnicity, and income level were similar in genotyped participants compared to the overall SICAS 1 and SICAS 2 cohorts (Table

E1). However, genotyped patients were on average 0.5 years younger than the overall cohort (mean [SD]; 8.0 [1.9] vs. 8.5 [1.9]; $P < 0.001$). The reference genotype (*Q/Q*) was identified in 119 children (27%), whereas the heterozygous *Q/R* genotype was seen in 186 children (43%), and the homozygous *R/R* genotype was observed in 128 children (30%). Overall, 314 (73%) of the children genotyped were found to have at least one *R* allele (*Q/R* or *R/R* genotypes). Children with at least one *R* allele were more likely to identify as Black than other races and ethnicities (37% versus 12.6%). AD was reported in 193 children (45%); FA was reported in 80 children (19%). The most commonly reported FA were: peanut (39/80, 49%), tree nuts (23/80, 29%), and shellfish (19/80, 24%; Table 1). Hives (49/80, 61%), skin redness (44/80, 55%), and itchy mouth (41/80, 51%) were the most prevalent FA symptoms reported. Severe FA reactions were reported in 47 participants (59%).

Association between genotype, FA and AD

There was no significant difference in the proportion of children with reported FA across genotypes (Table 1). In adjusted analysis, the number of *R* alleles was not associated with a statistically significant increase in odds of FA, although the effect size suggested increased risk (OR per *R* allele [95% CI], *P*; 1.11 [0.78 – 1.59], $P = 0.56$; Table E2). However, AD was more common in patients with at least one *R* allele (152/314 [48%] versus 41/119 [35%]), and adjusted analysis demonstrated increased risk of AD with each additional *R* allele (OR per *R* allele = 1.39 [1.03 – 1.87], $P = 0.03$). Further, in adjusted analysis, AD was shown to increase the odds of FA (OR = 3.67 [2.05 – 6.57], $P < 0.01$). These results held in sensitivity analyses when comparing participants with no risk alleles (*Q/Q* genotype) to those with at least one risk allele (*Q/R* or *R/R* genotypes; Table E2).

This series of findings supported the possibility it was along the relationship between genotype and FA was influenced AD. Specifically, our hypothesis was that there may be an indirect effect of genotype on FA that is mediated by the presence of AD, consistent with the dual-allergen hypothesis. To explore these findings further, causal mediation analysis was performed.

Causal mediation analysis allows the decomposition of the total effect of an exposure on an outcome, in the presence of a potential mediating factor, into a direct effect and an indirect effect. The indirect effect represents the portion of the total effect of the exposure on the outcome that is caused by the result of mediation from a third variable. In other words, the indirect effect determines the amount of the effect of the exposure on the outcome that is the result of the exposure making the mediating variable more likely and the mediator making the outcome more likely. In contrast, the direct effect represents the effect of the exposure on the outcome that is not the result of mediation through the hypothesized mediator variable (the portion of the effect of the exposure on the outcome after the effect of the mediated pathways has been accounted for). Mediation analysis was performed to determine the effect of number of risk alleles on FA, mediated by AD, modeling the outcome and the mediator, controlling for confounders of the exposure-outcome and exposure-mediator relationship (age, gender, income, and race and ethnicity).

Mediation Analysis: IL4RA^{R576} Allele Increases Odds of FA Mediated Via AD

Overall, the total effect of the number of risk alleles on risk of FA was not statistically significant, although the effect size indicated a trend towards increased risk (total effect; OR 1.13 [0.78 – 1.51]; $P = 0.59$). However, examining the direct and indirect effects of the mediation analysis further explains the mechanism by which genotype and AD may act upon risk of FA (Figure 1 and Table 2). The indirect effect demonstrates that number of *R* alleles increases risk of FA along the pathway mediated by AD (OR per *R* allele = 1.13 [1.02 – 1.23], $P = <0.01$). It is along this indirect pathway (number of *R* alleles increasing the risk of AD which in turn increases the risk of FA diagnosis) that a *Q/R* genotype had 1.13-times increased odds and a *R/R* genotype had 1.28-times increased odds of FA compared to a *Q/Q* genotype.

