Fungal Exposure, Atopy, and Asthma Exacerbations in Puerto Rican Children

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Abstract

Background: Glucan is a component of the fungal cell wall that is used as a marker of fungal exposure. Little is known about indoor glucan, atopy, and asthma exacerbations among children living in tropical environments such as Puerto Rico. Our objective was to examine whether glucan exposure is associated with degree of atopy or visits to the emergency department (ED)/urgent care for asthma in Puerto Rican children.

Methods: This was a cross-sectional study of 317 children aged 6 to 14 years with (cases, n = 160) and without (control subjects, n = 157) asthma in San Juan, Puerto Rico. Our primary outcomes were the number of positive skin tests to allergens (range, 0–15) and (in cases only) having had at least one visit to the ED/urgent care for asthma in the prior year. Levels of glucan, endotoxin, peptidoglycan, and five allergens (Der p 1, Bla g 2, Fel d 1, Can f 1, and Mus m 1) were measured in samples of house dust. Linear or logistic regression was used for the multivariate analysis.

Measurements and Main Results: In a multivariate analysis adjusting for case-control status, mouse allergen, and other covariates, children exposed to glucan levels in the second and third quartiles had approximately two more positive skin tests than those in the lowest quartile ($P < 0.01$ in both instances). Among children with asthma, exposure to the highest quartile of glucan was associated with nearly ninefold greater odds of one or more visits to the ED/urgent care for asthma (95% confidence interval for adjusted odds ratio, 2.7–28.4; $P < 0.001$).

Conclusions: Our results suggest that indoor fungal exposure leads to an increased degree of atopy and visits to the ED/urgent care for asthma in Puerto Rican children.

Keywords: fungi; asthma attacks; Puerto Ricans; children

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Fungal sensitization is increasingly recognized as a marker of asthma severity (1, 2), and recent findings suggest that antifungal treatment benefits subjects with severe asthma and fungal sensitization (but no allergic bronchopulmonary aspergillosis), with stronger evidence in adults (3) than in children (4). To date, the relation between fungal exposure and asthma or atopy is unclear.

Indoor fungal exposure can be assessed by questionnaires or, more directly, by measuring individual fungal species or β-D-glucan (which accounts for approximately half the weight of the fungal cell wall) in house dust (5–7). Glucan has been associated with lower risk of recurrent wheezing (8) in infancy and eczema at school age (9). On the other hand, glucan has been associated with lower respiratory illnesses in infancy (10), greater peak

flow variability at school age (11), and lower lung function in adulthood (12), and five studies reported no association between glucan and allergic diseases or atopy in childhood (13–16) or adulthood (12).

Childhood asthma is a major public health burden in Puerto Rico (17). Asthma exacerbations are also common in Puerto Rican children (17), in whom an increased degree of atopy is associated with greater asthma severity (18, 19).

Fungal spores are the most common outdoor biological particulates in Puerto Rico (20), and outdoor fungal concentrations are typically correlated with those of indoor fungi (21). Although indoor fungal exposure may contribute to atopy or asthma exacerbations in children living in tropical environments, no study has examined this question in Puerto Rico or elsewhere. We examined the relation between indoor glucan and the degree of atopy or visits to the emergency department (ED)/urgent care for asthma in 317 children living in San Juan, Puerto Rico.

Methods

Subject Recruitment

From March 2009 to June 2010, children in San Juan were recruited from randomly selected households, as previously described (10, 11). In brief, households were selected using a multistage probability sample design. Primary sampling units (PSUs) were randomly selected neighborhood clusters based on the 2000 U.S. Census, and secondary sampling units were randomly selected households within each primary sampling unit. On the basis of this design, 7,073 households were selected, and 6,401 (90.5%) were contacted: 1,111 households had one or more children who met inclusion criteria (age 6–14 yr, four Puerto Rican grandparents, and residence in the same household for $\geq$1 yr). Of these 1,111 households, 438 (39.4%) had one or more children with asthma (a case, defined as having physician-diagnosed asthma and wheeze in the prior year). From these 438 households, one child with asthma was selected (at random if there was more than one such child). Similarly, only one child without asthma (a control subject, having neither physician-diagnosed asthma nor wheeze in the prior year) was randomly selected from the remaining 673 households. To reach our target sample size ($\sim$700 children overall) we attempted to enroll 783 of the 1,111 eligible children. Parents of 105 (13.4%) of these 783 children refused to participate or could not be reached, leaving 678 participants (351 cases and 327 control subjects). There were no significant differences in age, sex, or area of residence between eligible children who did (n = 678) and did not (n = 105) agree to participate. After measuring a number of allergens in the dust collected from the homes of participants, there remained leftover dust to measure glucan from homes of 317 (46.8%) participants, who were thus included in the current analysis.

