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An interaction of the 17q12-21 locus with mold exposure in childhood asthma

To the Editor:

A plethora of large-scale genome-wide association studies have shown that the chromosome 17q12-21 locus is strongly and specifically associated with childhood asthma, but not with adult-onset asthma, and have highlighted that this locus interacts with early-life environmental exposures such as environmental tobacco smoke (ETS) and viral respiratory infection.^{1,2} However, these interaction findings did not fully replicate in different populations despite the consistent replication of the 17q12-21 region itself in patients with childhood asthma of various ethnicities.³ Moreover, asthma can be triggered and exacerbated by various environmental factors such as indoor and outdoor allergens, which have not been studied for interaction with this locus.³

Thus, we sought to comprehensively investigate the impact of various environmental exposures and clinical variables on the effect of rs8067378 on asthma risk, which was shown to be a risk single nucleotide polymorphism (SNP) for childhood asthma in the 17q12-21 region, based on over 7000 subjects across three populations.⁴

For our analysis, we selected asthmatic offspring trios from the family-based "Genetic Epidemiology of Asthma in Costa Rica" Study (GACRS).⁵ The corresponding whole-genome sequencing (WGS) data for SNP rs8067378 were generated as part of the National Heart, Lung, and Blood Institute Trans-Omics for Precision Medicine Program.⁶

REFERENCES

1. Global Initiative for Asthma. Global strategy for asthma management and prevention, 2019. Available from: www.ginasthma.org
2. Tan WC, Vollmer WM, Lamprecht B, Mannino DM, Jithoo A, Nizankowska-Mogilnicka E et al Worldwide patterns of bronchodilator responsiveness: results from the Burden of Obstructive Lung Disease study. *Thorax*. 2012;67(8):718–26.
3. Galant SP, Morphew T, Amaro S, Liao O. Value of the bronchodilator response in assessing controller naive asthmatic children. *J Pediatr*. 2007;151(5):457–62, 62 e1.
4. Tse SM, Gold DR, Sordillo JE, Hoffman EB, Gillman MW, Rifas-Shiman SL et al Diagnostic accuracy of the bronchodilator response in children. *J Allergy Clin Immunol*. 2013;132(3):554–559.e5.
5. Silva D, Severo M, Paciencia I, Rufo J, Martins C, Moreira P et al Setting definitions of childhood asthma in epidemiologic studies. *Pediatr Allergy Immunol*. 2019;30(7):708–15.
6. Billington CK, Penn RB, Hall IP. beta2 Agonists. *Handb Exp Pharmacol*. 2017;237:23–40.
7. Choudhry S, Ung N, Avila PC, Ziv E, Nazario S, Casal J et al Pharmacogenetic differences in response to albuterol between puerto ricans and mexicans with asthma. *Am J Respir Crit Care Med*. 2005;171(6):563–70.
8. Kavanagh J, Jackson DJ, Kent BD. Over- and under-diagnosis in asthma. *Breathe*. 2019;15(1):e20–e27.

Among the data collected by the protocol for GACRS, a total of 46 dichotomized clinical variables were selected for the interaction analysis and were classified into five categories: ① Birth condition and sex: bodyweight (>2.27 kg), breast feeding, prematurity, and sex; ② Allergy and allergen reactivity: doctor-diagnosed hay fever, doctor-diagnosed eczema, any positive skin test (>3mm), positive skin test to cat, dog, *D pteronyssinus*, *D farina*, *Alternaria tenuis*, *P americana*, *B germanica*, mixed grasses, and mixed trees, high eosinophil count (>500 cells), high total IgE (>100 IU/mL), and positive IgE to *Ascaris*, cockroach, and dust mite; ③ Family history: mother or father history of asthma, chronic bronchitis, eczema, and hay fever; ④ Residential environment: child shared room with others, daycare in the first year of life, humidity signs, mold or mildew signs, house animal, ETS at pregnancy, ETS at infancy, and ETS at present; and ⑤ Asthma symptom triggers: cold air, cold or flu, exercise, cat, dog, other animal, house dust, moldy places, and sleep. Our analyses were based on the family-based association test (FBAT) software (version 2.04) using the additive test model and the FBAT-Interaction (FBAT-I) methodology (R package fbati). The FBAT-I statistic accounts for the genetic main effect and tests for interaction of the genotype with the specified environmental variable.⁷ Clinical features were compared between groups, according to the exposure, using t-tests or chi-square tests, as appropriate.