Sensitivity analysis for unmeasured confounding was performed on mediation analysis results (Table E3). This analysis shows that for unmeasured confounding to nullify the results of the indirect effect, an unmeasured confounder would require a strength of association at least 1.35 times larger than the observed effect (E-value [95% CI lower limit], RR = 1.53 [1.17]). To further explore the possibility of interaction as well as mediation between genotype, AD and FA, a four-way decomposition of the mediation analysis was performed. This demonstrated no significant contribution of interaction or mediated interaction in explaining the total effect (Table E4). Sensitivity analyses modeling the genotype using the presence or absence of at least one risk allele (*Q/Q* vs. *Q/R* or *R/R*) demonstrated similar findings (Table E5). Given concern that subjects with purely a vegetable or fruit allergy may have pollen food allergy syndrome and not true FA, a sensitivity analysis was performed reclassifying subjects with solely a vegetable or fruit allergy as not having FA. Reclassifying those with exclusively a vegetable or fruit allergy as not having FA changes the FA assignment of eight patients (n = 72 from n = 80). This mediation analysis continued to demonstrate a significant indirect effect of number of risk alleles on FA mediated by AD (Table E6).

Patients With IL4RA^{R576} Allele Are More Likely to Report Severe FA

Subject FA symptoms, stratified by genotype, are displayed in Table E7. Children were more likely to report severe FA symptoms as number of *R* alleles increased (Figure 2). Adjusting for age, gender, income, and race and ethnicity, each *R* allele increased the odds of severe FA symptoms 2.68-fold [1.26 – 5.71] ($P = 0.01$; Figure 2). Increasing *R* alleles also predicted increased likelihood to report respiratory symptoms of wheezing (OR per *R* allele = 2.50 [1.14 – 5.47], $P = 0.02$) and difficulty breathing (OR per *R* allele = 2.34 [1.05 – 5.20], $P = 0.04$; Table E8).

Discussion

In this study, we demonstrate that AD is part of the causal pathway between a common *IL4RA* polymorphism and FA. There was a dose-response relationship between the number of risk alleles and patient-reported severe FA reactions. The mediation analysis provided valuable insight into the relationship between genotype, AD, and how they may impact FA. AD mediated the relationship between the genotype and FA. While genotype is immutable,

identification of AD as part of the causal pathway between genotype and FA suggests that interventions targeted towards AD may influence FA diagnosis in children born with the risk allele. This observation also provides further support for the dual-allergen hypothesis.

While the IL4Rα-R576 polymorphism has been associated with increased risk for atopic diseases, our work did not demonstrate a statistically significant increased risk of FA based on genotype alone (14). In the case of our analysis, the indirect and direct effects are not in the same direction, which likely explains why the total effect is not statistically significant, but the indirect effect is. Larger cohorts will be required to further elucidate the effects of *IL4RA* genotype on AD and atopic diseases independent of AD in the general population. Our results demonstrate that nearly all the excess risk for FA seen in those with at least one $IL4RA^{R576}$ allele is attributable to the increased risk of AD in this population. Sensitivity analysis demonstrates that unmeasured confounding is unlikely to explain the results of this AD-mediated pathway. Further, we demonstrate here that AD appears to act as a mediator in the link between genotype and risk for FA.

In addition to providing added support for the dual allergen hypothesis, this work also provides new insight into the *IL4RA* genotype as a risk factor for severity of FA reactions in a cohort of subjects with asthma. Previous work had shown that having at least one $IL4RA^{R576}$ allele was associated with an increased risk of asthma and asthma severity, although the association of this gene with other atopic diseases has been less clear (16, 17). Analogous to asthma, having at least one *IL4RA* risk allele was associated with severe FA symptoms, as well as respiratory FA symptoms. Consistent with a gain of function mutation, our work supports a dose response relationship between the number of $IL4RA^{R576}$ alleles and increasing likelihood of AD-mediated FA and severe FA reactions. The mechanism leading to increased severity of FA reactions based on genotype in this cohort of children with asthma may be the same or similar to that of the previously noted increased asthma severity based on *IL4RA* genotype, and it remains unclear if this result would also be found in patients without asthma.

Prior literature suggests that polymorphisms in the IL4R can alter the risk of FA by impairing oral tolerance and promoting FA (30, 31). Similar to our study's $IL4RA^{R576}$ polymorphism, which results in a gain of function mutation at the IL4R causing increased IL-4 pathway signaling, a well-characterized murine transgenic model of FA has been studied involving a gain of function IL-4Rα chain polymorphism causing enhanced IL-4 receptor signaling. In studies of these mice carrying inactivating mutation in the IL-4Rα subunit's immunotyrosine inhibitory motif ($Il4ra^{F709}$), impaired generation and function of mucosal allergen-specific Treg cells was observed via a STAT6-dependent mechanism. This led to selective augmentation of IL4R signaling in Treg cells to induce their reprogramming into T_H2-like cells and FA susceptibility (31).