Study Procedures

Study participants completed a protocol that included a questionnaire on respiratory and general health (22), spirometry, allergy skin testing, and collection of dust samples.

Spirometry was conducted with the EasyOne spirometer (NDD Medical Technologies, Andover, MA). Subjects had to be free of respiratory infections for 4 or more weeks before testing, and they were also instructed (when possible) to avoid use of inhaled short- and long-acting bronchodilators for 4 or more and 12 or more hours before testing, respectively. Expiratory maneuvers were judged acceptable if they met or exceeded American Thoracic Society criteria for children (23). The best FEV_1 and FVC were selected for analysis.

A global dust sample was obtained by combining dust collected from three areas in the home: the one in which the child sleeps (where a mattress is located, usually the bedroom), the living room/television room, and the kitchen. The dust was sifted through a 50-mesh metal sieve. The fine dust was reweighed, extracted, and aliquoted for analysis of five allergens (cockroach [Bla g 2], dog [Can f 1] and cat [Fel d 1] dander, dust mite [Der p 1], and mouse urinary protein [Mus m 1]) using monoclonal antibody Multiplex array assays that used the same reagents as in the established ELISA (24).

After measuring allergens, glucan was measured in leftover dust. Sieved dust samples were weighed and aliquoted in a gravimetrics laboratory with a filtered air supply and precise temperature and humidity control. Samples were weighed to the nearest microgram using a dual chamber microbalance (Model XP26; Mettler, Columbus, OH). To extract (1→3, 1→6)-β-D-glucan, samples were mixed with phosphate-buffered saline and then autoclaved and centrifuged. These samples were subsequently analyzed by a sandwich ELISA using anti-(1→3, 1→6)-β-D-glucan monoclonal antibody for capture, followed by administration of a rabbit antiscleroglucan polyclonal antibody (25). Endotoxin was measured using the kinetic chromogenic Limulus amebocyte lysate assay, using multiple dilutions and a 12-point standard curve based on control standard endotoxin from *Escherichia coli* ranging from 0.0244 to 50.0 EU/ml (26). Peptidoglycan was extracted from samples using an ELISA with monoclonal antibodies that bind soluble bacterial peptidoglycan (27). High-affinity receptor was used as a capture reagent, and murine monoclonal antibodies to peptidoglycan were used for detection. Galactosyl ceramide dissolved in alcohol was used for coating, and buffered bovine serum albumin was used for blocking.

Skin test reactivity to aeroallergens was assessed using a Multi Test device (Lincoln Diagnostics, Decatur, IL). In addition to histamine and saline solution, extracts from *Blomia tropicalis*, German cockroach (*Blattella germanica*), cat dander, dog dander, dust mite mix, dust mix, mixed grass pollen, mugwort/sage, ragweed, weed mix, mixed tree pollen, mold mix, *Penicillium*, *Alternaria tenuis*, and mouse pelt were applied to the skin of the forearm (Alk-Abello, Round Rock, TX). A test was considered positive if the maximum diameter of the wheal was greater than or equal to 3 mm after subtraction of the maximum diameter of the negative control.

Written parental consent was obtained for participating children, from whom written assent was also obtained. The study was approved by Institutional Review Boards of the University of Puerto Rico (San Juan, PR), Brigham and Women's Hospital (Boston, MA), and the University of Pittsburgh (Pittsburgh, PA).

Statistical Analysis

Nondetectable levels of glucan, endotoxin, peptidoglycan, or allergens were assigned a constant value (half the lowest detectable level). Glucan, endotoxin, peptidoglycan, and allergen levels were first analyzed as quartiles of exposure (after log10 transformation) and then as continuous (if a linear trend was present). Our two primary

outcomes were degree of atopy (the sum of positive skin tests to the allergens tested [0–15]) and having had at least one visit to the ED or urgent care for asthma in the previous year.