TABLE 1 Demographic characteristics and clinical features of study subjects (N = 894), and comparison between groups according to moldy place trigger factor. Mean (SD) or N (%) is shown

	Costa Rican Study subjects	Is moldy place trigger factor?		<i>p</i> -value
		No	Yes	
N	894	186 (20.81)	671 (75.06)	
Age	9.28 ± 1.86	9.33 ± 1.91	9.28 ± 1.85	.7531
Sex (male)	528 (59.06)	111 (59.68)	399 (59.46)	1
Height, m	1.33 ± 0.12	1.33 ± 0.11	1.33 ± 0.12	.8224
Body mass index, kg/m ²	18.45 ± 3.92	18.20 ± 3.99	18.59 ± 3.94	.2297
No. of older siblings	1.14 ± 1.31	1.15 ± 1.33	1.13 ± 1.29	.8279
Spirometric measure				
FEV ₁ , L	1.80 ± 0.52	1.82 ± 0.54	1.80 ± 0.51	.6017
FEV ₁ (% of predicted)	98.87 ± 16.84	98.89 ± 15.91	98.79 ± 16.97	.9387
FEV ₁ /FVC, %	84.36 ± 7.79	84.48 ± 7.61	84.27 ± 7.84	.7376
FEV ₁ /FVC (% of predicted)	94.87 ± 8.70	95.00 ± 8.55	94.79 ± 8.75	.7714
Bronchodilator response as % of baseline FEV1	5.58 ± 10.20	5.01 ± 8.32	5.65 ± 10.54	.3841
Log ₁₀ Dose response slope from saline	1.15 ± 0.54	1.12 ± 0.57	1.16 ± 0.53	.4022
Blood tests				
Total serum IgE, IU/mL	725.26 ± 898.96	632.62 ± 844.50	761.11 ± 925.26	.0745
Positive IgE to dust mite	663 (74.16)	131 (70.43)	507 (75.56)	.2115
Positive IgE to cockroach	387 (43.29)	71 (38.17)	304 (45.31)	.1066
Positive IgE to Ascaris	334 (37.36)	58 (31.18)	262 (39.05)	.0725
At least 1 positive specific IgE	707 (79.08)	140 (75.27)	540 (80.48)	.1720
Eosinophil, count/mm ²	553.84 ± 403.93	535.03 ± 391.80	558.61 ± 405.59	.4757
No. of positive skin tests to allergens	3.08 ± 1.82	2.73 ± 1.82	3.19 ± 1.80	.0027
Household mold or mildew exposure	549 (61.41)	91 (48.92)	438 (65.28)	4.80 × 10 ⁻⁵
ETS exposure	217 (24.27)	41 (22.04)	165 (24.59)	.5545

Note: Data are presented as mean ± standard deviation or numbers (percentages).

Abbreviations: FEV1, forced expiratory volume in the first one second; FVC, forced vital capacity; IgE, immunoglobulin E; ETS, environmental tobacco smoke.

The statistical software R (<http://www.R-project.org>) was used to evaluate these tests.

After confirming the pedigree structure using KING (Kinship-Based INference for Genome-wide Association Studies), we kept 894 GACRS asthmatic trios for the analysis.⁸ In the 17q12-21 region, rs8067378 with a SNP missing rate of zero, no Mendelian errors, and no significant Hardy-Weinberg deviation (HWE, $p = .698$) showed genome-wide significance ($p = 7.38 \times 10^{-10}$) in the FBAT analysis, using asthma affection status as the phenotype. The characteristics of the study subjects are summarized in Table 1.

"A moldy place as asthma symptom trigger" showed the most significant interaction ($p = 6.66 \times 10^{-4}$, Figure 1) that remained significant after the Bonferroni correction ($\alpha = 0.05$) for a total of 46 tests, that is $p < 0.001$. With respect to this binary variable, both groups in the GACRS were well-balanced regarding the demographic factors, lung function, smoking exposure, and other clinical variables, except for household mold exposure and the number of allergens in the skin prick tests (Table 1). To dissect the interaction, we considered

the following subgroup analysis. We performed the standard FBAT association test for asthma affection in the "yes" and the "no" group separately. In the "yes" group, we obtained a highly significant p -value of $p = 6.67 \times 10^{-12}$, whereas in the "no" group the p -value was not significant ($p = .824$). Based on the other categories, including ETS and family history, there was no significant interaction after the Bonferroni correction (Figure 1).