Group 2 innate lymphoid cells (ILC2), due to their secretion of IL-4, were also found to play a pivotal role in inducing FA in this murine model by increasing mucosal mast cell activation and hindering allergen-specific Treg cell induction and suppressive functions (30). Our study is consistent with observations made in these mechanistic models and suggests a role for immunomodulators, such as an anti-IL4R antibody, as a possible therapy for FA. For

children with risk alleles that result in increased IL4R signaling, anti-IL4R treatment may prove helpful in treating both AD and FA by resetting the circuit of impaired induction of allergen-specific Treg cells, which leads to T_H2 cell type skewing (30).

Given that there is no current cure for FA, there has been increased interest exploring the use of biologic agents for FA. Dupilumab, a human monoclonal antibody against the IL4Rα subunit that acts as a dual inhibitor of IL-4 and IL-13 signaling, is currently approved for use for AD, asthma, and chronic rhinosinusitis with nasal polyposis (32). Given its T_H2 suppressive mechanism of action, dupilumab is also being investigated as a potential biologic treatment option for FA in several ongoing clinical trials evaluating its use either as an adjunct to oral immunotherapy for peanut allergy, milk allergy, or as monotherapy (33–35). Our findings support the impetus of the ongoing work that aims to translate the dual allergen hypothesis into disease prevention by seeking to treat AD as an approach to prevent FA. Our work also supports ongoing trials using IL-4 monoclonal antibodies (such as dupilumab) as treatment for atopic diseases generally, but specifically for those with *IL4RA* polymorphisms and those with both AD and FA (36, 37).

This study has several strengths. Our sample size included 433 children and represented a diverse group of subjects especially with regards to race and ethnicity. The number of *R* alleles was well distributed in our cohort, with an approximate 1:2:1 ratio of genotypes. While this is unlikely the community prevalence of the risk allele, it did allow for higher power in detecting relationships between genotype, disease prevalence, and symptoms. We leveraged causal inference methods in order to identify mechanistic relationships between the genotype, AD, and FA.

There are some limitations worth noting. The study population consists of children with asthma, and thus is biased toward atopic diseases with a higher prevalence of AD, FA, and *IL4RA* polymorphisms than is reported in the general population (38–40). Our participants are reflective of an inner-city population with asthma, thus generalizability to patients in other settings may be limited. Consistent with prior work, genotype and AD varied by race and ethnicity in our cohort, and while adjusted analysis treated race and ethnicity as a possible confounder, unmeasured confounding is possible (39, 41). This study was retrospective, and performing an analysis not *a priori* specified for in the aims of the original studies. AD, FA diagnoses, and FA symptoms were reported by parents, and FA diagnosis was not confirmed by the gold standard of double-blind placebo-controlled food challenge, although FA case determination was independently assessed by study physicians. FA cases met clinical criteria, including reported symptoms occurring within one hour that were consistent with IgE-mediated FA, and modeled from other studies to allow judicious diagnosis based on clinical algorithms (42–44).

Conclusion

These results strengthen support for the dual-allergen hypothesis by demonstrating that AD is part of the causal pathway between the IL4Rα-R576 polymorphism and FA in patients with asthma and provides further evidence for the use of biologics such as dupilumab in the blockade of IL-4 mediated pathways as a potential therapy in FA.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgement:

For the SICAS 1 and SICAS 2 studies, we thank the following companies for their generous donations. Lincoln Diagnostics, Inc., Decatur, IL, USA, Multi-Test II devices; Greer, Inc, Lenoir, NC allergenic extracts for skin testing. Thermo Fisher, Inc. ImmunoCAP® testing. Monaghan Medical, Inc aerochambers, and Aeorcrine, Inc., NiOx Machines. The content is solely the responsibility of the authors and does not necessarily represent the official views of Harvard Catalyst, Harvard University and its affiliated academic healthcare centers, or the National Institutes of Health. We also thank the Community Schools, the participants, and their families for the support they provided for the study. Graphical abstract created with BioRender.com.

Funding:

Dr. Banzon is supported by T32 AI 007512 from the National Institutes of Health. Dr. Kelly is supported by R38 HL150212–01 from the National Institutes of Health. Dr. Bartnikas is supported by K23 AI143962 from the National Institutes of Health. Dr. Chatila is supported by R01 AI065617 and R0 AI126915 from the National Institutes of Health. Dr. Phipatanakul is supported by the grants U01 AI 152033 and K24 AI 106822 from the National Institutes of Health and Allergy Asthma Awareness Initiative, Inc. Dr. Lai is supported by R01 AI144119 from the National Institutes of Health. This work was conducted with support from Harvard Catalyst, The Harvard Clinical and Translational Science Center (National Center for Research Resources and the National Center for Advancing Translational Sciences, National Institutes of Health Award UL1 TR002541) and financial contributions from Harvard University and its affiliated academic healthcare centers.