Bivariate analyses were conducted using two-tailed *t* tests or exact Cochran-Armitage tests for continuous variables, and Chi-square or Cochran-Armitage tests for categorical variables. A stepwise approach was used to build the linear (for degree of atopy) or logistic (for one or more visits to the ED or urgent care for asthma in the previous year) regression multivariate models. Negative binomial regression with robust error calculation was used in a confirmatory analysis for degree of atopy. All final models included age, sex, and household income (less than vs. greater than or equal to $15,000/yr [near the median income for Puerto Rican households in 2008–2009] [28]); models for atopy also included case-control status (asthma vs. no asthma), and those for ED/urgent care visits for asthma (in cases only) also included parental history of asthma and current exposure to environmental tobacco smoke (ETS). The following covariates were considered for inclusion in the initial multivariate models: body mass index as a *z*-score (based on 2000 Centers for Disease Control and Prevention growth charts [29]), use of inhaled corticosteroids in the prior 6 months, season of dust collection, and indoor allergens. Such covariates remained in the final models if they were statistically significant ($P < 0.05$) or changed the parameter estimate (β coefficient) by 10% or more. After the final models were built, we tested for first-order interactions between glucan and other covariates. All analyses were performed using SAS version 9.3 (SAS Institute, Cary, NC).

Results

Table E1 in the online supplement shows a comparison of the 317 participants (46.8% of the earlier cohort) and those not included in this analysis because they did not have glucan measurements. Compared with children who were not included in this analysis, those included were more likely to be exposed to ETS and to have lower household income and higher levels of three allergens (cockroach, dust mite, and cat). There was no significant difference in the degree of atopy or in ED/urgent care visits for asthma between children who were and were not included in this analysis.

Table 1 shows the characteristics of children with (cases) and without (control subjects) asthma included in this analysis. Compared with control subjects, cases were significantly younger and more likely to be male and exposed to ETS and to have lower household income, a parental history of asthma, and higher mouse allergen levels but lower FEV_1, FVC, and FEV_1/FVC.

Figure E1 shows the correlation between glucan and markers of bacterial exposure or allergens. Glucan was significantly and positively correlated with endotoxin and peptidoglycan, and significantly but negatively correlated with dust mite allergen. We found no significant correlation between glucan and allergens other than dust mite.

Table 2 shows the results of the bivariate analyses of glucan and the covariates or outcomes of interest. Glucan was significantly and negatively associated with BMI and FEV_1 but significantly and positively associated with current ETS. We found no significant association between glucan and current diagnosis of asthma or one or more hospitalizations for asthma in the previous year. Among children with asthma, higher glucan levels were significantly associated with one or more visits to the ED/urgent care for asthma in the previous year.

Table 1. Baseline characteristics of participating children

Characteristics	Cases (*n = 160*)	Control Subjects (*n = 157*)	All Children (*n = 317*)
Age, yr	10.1 (2.6)	10.7 (2.8)*	10.4 (2.7)
Female sex	66 (41%)	83 (53%)*	149 (47%)
BMI, *z*-score	0.53 (1.18)	0.48 (1.03)	0.51 (1.11)
Current exposure to second-hand tobacco smoke	82 (51%)	62 (39%)*	144 (45%)
Household income ⩾ $15,000/yr	29 (18%)	49 (32%)†	78 (25%)
Parental history of asthma	110 (69%)	42 (27%)†	152 (48%)
Glucan, μg/mg dust‡	0.15 (3.89)	0.14 (3.65)	0.14 (3.76)
Endotoxin, EU/mg dust‡	34.9 (2.52)	32.4 (2.45)	33.6 (2.49)
Peptidoglycan, μg/mg dust‡	0.02 (13)	0.02 (16)	0.02 (14)
Bla g in house dust, U/g‡	2.6 (5.5)	2.1 (4.6)	2.3 (5.0)
Can f in house dust, μg/g‡	0.18 (8.3)	0.16 (6.8)	0.17 (7.6)
Der p in house dust, μg/g‡	5.1 (3.3)	4.8 (3.0)	5.0 (3.2)
Fel d in house dust, μg /g‡	0.04 (7.9)	0.06 (12)	0.05 (10)
Mus m in house dust, ng/g‡	12 (11)	6.3 (8.5)*	8.7 (10)
STR to ⩾ 1 indoor allergen§	108 (77%)	94 (70%)	202 (73%)
STR to ⩾ 1 outdoor allergen‖	78 (56%)	73 (57%)	151 (57%)
At least one positive skin test	112 (78%)	101 (75%)	213 (77%)
Total number of positive skin tests	4.62 (4.31)	4.08 (4.14)	4.36 (4.23)
FEV_1, L	1.82 (0.63)	2.07 (0.75)†	1.94 (0.70)
FVC, L	2.24 (0.74)	2.50 (0.87)†	2.38 (0.82)
FEV_1/FVC	0.81 (0.098)	0.83 (0.093)*	0.82 (0.096)
⩾1 ED or urgent care visit in the last year¶	82 (51%)		
⩾1 Hospitalization for asthma in the last year¶	38 (23.4%)		

