Gene-environment interaction between an *IL4R* variant and school endotoxin exposure contributes to asthma symptoms in inner-city children

Peggy S. Lai, MD, MPH[a,b,c], **Amir H. Massoud, MD**[c,d], **Mingcan Xia, MD, PhD**[c,d], **Carter R. Petty, MA**[e], **Amparito Cunningham, MD, MPH**[c], **Talal A. Chatila, MD, MSc**[c,d], and **Wanda Phipatanakul, MD, MS**[c,d]

[a]Division of Pulmonary and Critical Care, Massachusetts General Hospital, Boston, Massachusetts

[b]Department of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, Massachusetts

[c]Harvard Medical School, Boston, Massachusetts

[d]Allergy and Immunology, Boston Children's Hospital, Boston, Massachusetts

[e]Institutional Centers for Clinical and Translational Research, Boston Children's Hospital, Boston, Massachusetts

Keywords

asthma; gene-environment interaction; endotoxin; school; health disparities; Q551R; Q576R

To the Editor

Gene-environment interactions are thought to play a critical role in determining asthma incidence and severity. A human interleukin 4 receptor alpha chain gene (*IL4R*) variant that results in a glutamine to arginine substitution at amino acid residue 576 (IL4Rα-Q576R polymorphism) is associated with asthma diagnosis and severity [1]. We have recently shown that the IL4Rα[R576] variant promotes severe airway inflammation by uniquely linking the IL4R to a growth-factor-receptor-bound protein 2 (GRB2)-dependent intracellular signaling pathway that destabilizes allergen-specific regulatory T cells to drive mixed T_H2/T_H17 cell inflammation [2]. School endotoxin exposure is associated with asthma morbidity [3]. High endotoxin exposure is associated with increased T_H17 cell skewing [4], leading us to test the

hypothesis that there is an interaction between the IL4Rα-Q576R polymorphism, school endotoxin exposure, and asthma symptoms.

The School Inner-city Asthma Study (SICAS) is a single-center prospective cohort study conducted between 2008 and 2013 of children with persistent asthma attending inner-city elementary schools in a northeastern United States city (Online Repository). Repeated assessment of asthma symptom days over a 14-day period was assessed by parental or caretaker survey at three month intervals. One-week classroom air samples were collected twice during the school year using charged particle samplers (Quadra; Sharper Image/ Camelot Venture Group); endotoxin levels were assessed using the Limulus Amebocyte Lysate assay [3]. Genotyping of the $IL4R^{Q576}$ and $IL4R^{R576}$ alleles was carried out using the amplification resistance mutation screen PCR method [2] on DNA was extracted from either whole blood (Gentra Puregene Blood Kit; Qiagen) or saliva (prepIT L2P; DNA Genotek).

The IL4Rα-Q576R genotypes were modeled as a three-group categorical variable (*Q/Q*, *Q/R*, or *R/R* genotypes, with the *Q/Q* genotype as the reference group). To test the hypothesis that the genotypes differed by self-reported race, Fisher's exact tests were performed. To test the hypothesis that a gene-environment interaction was present, a multiplicative term between classroom endotoxin levels (continuous) and genotype (3-level categorical variable) was included in binomial family generalized estimating equations with a logit link and an overdispersion parameter. These models included only observations during the school year, and adjusted for age, gender, self-reported race, allergic sensitization, baseline asthma severity, use of asthma controller medications, annual income, and season (using linear and quadratic terms for days since school start). All statistical analyses were performed in Stata version 13.1 (StataCorp). Two-sided p-values of <0.05 were considered statistically significant for main effects; p-values of <0.10 were considered statistically significant for interactions.