Abbreviations:

AD	Atopic dermatitis
FA	Food allergy
IL4RA	Human interleukin 4 receptor alpha chain gene
IL4Rα	Human interleukin 4 receptor alpha chain protein

References:

1. Kulig M, Bergmann R, Klettke U, Wahn V, Tacke U, Wahn U. Natural course of sensitization to food and inhalant allergens during the first 6 years of life. Journal of Allergy and Clinical Immunology 1999;103(6):1173–9. [PubMed: 10359902]
2. Spergel JM, Paller AS. Atopic dermatitis and the atopic march. Journal of Allergy and Clinical Immunology 2003;112(SUPPL. 6).
3. Hill DJ, Sporik R, Thorburn J, Hosking CS. The association of atopic dermatitis in infancy with immunoglobulin E food sensitization. Journal of Pediatrics 2000;137(4):475–9. [PubMed: 11035824]
4. Lack G, Fox D, Northstone K, Golding J. Factors Associated with the Development of Peanut Allergy in Childhood. New England Journal of Medicine 2003;348(11):977–85. [PubMed: 12637607]
5. Schroeder A, Kumar R, Pongracic JA, Sullivan CL, Caruso DM, Costello J, et al. Food Allergy is Associated with an Increased Risk of Asthma. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology 2009;39(2):261-. [PubMed: 19187334]
6. Paller AS, Spergel JM, Mina-Osorio P, Irvine AD. The atopic march and atopic multimorbidity: Many trajectories, many pathways. Journal of Allergy and Clinical Immunology 2019;143(1):46–55. [PubMed: 30458183]
7. Lack G Epidemiologic risks for food allergy. Journal of Allergy and Clinical Immunology 2008;121(6):1331–6. [PubMed: 18539191]

8. Strid J, Hourihane J, Kimber I, Callard R, Strobel S. Epicutaneous exposure to peanut protein prevents oral tolerance and enhances allergic sensitization. Clinical and Experimental Allergy 2005;35(6):757–66. [PubMed: 15969667]
9. Strid J, Hourihane J, Kimber I, Callard R, Strobel S. Disruption of the stratum corneum allows potent epicutaneous immunization with protein antigens resulting in a dominant systemic Th2 response. European Journal of Immunology 2004;34(8):2100–9. [PubMed: 15259007]
10. Bartnikas LM, Gurish MF, Burton OT, Leisten S, Janssen E, Oettgen HC, et al. Epicutaneous sensitization results in IgE-dependent intestinal mast cell expansion and food-induced anaphylaxis. Journal of Allergy and Clinical Immunology 2013;131(2).
11. Sampson HA, O'Mahony L, Burks AW, Plaut M, Lack G, Akdis CA. Mechanisms of food allergy. J Allergy Clin Immunol 2018;141(1):11–9. [PubMed: 29307410]
12. Brough HA, Nadeau KC, Sindher SB, Alkotob SS, Chan S, Bahnson HT, et al. Epicutaneous sensitization in the development of food allergy: What is the evidence and how can this be prevented? Allergy 2020;75(9):2185–205. [PubMed: 32249942]
13. Khan SJ, Dharmage SC, Matheson MC, Gurrin LC. Is the atopic march related to confounding by genetics and early-life environment? A systematic review of sibship and twin data. Allergy; 2018. p. 17–28.
14. Hershey GKK, Friedrich MF, Esswein LA, Thomas ML, Chatila TA. The Association of Atopy with a Gain-of-Function Mutation in the α Subunit of the Interleukin-4 Receptor. New England Journal of Medicine 1997;337(24):1720–5. [PubMed: 9392697]
15. Chatila TA. Interleukin-4 receptor signaling pathways in asthma pathogenesis. Trends Mol Med; 2004. p. 493–9. [PubMed: 15464449]
16. Rosa-Rosa L, Zimmermann N, Bernstein JA, Rothenberg ME, Khurana Hershey GK. The R576 IL-4 receptor allele correlates with asthma severity. Journal of Allergy and Clinical Immunology 1999;104(5):1008–14. [PubMed: 10550746]
17. Massoud AH, Charbonnier LM, Lopez D, Pellegrini M, Phipatanakul W, Chatila TA. An asthma-associated IL4R variant exacerbates airway inflammation by promoting conversion of regulatory T cells to TH17-like cells. Nature Medicine 2016;22(9):1013–22.
18. Deeks ED. Dupilumab: A Review in Moderate to Severe Asthma. Drugs 2019;79(17):1885–95. [PubMed: 31728838]
19. Gooderham MJ, Hong HCh, Eshtiaghi P, Papp KA. Dupilumab: A review of its use in the treatment of atopic dermatitis. Journal of the American Academy of Dermatology 2018;78(3 Suppl 1):S28–S36. [PubMed: 29471919]
20. Sheehan WJ, Permaul P, Petty CR, Coull BA, Baxi SN, Gaffin JM, et al. Association between allergen exposure in inner-city schools and asthma morbidity among students. JAMA Pediatrics 2017;171(1):31–8. [PubMed: 27893060]
21. Phipatanakul W, Koutrakis P, Coull BA, Petty CR, Gaffin JM, Sheehan WJ, et al. Effect of School Integrated Pest Management or Classroom Air Filter Purifiers on Asthma Symptoms in Students With Active Asthma: A Randomized Clinical Trial. Jama 2021;326(9):839–50. [PubMed: 34547084]
22. Wang J Management of the patient with multiple food allergies. Curr Allergy Asthma Rep 2010;10(4):271–7. [PubMed: 20431971]
23. Sampson HA. Anaphylaxis and emergency treatment. Pediatrics 2003;111(6 Pt 3):1601–8. [PubMed: 12777599]
24. VanderWeele TJ. A unification of mediation and interaction: a 4-way decomposition. Epidemiology 2014;25(5):749–61. [PubMed: 25000145]
25. Shi B, Choirat C, Coull BA, VanderWeele TJ, Valeri L. CMAverse: A Suite of Functions for Reproducible Causal Mediation Analyses. Epidemiology 2021;32(5):e20–e2. [PubMed: 34028370]
26. Baron RM, Kenny DA. The moderator-mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. J Pers Soc Psychol 1986;51(6):1173–82. [PubMed: 3806354]
27. Agler R, De Boeck P. On the Interpretation and Use of Mediation: Multiple Perspectives on Mediation Analysis. Front Psychol 2017;8:1984. [PubMed: 29187828]