Definition of abbreviations: BMI = body mass index; ED = emergency department; STR = skin test reactivity.
Data are presented as n (%) for categorical variables and mean (SD) for continuous variables. Percentages expressed as a proportion of subjects with available data.
*$P < 0.05$ for comparison of cases and control subjects.
†$P < 0.01$ for comparison of cases and control subjects.
‡Glucan, endotoxin, peptidoglycan, and allergen levels analyzed on a log10 scale and then exponentiated.
§STR to one or more of the following allergens: *Alternaria*, *Blomia tropicalis*, cat, cockroach, dog, dust mix, dust mite mix, mold mix, mouse, and *Penicillium*.
‖STR to one or more of the following allergens: grass mix, mugwort/sage, ragweed, tree mix, weed mix.
¶Exacerbation data apply to children with asthma only.

Table 2. Glucan, selected covariates, and measures of allergy and asthma

Characteristics	Quartiles of Glucan Level (μg/mg of Dust)				*P* Value for Trend*
	1st Quartile (0.01–0.05)	2nd Quartile (0.05–0.14)	3rd Quartile (0.14–0.29)	4th Quartile (0.30–23.0)	
Participants, n	80	78	79	80	
Age, yr	10.6 (2.6)	10.6 (2.9)	10.4 (2.7)	9.9 (2.6)	0.07
Female sex	37 (46%)	33 (42%)	41 (52%)	38 (48%)	0.62
BMI, *z*-score	0.64 (1.10)	0.76 (1.04)	0.31 (1.11)	0.33 (1.14)	0.01
Asthma	37 (46%)	41 (53%)	38 (48%)	44 (55%)	0.40
Current exposure to second-hand tobacco smoke	31 (39%)	30 (38%)	36 (46%)	47 (59%)	<0.01
Household income ⩾ $15,000/yr	21 (27%)	26 (35%)	13 (17%)	18 (23%)	0.18
Parental history of asthma	38 (48%)	32 (41%)	39 (49%)	43 (54%)	0.29
Endotoxin, EU/mg†	24.4 (2.4)	27.6 (2.4)	35.5 (2.3)	53.3 (2.3)	<0.01
Peptidoglycan, μg/mg†	0.01 (20)	0.02 (16)	0.02 (11)	0.02 (10)	0.07
Bla g in house dust, U/g†	1.6 (4.0)	2.5 (6.1)	2.7 (4.8)	2.8 (5.0)	0.04
Can f in house dust, μg/g†	0.16 (6.7)	0.20 (11)	0.21 (6.2)	0.13 (7.0)	0.60
Der p in house dust, μg /g†	6.0 (2.9)	6.1 (2.7)	5.4 (3.3)	3.1 (3.3)	<0.01
Fel d in house dust, μg/g†	0.05 (9.5)	0.05 (12)	0.04 (9.5)	0.05 (9.2)	0.96
Mus m in house dust, ng/g†	7.2 (6.1)	6.1 (9.5)	12 (13)	11 (11)	0.12
STR to indoor allergens‡	47 (64%)	53 (79%)	53 (83%)	49 (70%)	0.31
Alternaria	10 (13%)	18 (27%)	18 (28%)	19 (27%)	0.06
Blomia tropicalis	34 (47%)	39 (59%)	33 (52%)	34 (49%)	0.96
Cat	19 (25%)	27 (40%)	29 (45%)	20 (28%)	0.62
Cockroach	15 (20%)	24 (36%)	31 (48%)	22 (31%)	0.06
Dog	16 (21%)	21 (31%)	23 (35%)	13 (18%)	0.86
Dust mix	18 (24%)	28 (42%)	23 (36%)	22 (31%)	0.50
Dust mite mix	27 (36%)	39 (58%)	39 (60%)	27 (38%)	0.75
Mold mix	7 (9%)	9 (13%)	8 (12%)	11 (15%)	0.34
Mouse	12 (16%)	16 (24%)	18 (28%)	20 (28%)	0.07
Penicillium	10 (13%)	16 (24%)	19 (30%)	15 (21%)	0.18
STR to outdoor allergens§	33 (44%)	41 (61%)	41 (66%)	36 (57%)	0.08
Grass mix	9 (12%)	12 (18%)	10 (20%)	12 (23%)	0.14
Mugwort/sage	9 (12%)	14 (22%)	9 (18%)	12 (24%)	0.14
Ragweed	22 (29%)	31 (46%)	34 (52%)	30 (42%)	0.09
Tree mix	16 (21%)	28 (42%)	25 (38%)	19 (27%)	0.57
Weed mix	12 (16%)	22 (33%)	21 (32%)	16 (23%)	0.40
At least one positive skin test	51 (68%)	54 (81%)	55 (85%)	53 (75%)	0.29
Total no. of positive skin tests	3.1 (3.8)	5.1 (4.4)	5.2 (4.3)	4.1 (4.2)	0.17
FEV_1, L	2.03 (0.80)	2.06 (0.76)	1.92 (0.68)	1.78 (0.53)	0.02
FEV_1/FVC	0.82 (0.11)	0.83 (0.08)	0.82 (0.11)	0.81 (0.08)	0.39
Use of inhaled corticosteroids in the last 6 mo‖	14 (18%)	14 (18%)	9 (11%)	8 (10%)	0.11
⩾1 ED or urgent care visit for asthma in the previous yr‖	16 (48%)	12 (29%)	20 (53%)	32 (74%)	<0.01
⩾1 Hospitalization for asthma in the previous yr‖	10 (30%)	8 (20%)	9 (24%)	11 (26%)	0.87