To our knowledge, there are no interaction studies of mold exposure with the 17q12-21 locus. However, evidence has been accumulated that infant exposure to mold correlates with childhood asthma development after controlling for other potential risk factors.⁹ Interestingly, not household mold exposure ($p = .351$), but mold exposure as a trigger factor of respiratory symptoms showed a significant interaction with the 17q12-21 region in our analysis. The questionnaire for assessment of household mold exposure has limitations because parents were asked about housemold mold exposure during the last 12 months, contrary to the questionnaire about asthma symptom triggers since birth. Moreover, outdoor exposure is generally more relevant in terms of sensitization and disease

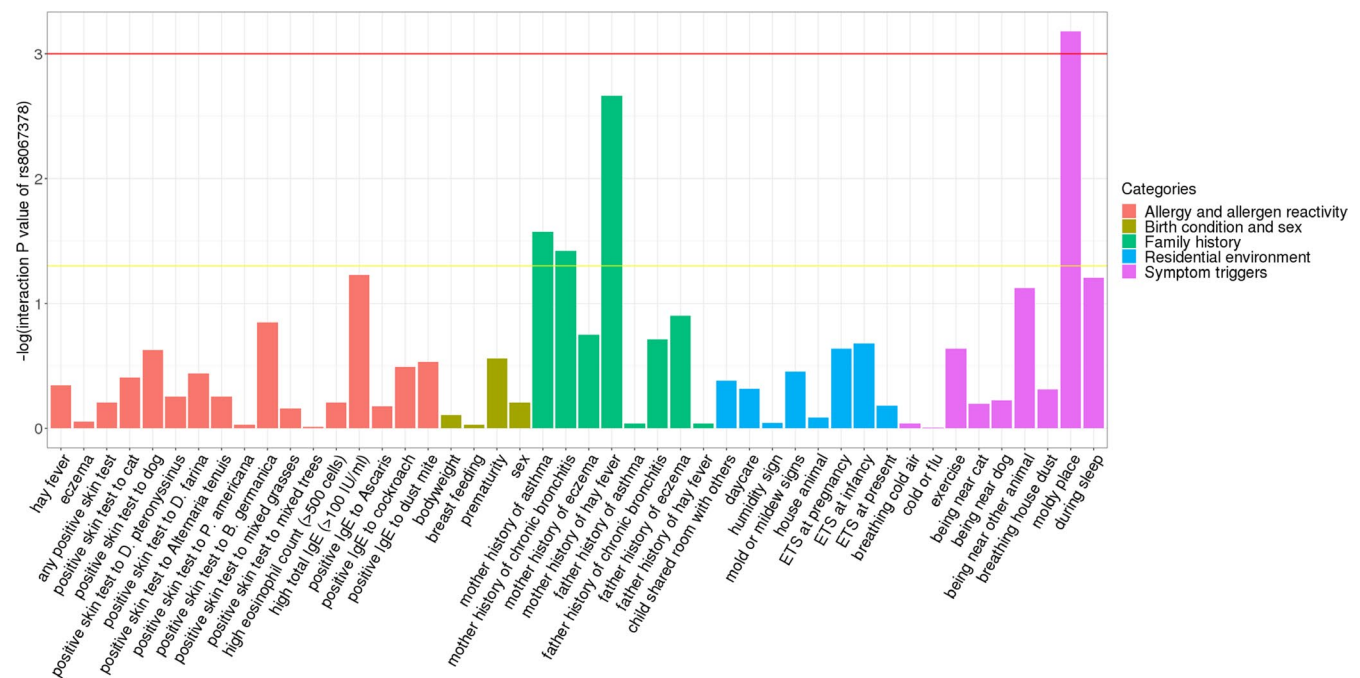


FIGURE 1 Family-Based Association Test-Interaction (FBAT-I) p-values for rs8067378 across all 46 clinical variables classified into five categories. The red line represents the Bonferroni-corrected threshold ($\alpha = 0.05$) for 46 tests, that is a p-value of $p = .001$, and the yellow line represents a p-value of $p = .05$

expression compared to indoor exposure.¹⁰ Therefore, we suggest that the question about a trigger captures the mold exposure better than parental report of household mold or mildew exposure.