28. O'Rourke HP, MacKinnon DP. Reasons for Testing Mediation in the Absence of an Intervention Effect: A Research Imperative in Prevention and Intervention Research. J Stud Alcohol Drugs 2018;79(2):171–81. [PubMed: 29553343]
29. VanderWeele TJ, Ding P. Sensitivity Analysis in Observational Research: Introducing the E-Value. Ann Intern Med 2017;167(4):268–74. [PubMed: 28693043]
30. Noval Rivas M, Burton OT, Oettgen HC, Chatila T. IL-4 production by group 2 innate lymphoid cells promotes food allergy by blocking regulatory T-cell function. J Allergy Clin Immunol 2016;138(3):801–11.e9. [PubMed: 27177780]
31. Noval Rivas M, Burton OT, Wise P, Charbonnier LM, Georgiev P, Oettgen HC, et al. Regulatory T cell reprogramming toward a Th2-cell-like lineage impairs oral tolerance and promotes food allergy. Immunity 2015;42(3):512–23. [PubMed: 25769611]
32. Macdougall JD, Burks AW, Kim EH. Current Insights into Immunotherapy Approaches for Food Allergy. Immunotargets Ther 2021;10:1–8. [PubMed: 33537245]
33. Study to Evaluate Dupilumab Monotherapy in Pediatric Patients With Peanut Allergy [Available from: https://ClinicalTrials.gov/show/NCT03793608.
34. Study in pediatric subjects with peanut allergy to evaluate efficacy and safety of dupilumab as adjunct to AR101 (peanut oral immunotherapy [Available from: https://ClinicalTrials.gov/show/NCT03682770.
35. Dupilumab and Milk OIT for the Treatment of Cow's Milk Allergy [Available from: https://ClinicalTrials.gov/show/NCT04148352.
36. Investigating Dupilumab's Effect in Asthma by Genotype (IDEA) [Available from: https://www.clinicaltrials.gov/ct2/show/NCT03694158.
37. Effectiveness of Dupilumab in Food Allergic Patients With Moderate to Severe Atopic Dermatitis [Available from: https://clinicaltrials.gov/ct2/show/NCT04462055.
38. Langan SM, Irvine AD, Weidinger S. Atopic dermatitis. Lancet 2020;396(10247):345–60. [PubMed: 32738956]
39. Wu X, Di Rienzo A, Ober C. A population genetics study of single nucleotide polymorphisms in the interleukin 4 receptor α (IL4RA) gene. Genes and Immunity 2001;2(3):128–34. [PubMed: 11426321]
40. Warren CM, Jiang J, Gupta RS. Epidemiology and Burden of Food Allergy. Curr Allergy Asthma Rep 2020;20(2):6. [PubMed: 32067114]
41. Brunner PM, Guttman-Yassky E. Racial differences in atopic dermatitis. Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology 2019;122(5):449–55.
42. Gupta RS, Springston EE, Smith B, Pongracic J, Holl JL, Warrier MR. Parent report of physician diagnosis in pediatric food allergy. J Allergy Clin Immunol 2013;131(1):150–6. [PubMed: 22947345]
43. Stutius LM, Sheehan WJ, Rangsithienchai P, Bharmanee A, Scott JE, Young MC, et al. Characterizing the relationship between sesame, coconut, and nut allergy in children. Pediatr Allergy Immunol 2010;21(8):1114–8. [PubMed: 21073539]
44. Gaffin JM, Sheehan WJ, Morrill J, Cinar M, Borras Coughlin IM, Sawicki GS, et al. Tree nut allergy, egg allergy, and asthma in children. Clin Pediatr (Phila) 2011;50(2):133–9. [PubMed: 21098525]