Definition of abbreviations: BMI = body mass index; ED = emergency department; STR = skin test reactivity.
Data are presented as n (%) for categorical variables and mean (SD) for continuous variables. Percentages expressed as a proportion of subjects with available data.
**P* values for continuous variables are calculated as linear trend across quartiles using analysis of variance with orthogonal linear contrast. The two-sided exact Cochran-Armitage trend test was used for categorical variables.
†Glucan, endotoxin, peptidoglycan, and allergen levels analyzed on a log10 scale and then exponentiated.
‡STR to one or more of the following allergens: *Alternaria*, *Blomia tropicalis*, cat, cockroach, dog, dust mix, dust mite mix, mold mix, mouse, and *Penicillium*.
§STR to one or more of the following allergens: grass mix, mugwort/sage, ragweed, tree mix, weed mix.
‖Exacerbation data apply to children with asthma only.

Table 3 shows the results of the analysis of glucan and degree of atopy in all subjects and separately for cases and control subjects. In bivariate analyses among all subjects, children exposed to glucan levels in the second and third quartiles had approximately two more positive skin tests to allergens than those exposed to glucan levels in the first (lowest) quartile ($P < 0.01$ in both instances). There was no significant association between exposure to the highest (fourth) quartile of glucan and the degree of atopy. In a multivariate analysis adjusting for mouse allergen and other covariates among all subjects, children exposed to glucan levels in the second and third quartiles had approximately 1.7 and 2.2 more positive

Table 3. Analysis of glucan and degree of atopy in children with and without asthma

Variable	No. of Positive Skin Tests,* β (95% CI), *P* Value		
	Cases	Control Subjects	All Subjects
Unadjusted			
Glucan			
Quartile 1	Reference		
Quartile 2	1.82 (−0.26 to 3.90), 0.09	1.79 (−0.16 to 3.74), 0.07	1.90 (0.49 to 3.32), 0.008
Quartile 3	1.29 (−0.88 to 3.47), 0.2	2.60 (0.71 to 4.49), 0.007	2.00 (0.58 to 3.42), 0.006
Quartile 4	0.02 (−2.01 to 2.05), 1.0	1.58 (−0.39 to 3.54), 0.12	0.83 (−0.57 to 2.22), 0.2
Multivariate model*			
Glucan			
Quartile 1	Reference		
Quartile 2	1.57 (−0.58 to 3.71), 0.15	1.86 (−0.13 to 3.84), 0.07	1.69 (0.25 to 3.12), 0.02
Quartile 3	1.76 (−0.44 to 3.96), 0.12	2.82 (0.88 to 4.76), 0.005	2.18 (0.74 to 3.61), 0.003
Quartile 4	0.44 (−1.61 to 2.50), 0.7	2.46 (0.37 to 4.55), 0.02	1.13 (−0.29 to 2.55), 0.12
Household income ⩾ $15,000/yr	−0.51 (−2.41 to 1.39), 0.6	−0.05 (−1.59 to 1.49), 1.0	−0.19 (−1.38 to 1.0), 0.8
Case (vs. control)			0.71 (−0.47 to 1.89), 0.2
Mus m in house dust, ng/g†	−0.87 (−1.57 to −0.18), 0.01	−0.32 (−1.12 to 0.47), 0.4	−0.72 (−1.24 to −0.21), 0.006