Baseline characteristics of the 236 children included in the analysis are depicted in Table 1; 51.7% were male, the average age was 8.0 ± 1.9 years, and 5.1% self-identified as being Caucasian. The distribution of genotypes differed by race; 75.0%, 13.1%, and 31.7% of Caucasian, African-American, and Hispanic children had the *Q/Q* genotype, whereas 0%, 47.6%, and 14.6% of Caucasian, African-American, and Hispanic children had the *R/R* genotype (p < 0.001). The frequency of the *Q* allele was highest in children of Caucasian race, whereas the frequency of the *R* allele was the highest in children of African-American or mixed race (Supplementary Table 1). Median measured classroom endotoxin levels were 27.5 EU/m^3 [interquartile range 7.4 – 71.8 EU/m^3]. In univariate analyses, the main effects of the genotype and of classroom endotoxin levels on asthma symptoms did not reach statistical significance. In a multivariate model, the effect of classroom endotoxin levels on asthma symptom days varied by genotype (overall interaction p-value = 0.09; pairwise interaction p-values for *Q/R* vs. *Q/Q* = 0.115, *R/R* vs. *Q/Q* = 0.049). Higher classroom endotoxin levels were associated with fewer asthma symptoms for children with the *Q/Q* genotype (OR 0.76 [0.55 – 1.04]), an equivocal effect for the *Q/R* genotype (OR 1.10 [0.78 – 1.55]), and increased asthma symptoms for the *R/R* genotype (OR 1.34 [0.85 – 2.13], Figure 1, Supplementary Table 2).

In this study, we identify a gene-environment interaction associated with pediatric asthma symptoms. Few gene-environment interaction studies involving the IL4Rα-Q576R polymorphism have been performed. In a humanized mouse model of the IL4Rα-R576 variant (*Il4ra*R576 mice), exposure of the mice to fine and ultrafine particles from vehicular exhaust exacerbated allergic inflammation in association with augmented mixed Th2/Th17 airway cell responses [5], demonstrating the plausibility that interactions between this allele and environmental exposures contribute to asthma morbidity. Similarly, an exaggerated mixed Th2-Th17 cell inflammation may underlie the interaction between the IL4Rα-R576 variant and endotoxin exposure.

Of note, high endotoxin exposure in the presence of the *Q/Q* genotype was associated with a protective effect against asthma symptoms. A pro-con effect of endotoxin exposure on asthma has been well described in epidemiologic studies. For example, Tischer *et al* [6], in an analysis of three large European birth cohorts, reported a protective effect of early life endotoxin exposure on later asthma development in the Spanish cohort, an equivocal effect in the German cohort, and a harmful effect in the Dutch cohort. Our findings support the possibility that the *IL4R Q* versus *R* variants influence the outcome of endotoxin exposure on asthma.

Our study has a few limitations. Our sample size was modest, thus we had limited power to detect associations, and we were not able to account for possible confounding due to population stratification by performing stratified analyses using self-reported race. We did not validate our findings in an independent cohort, and there is linkage disequilibrium between the polymorphism studied and other *IL4R* polymorphisms; thus, our findings are preliminary and would need to be verified in larger studies. However, it has been suggested that gene-environment interactions contribute to racial disparities in asthma outcomes [7]. United States poverty rates are highest in ethnic minorities [8]; a prior nationwide study found that poverty is associated with higher home endotoxin exposure [9]. It is possible that the gene-environment interaction we observed contributes to racial disparities in pediatric asthma. While it may be difficult to address individual home environmental conditions, a public health initiative addressing the environment of inner-city schools, where there is a high proportion of ethnic minorities, may improve observed disparities in asthma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

REFERENCES CITED

1. Rosa-Rosa L, Zimmermann N, Bernstein JA, Rothenberg ME, Khurana Hershey GK. The R576 IL-4 receptor alpha allele correlates with asthma severity. J Allergy Clin Immunol. 1999; 104(5): 1008–14. [PubMed: 10550746]

2. 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. Nat Med. 2016; 22(9):1013–22. [PubMed: 27479084]

3. Lai PS, Sheehan WJ, Gaffin JM, Petty CR, Coull BA, Gold DR, Phipatanakul W. School Endotoxin Exposure and Asthma Morbidity in Inner-city Children. Chest. 2015; 148(5):1251–8. [PubMed: 26087201]

4. Glader P, Smith ME, Malmhall C, Balder B, Sjostrand M, Qvarfordt I, Linden A. Interleukin-17-producing T-helper cells and related cytokines in human airways exposed to endotoxin. Eur Respir J. 2010; 36(5):1155–64. [PubMed: 20185422]