Highlights box:

What is already known about this topic?

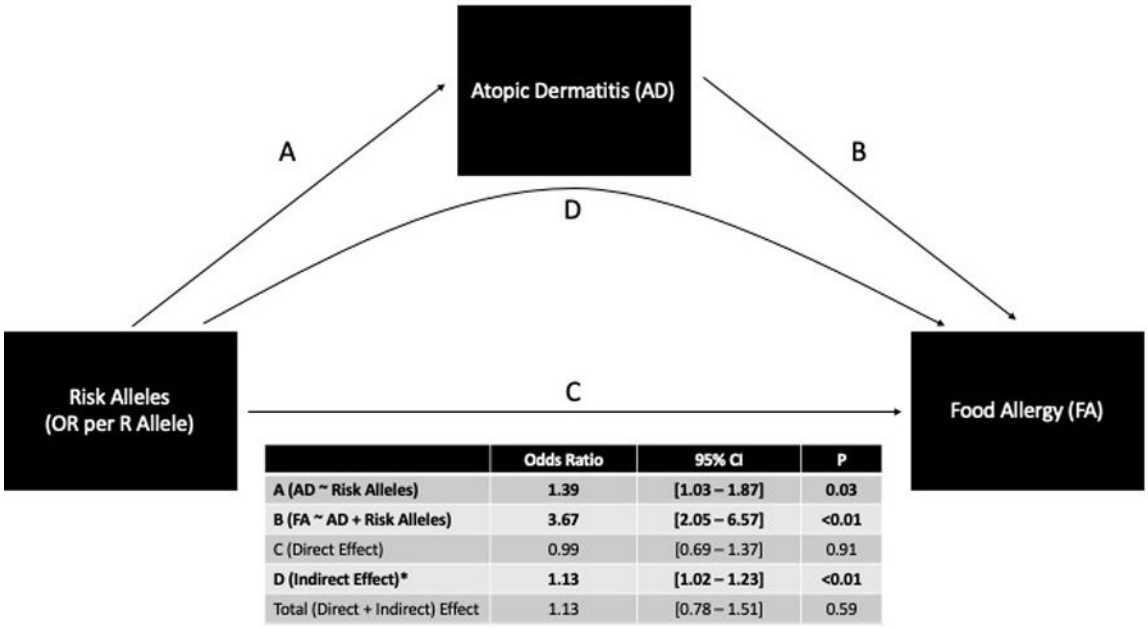
AD and FA likely share genetic risk factors. The *IL4RA*^{R576} variant has been associated with increased risk and severity of allergic diseases such as asthma.

What does this article add to our knowledge?

AD is part of the causal pathway between a common *IL4RA* variant and FA. *IL4RA*^{R576} is also associated with a higher risk of severe food allergy reactions. These results support the epicutaneous exposure hypothesis in the development of FA and identify a vulnerable subgroup of children at risk for severe food allergy reactions.

How does this study impact current management guidelines?

Disease modifying treatments for AD may concurrently reduce risk for future development of FA in children with genetic risk factors and warrant further investigation.



	Odds Ratio	95% CI	P
A (AD ~ Risk Alleles)	**1.39**	**[1.03 – 1.87]**	**0.03**
B (FA ~ AD + Risk Alleles)	**3.67**	**[2.05 – 6.57]**	**<0.01**
C (Direct Effect)	0.99	[0.69 – 1.37]	0.91
D (Indirect Effect)*	**1.13**	**[1.02 – 1.23]**	**<0.01**
Total (Direct + Indirect) Effect	1.13	[0.78 – 1.51]	0.59

Figure 1.