Definition of abbreviations: CI = confidence interval.
*Model additionally adjusted for age, sex, and parental history of asthma.
†Allergen level was transformed to a logarithmic (log10) scale.

skin tests, respectively, than those exposed to the first quartile of glucan ($P < 0.05$ in both instances). In this multivariate model, mouse allergen was significantly and inversely associated with the degree of atopy. There was no significant modification of the estimated effect of glucan exposure on the degree of atopy by any covariate. Similar results for the analysis of degree of atopy in all subjects were obtained in a confirmatory analysis using negative binomial regression with robust error calculation (Table E2). Consistent with a nonsignificant interaction between case-control status and glucan level on degree of atopy, our results were generally similar in cases and control subjects, with the exception of a stronger and more significant association between the highest quartile of glucan and the degree of atopy in control subjects than in cases (Table 3).

Table 4 shows the results of the analysis of glucan and one or more ED/urgent care visits for asthma in the previous year. In bivariate analyses, children exposed to the highest (fourth) quartile of glucan levels had greater odds of one or more ED/urgent care visits for asthma. In a multivariate analysis, children exposed to the highest quartile of glucan had nearly ninefold higher odds of one or more visits to the ED/urgent care for asthma than those exposed to the first quartile of glucan. In this analysis, peptidoglycan was significantly and inversely associated with one or more ED/urgent care visits for asthma. We found no significant modification of the estimated effect of glucan exposure on ED/urgent care visits for asthma by atopic status or any covariate in the multivariate model.

Discussion

To our knowledge, this is the first study to report an association between glucan and an increased degree of atopy or visits to the ED/urgent care for asthma in school-aged children. This is also the first study to examine glucan and asthma exacerbations in children living in a tropical environment.

Fungal exposure (assessed by questionnaires or markers other than glucan) has been associated with asthma symptoms or atopy in some but not all previous reports. In studies conducted in Europe (30–32), Costa Rica (33), and

Table 4. Analysis of glucan and visits to the emergency department or urgent care for asthma

Variable	⩾1 ED/Urgent Care Visit for Asthma
Unadjusted	OR (95% CI), *P* value
Glucan	
Quartile 1	1.00
Quartile 2	0.44 (0.17–1.11), 0.08
Quartile 3	1.17 (0.47–2.90), 0.7
Quartile 4	2.82 (1.12–7.10), 0.03
Multivariate model*	
Glucan	
Quartile 1	1.00
Quartile 2	0.67 (0.22–1.98), 0.5
Quartile 3	2.32 (0.75–7.14), 0.14
Quartile 4	8.76 (2.70–28.4), <0.001
Household income ⩾ $15,000/yr	0.12 (0.04–0.36), <0.001
Use of inhaled corticosteroids in the prior 6 mo	3.74 (1.50–9.35), 0.005
Peptidoglycan in house dust, μg/mg†	0.61 (0.42–0.89), 0.009

Definition of abbreviations: CI = confidence interval; ED = emergency department; OR = odds ratio.
*Model additionally adjusted for age, sex, parental history of asthma, and exposure to ETS.
†Peptidoglycan level was transformed to a logarithmic (log10) scale.

the U.S. Northeast (34, 35) or Midwest (36), parental report of visible mold/dampness/water damage (30, 32, 33, 36) or spore count of viable mold (e.g., *Penicillium*) (31, 34, 35) has been associated with asthma symptoms (in early life [30, 32, 34, 36] or [in children sensitized to mold] at school age [34]), as well as with increased airway responsiveness (33) or atopy (31) at school age. In contrast, spore count of *Penicillium* in house dust was not significantly associated with asthma or atopy in a study of school-aged children in European farms with high microbial exposure (37). In that study, the spore count of another fungal species (*Eurotium*) was significantly and inversely associated with asthma but not with atopy (37).