An interaction of ETS exposure within the 17q12-21 locus, that has been previously reported, was not found in our study. However, in our analysis, ETS information was based on parental self-reporting of smoking behavior which could weaken the effect due to reporting errors. On the other hand, the interaction with mother's history of hay fever was significant ($p = 2.17 \times 10^{-3}$ in Figure 1), while the father's history was not ($p = .914$). This is a potential support of the result in a previous meta-analysis that maternal asthma history increases offspring asthma risk to a greater extent than paternal one.¹¹

It is important to consider the strengths and potential limitations of our work. Our study design, including only affected trios, overcomes spurious findings as a result of confounding factors such as misspecified environmental main effects and population stratification. Our analysis utilized the FBAT-I methodology with an additive model and 10^6 Monte-Carlo iterations, while previous gene-environment interaction analyses were based on the likelihood ratio test.¹ We considered only the SNP rs8067378 within the 17q12-21 region but linkage disequilibrium is very strong between SNPs at the locus, with an extended haplotype spanning the entire core region in populations of European, Latinx, and East Asian ancestry.³ However, this approach also might ignore other SNPs that are associated with childhood asthma independently of rs8067378, but the identification of causal variants within the locus is still incomplete. Further research is needed to address this issue. Finally, childhood

asthma has heterogenic subtypes such as atopic or non-atopic, and this could weaken the association and interaction with rs8067378 in our analysis purely based on asthma affection status. In conclusion, our interaction analysis of the 17q12-21 locus discovers a new significant factor, the mold exposure as a trigger factor. We hope that this finding fosters a better understanding of the pathophysiology underlying childhood asthma.

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CONFLICT OF INTEREST

Dr Celedón has received research materials from Merck and GSK (inhaled steroids) and Pharmavite (vitamin D and placebo capsules),

in order to provide medications free of cost to participants in NIH-funded studies, unrelated to the current work. The other authors have nothing to disclose.

AUTHOR CONTRIBUTION

Sanghun Lee: Conceptualization (lead); Writing-original draft (lead). **Jessica Lasky-Su:** Writing-review & editing (supporting). **Wonji Kim:** Data curation (supporting). **Sungho Won:** Conceptualization (supporting). **Cecelia Laurie:** Data curation (supporting). **Juan Celedón:** Writing-review & editing (supporting). **Christoph Lange:** Conceptualization (supporting); Funding acquisition (supporting); Supervision (supporting); Writing-review & editing (supporting). **Scott T. Weiss:** Funding acquisition (supporting); Writing-review & editing (supporting). **Julian Hecker:** Data curation (supporting); Investigation (supporting); Methodology (supporting); Supervision (supporting); Writing-review & editing (supporting).

PEER REVIEW

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REFERENCES

- Bouzigon E, Corda E, Aschard H, et al. Effect of 17q21 variants and smoking exposure in early-onset asthma. *N Engl J Med*. 2008;359(19):1985-1994.
- Çalışkan M, Bochkov YA, Kreiner-Møller E, et al. Rhinovirus wheezing illness and genetic risk of childhood-onset asthma. *N Engl J Med*. 2013;368(15):1398-1407.
- Stein MM, Thompson EE, Schoettler N, et al. A decade of research on the 17q12-21 asthma locus: Piecing together the puzzle. *J Allergy Clin Immunol*. 2018;142(3):749-764.
- Moffatt MF, Kabesch M, Liang L, et al. Genetic variants regulating ORMDL3 expression contribute to the risk of childhood asthma. *Nature*. 2007;448(7152):470-473.
- Hunninghake GM, Soto-Quiros ME, Avila L, et al. Sensitization to *Ascaris lumbricoides* and severity of childhood asthma in Costa Rica. *Journal of Allergy and Clinical Immunology*. 2007;119(3):654-661. <http://dx.doi.org/10.1016/j.jaci.2006.12.609>
- Taliun D, Harris DN, Kessler MD, Carlson J, Szpiech ZA. Sequencing of 53,831 diverse genomes from the NHLBI TOPMed Program. *J bioRxiv*. 2019;563866:1. <https://www.biorxiv.org/content/10.1101/563866v1>
- Hoffmann TJ, Lange C, Vansteelandt S, Laird NM. Gene-environment interaction tests for dichotomous traits in trios and sibships. *Genet Epidemiol*. 2009;33(8):691-699.
- Manichaikul A, Mychaleckyj JC, Rich SS, Daly K, Sale M, Chen W. Robust relationship inference in genome-wide association studies. *Bioinformatics*. 2010;26(22):2867-2873. <http://dx.doi.org/10.1093/bioinformatics/btq559>
- Reponen T, Lockey J, Bernstein DI, et al. Infant origins of childhood asthma associated with specific molds. *J Allergy Clin Immunol*. 2012;130(3):639-644.
- Bush RK, Portnoy JM, Saxon A, Terr AI, Wood RA. The medical effects of mold exposure. *Journal of Allergy and Clinical Immunology*. 2006;117(2):326-333. <http://dx.doi.org/10.1016/j.jaci.2005.12.001>
- Lim RH, Kobzik L, Dahl M. Risk for asthma in offspring of asthmatic mothers versus fathers: a meta-analysis. *PLoS One*. 2010;5(4):e10134.