Mediation analysis predicting food allergy based on *IL4RA* genotype, mediated by atopic dermatitis.

Mediation analysis performed using two logistic regression models: atopic dermatitis (AD) predicted from genotype (*Q/Q* versus *Q/R* or *R/R*), and food allergy (FA) predicted from atopic dermatitis and genotype. All genotype results are relative to the *Q/Q* genotype. Patients with at least one *R* allele were more likely to have AD; having AD was strongly association with FA. Genotype was shown in increase odds of FA when mediated by AD (D, indirect effect; OR / risk allele = 1.13 [1.02 – 1.22], $P = <0.01$). Once the mediating effect of AD was removed, genotype was not shown to increase the odds of FA (C, direct effect; OR = 0.99 [0.78 – 1.51], $P = 0.59$). All models calculated using age, sex, income level (< $25,000) and race and ethnicity as potential covariates. Odds ratios and confidence intervals calculated using CMAverse package in R using 1000 bootstraps and missing data (income) addressed utilizing multiple imputation using ten imputations.

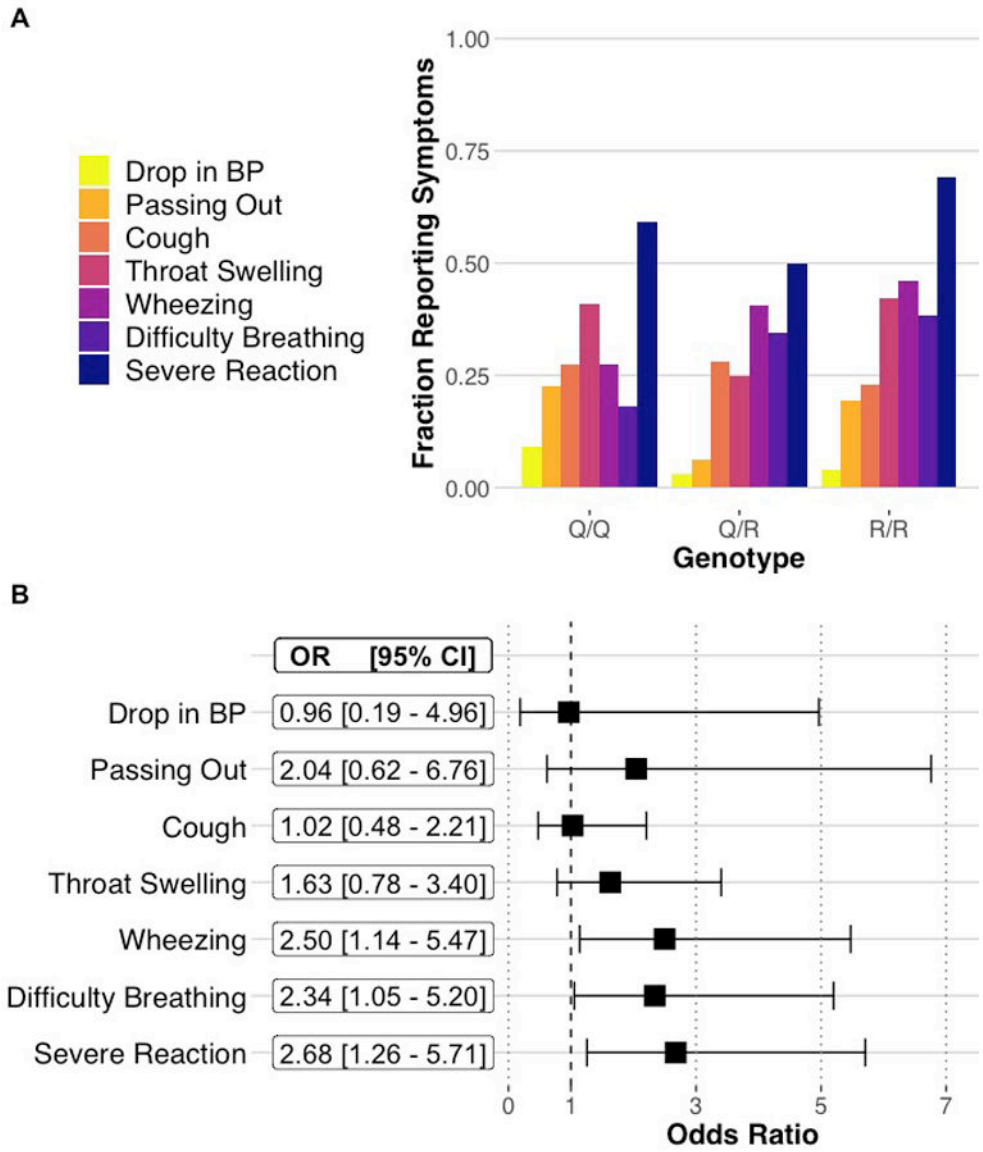


Figure 2.