5. Xia M, Viera-Hutchins L, Garcia-Lloret M, Noval Rivas M, Wise P, McGhee SA, Chatila ZK, Daher N, Sioutas C, Chatila TA. Vehicular exhaust particles promote allergic airway inflammation through an aryl hydrocarbon receptor-notch signaling cascade. J Allergy Clin Immunol. 2015; 136(2):441–53. [PubMed: 25825216]

6. Tischer C, Casas L, Wouters IM, Doekes G, Garcia-Esteban R, Gehring U, Hyvarinen A, Oldenwening M, Kerkhof M, Sunyer J, Standl M, Thiering E, Torrent M, Heinrich J. group Hs. Early exposure to bio-contaminants and asthma up to 10 years of age: results of the HITEA study. Eur Respir J. 2015; 45(2):328–37. [PubMed: 25186271]

7. Forno E, Celedon JC. Asthma and ethnic minorities: socioeconomic status and beyond. Curr Opin Allergy Clin Immunol. 2009; 9(2):154–60. [PubMed: 19326508]

8. Macartney, S., Bishaw, A., Fontenot, K. Poverty Rates for Selected Detailed Race and Hispanic Groups by State and Place: 2007–2011. U.S. Census Bureau; 2013. Contract No.: Document Number|

9. Thorne PS, Mendy A, Metwali N, Salo P, Co C, Jaramillo R, Rose KM, Zeldin DC. Endotoxin Exposure: Predictors and Prevalence of Associated Asthma Outcomes in the United States. Am J Respir Crit Care Med. 2015; 192(11):1287–97. [PubMed: 26258643]
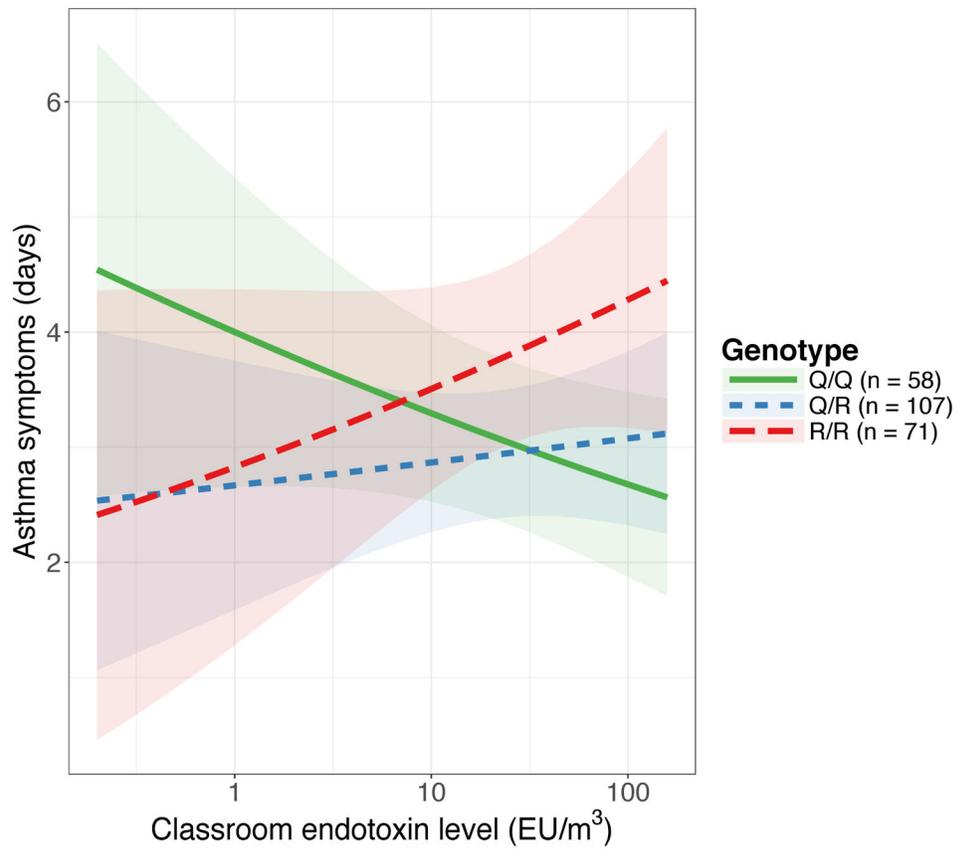
Figure 1.
Predicted asthma symptom days per two-week period associated with classroom endotoxin levels (log-transformed) stratified by IL4Rα-Q576R genotype demonstrates a gene by environment (G×E) interaction. The overall interaction p-value = 0.09; pairwise interaction p-values for *Q/R* vs. *Q/Q* = 0.115, *R/R* vs. *Q/Q* = 0.049.