Unlike previous studies, ours simultaneously assessed glucan, five allergens (including Mus m 1), and two markers of bacterial exposure in house dust. Our study also differs from prior reports regarding factors including sample size, geographic location, level of glucan, and assessment of microbial or fungal diversity. The only previous study of glucan and atopy in the tropics was conducted in a convenience sample of 98 children attending an otolaryngology clinic in Singapore (38). Although there was no significant association between glucan and atopy, that study had small sample size and no data for any covariate other than endotoxin.

Glucan level in house dust varies across European countries, where sampling season and other factors affect glucan measurements (39) (e.g., glucan concentrations are 50% higher in samples collected in summer as compared with those in winter). The geometric mean glucan level among control subjects in a multicenter European study with a large number of farm dwellers was 2,959 μg/g of mattress dust, approximately 20 times higher than that in our study (16). An effect of glucan may not be evident in children exposed to a heavy and diverse microbial burden (e.g., living in farms in central Europe) (37). Compared with such children, those in urban San Juan are much less exposed to feces from large mammals but perhaps comparably exposed to mice.

We found no significant association between endotoxin and atopy, but the endotoxin levels in our study (geometric mean = 33.6 EU/mg dust) were more than four times lower than those in farms in Germany and Switzerland (40). In contrast to our findings for endotoxin, mouse allergen was inversely associated with the degree of atopy in study participants. Together with previous results (41, 42), our findings suggest that microbial exposures correlated with mouse allergen may protect against atopy. We also noted that peptidoglycan, a toll-like receptor 2 ligand with immunomodulatory effects (43, 44), was inversely associated with ED/urgent care visits for asthma (but not with atopy). Consistent with our findings, a study of 553 European children living in farming and nonfarming environments reported that peptidoglycan exposure was inversely associated with wheezing but not with atopy, suggesting that peptidoglycan may influence asthma symptoms or control by affecting innate immune responses (45).

Glucans have insufficiently characterized but complex effects on immune responses. Glucans have been shown to competitively inhibit Th2 responses to dust mite allergen (46) in experimental studies. In humans, exposure to high levels of airborne glucan may increase the ratio of IFN-γ to IL-4 in nonatopic subjects (47) and have been associated with increased levels of multiple serum cytokines, including tumor necrosis factor-α, IL-6, and IL-8 (48). In rodents, inhaled glucan increases pulmonary eosinophilia (49, 50) and allergic responses to ovalbumin (51).

Our findings may be explained not only by the immune-modulatory effects of fungi but also by their mediation of responses to other allergens (7). Fungal proteases can injure the airway epithelium, thereby increasing allergen access (2). An adjuvant effect, with increased sensitization to allergens delivered alongside glucan, has been noted in a murine model (52). In another mouse model, repeated allergen exposure was noted to be more likely to produce airway eosinophilia in the presence of glucan (53).

We recognize several limitations to our findings. First, this is a cross-sectional study, and thus we cannot assess a temporal relationship between glucan and the degree of atopy. However, our findings are likely to reflect effects of fungal exposure before at least one ED/urgent care visit for asthma. Although seasonal variability can affect glucan levels (31), we obtained similar results after additional adjustment of our multivariate models for month of sample collection, suggesting that our single measurement adequately reflects chronic and clinically relevant glucan exposure.

Second, selection bias and confounding are possible in any observational study. However, selection bias is unlikely to explain our results, because there were no significant differences in the outcomes of interest between children who were and were not included in this analysis. Confounding by factors such as poverty is also improbable, as we obtained similar results in confirmatory analyses adjusting for measures of socioeconomic status other than income (Tables E3 and E4). In addition, if the association between glucan and atopy were mostly explained by SES, one would expect that the direction of the association between mouse allergen (correlated with lower income) and atopy would be the same as that for glucan (not the case). Third, we cannot separate the effects of fungal species. However, glucan is a good predictor of fungal burden and superior to parental report (54). Finally, we had limited statistical power to detect a potential threshold for attenuation of an effect of glucan on the degree of atopy or an effect of glucan exposure on hospitalizations in children with asthma. We also had limited power to detect interactions between glucan level and relevant covariates (e.g., allergen levels or atopic status).

In summary, our results suggest that indoor fungal exposure increases the degree of atopy and ED/urgent care visits for asthma in children in Puerto Rico. Fungal exposure may have more detrimental effects on asthma or atopy in tropical environments where indoor bacterial exposure is sparser and less diverse. ■

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