Food Allergy Symptoms by *IL4RA* Genotypes.

(A) Unadjusted percent of patients with food allergy who reports various food allergy symptoms stratified by number of $IL4RA^{R576}$ alleles. Severe food allergy symptoms were defined in as the presence of any of the following symptoms: throat tightness, difficulty breathing, coughing, wheezing, drop in blood pressure or passing out. (B) The results of the multivariable logistic regression predicting food allergy symptoms based on presence of at least one risk allele (genotypes *Q/Q* versus *Q/R* or *R/R*). All models are adjusted for age, sex, income level (< $25,000) and race and ethnicity.

Table 1.

Baseline characteristics of study population. Summary statistic presented as N (%) or mean (SD)

Overall Cohort	**All (n=433)**	***QQ* (n=119)**	***QR* (n=186)**	***RR* (n=128)**	***P***
Age, mean (SD)	8.0 (1.9)	8.3 (1.9)	7.9 (2.0)	7.9 (1.8)	0.19
Female, n (%)	203 (47)	58 (49)	81 (44)	64 (50)	0.47
Race and ethnicity, n (%)					<0.001
Hispanic/Latino	192 (44)	68 (57)	87 (47)	37 (29)	
Black	130 (30)	15 (13)	55 (30)	60 (47)	
Other/mixed	80 (18)	18 (15)	34 (18)	28 (22)	
White	31 (7)	18 (15)	10 (5)	3 (2)	
Income <$25K[a], *n (%)*	145 (44)	33 (35)	59 (43)	53 (52)	0.05
Atopic dermatitis[b], *n (%)*	193 (45)	41 (35)	86 (47)	66 (52)	0.02
Food allergy[c], *n (%)*	80 (19)	22 (20)	32 (18)	26 (21)	0.73
Specific Food Allergies	**All (n=80)**	**QQ (n=22)**	**QR (n=32)**	**RR (n=26)**	**P**
Peanut, n (%)	39 (48.8)	8 (36.4)	19 (59.4)	12 (46.2)	0.24
Fish, n (%)	23 (28.7)	7 (31.8)	9 (28.1)	7 (26.9)	0.93
Fruits, n (%)	19 (23.8)	5 (22.7)	7 (21.9)	7 (26.9)	0.90
Grains, n (%)	16 (20)	5 (22.7)	8 (25)	3 (11.5)	0.41
Soy, n (%)	15 (18.8)	2 (9.1)	4 (12.5)	9 (34.6)	0.04
Eggs, n (%)	12 (15)	4 (18.2)	3 (9.4)	5 (19.2)	0.51
Tree nut, n (%)	11 (13.8)	2 (9.1)	2 (6.2)	7 (26.9)	0.06
Shellfish, n (%)	8 (10)	3 (13.6)	2 (6.2)	3 (11.5)	0.64
Vegetables, n (%)	6 (7.5)	4 (18.2)	0 (0)	2 (7.7)	0.05
Seeds, n (%)	5 (6.2)	3 (13.6)	2 (6.2)	0 (0)	0.15
Milk, n (%)	5 (6.2)	1 (4.5)	2 (6.2)	2 (7.7)	0.90
Meats, n (%)	3 (3.8)	1 (4.5)	0 (0)	2 (7.7)	0.30
Other, n (%)	2 (2.5)	1 (4.5)	1 (3.1)	0 (0)	0.58

[a] n=101 missing as participant's caregiver declined to answer questions about household income

[b] n=4 missing

[c] n=20 missing

Table 2.

Mediation analysis of risk alleles predicting food allergy mediated by atopic dermatitis.

Predictor = Number of R alleles (OR per R allele)			
	Odds Ratio	**95% CI**	***P***
Direct Effects			
Pure Natural Direct Effect	0.99	[0.69 – 1.37]	0.91
Total Natural Direct Effect	0.99	[0.70 – 1.37]	0.91
Controlled Direct Effect	0.99	[0.67 – 1.40]	0.91
Indirect (Mediated) Effects			
Pure Natural Indirect Effect	1.13	[1.02 – 1.24]	<0.01
Total Natural Indirect Effect	1.13	[1.02 – 1.23]	<0.01
Total Effect	1.13	[0.78 – 1.51]	0.59

All models controlled for possible confounding by age, sex, income and race and ethnicity.

Proportion mediated represents the proportion of the total effect that is the result of the indirect (mediated) effect.