Table 1

Baseline characteristics of study participants obtained during initial study visit, overall and stratified by IL4Rα-Q576R genotype. P-values represent univariate testing between genotype. Distribution of genotype differs by self-reported race, p < 0.001.

Characteristic	Overall	Q/Q genotype	Q/R genotype	R/R genotype	p-value
n	236	58	107	71	
Age in years (mean ± sd)	7.96 ± 1.91	8.00 ± 1.84	7.84 ± 2.01	8.11 ± 1.82	0.641
Male gender, n (%)	122 (51.7%)	27 (46.6%)	62 (57.9%)	33 (46.5%)	0.216
Self-reported race, n (%)					<0.001
Caucasian	12 (5.1%)	9 (15.5%)	3 (2.8%)	0 (0.0%)	
African-American	84 (35.6%)	11 (19.0%)	33 (30.8%)	40 (56.3%)	
Hispanic	82 (34.7%)	26 (44.8%)	44 (41.1%)	12 (16.9%)	
Other	42 (17.8%)	11 (19.0%)	19 (17.8%)	12 (16.9%)	
Mixed	16 (6.8%)	1 (1.7%)	8 (7.5%)	7 (9.9%)	
Annual income >$25,000, n (%)	104 (44.1%)	30 (51.7%)	46 (43.0%)	28 (39.4%)	0.359
Family history of asthma, n (%)	187 (79.2%)	43 (74.1%)	88 (82.2%)	56 (78.9%)	0.470
Physician diagnosis of hayfever/allergic rhinitis, n (%)	35 (14.9%)	7 (12.3%)	16 (15.0%)	12 (16.9%)	0.766
Physician diagnosis of eczema, n (%)	108 (45.8%)	19 (32.8%)	52 (48.6%)	37 (52.1%)	0.065
Allergic sensitization [a] (%)	159 (67.4%)	40 (69.0%)	77 (72.0%)	42 (59.2%)	0.195
Asthma severity [b] (%)					0.742
mild	20 (8.5%)	2 (3.4%)	11 (10.3%)	7 (9.9%)	
intermittent	47 (19.9%)	10 (17.2%)	21 (19.6%)	16 (22.5%)	
moderate	69 (29.2%)	18 (31.0%)	31 (29.0%)	20 (28.2%)	
severe	100 (42.4%)	28 (48.3%)	44 (41.1%)	28 (39.4%)	
Controller medication [c] use, n (%)	129 (54.7%)	30 (51.7%)	59 (55.1%)	40 (56.3%)	0.864
Use of rescue inhaler > 1x/week (%)	51 (21.6%)	11 (19.0%)	21 (19.6%)	19 (26.8%)	0.449
Asthma symptom days (mean ± sd) [d]	2.86 ± 4.09	2.60 ± 4.05	2.79 ± 4.05	3.18 ± 4.20	0.703

[a] Allergic sensitization was defined as any positive skin prick test to 14 common aeroallergens or specific IgE level   0.35kU/L on serologic testing

[b] Asthma severity is graded based on recommendations from the National Asthma Education and Prevention Program, Third Expert Panel on the Diagnosis and Management of Asthma.

[c] Controller medications are defined as use of an inhaled corticosteroid or leukotriene modifier, as ascertained by review of active prescriptions during baseline study visit.

[d] Number of days with asthma symptoms noted by parent or caretaker in 14 days prior to initial clinic visit