Vitamin D Insufficiency and Severe Asthma Exacerbations in Puerto Rican Children

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Rationale: **Vitamin D insufficiency (a serum 25(OH)D <30 ng/ml) has been associated with severe asthma exacerbations, but this could be explained by underlying racial ancestry or disease severity. Little is known about vitamin D and asthma in Puerto Ricans.**

Objectives: **To examine whether vitamin D insufficiency is associated with severe asthma exacerbations in Puerto Rican children, independently of racial ancestry, atopy, and time outdoors.**

Methods: **A cross-sectional study was conducted of 560 children ages 6–14 years with (n = 287) and without (n = 273) asthma in San Juan, Puerto Rico. We measured plasma vitamin D and estimated the percentage of African racial ancestry among participants using genome-wide genotypic data. We tested whether vitamin D insufficiency is associated with severe asthma exacerbations, lung function, or atopy (greater than or equal to one positive IgE to allergens) using logistic or linear regression. Multivariate models were adjusted for African ancestry, time outdoors, atopy, and other covariates.**

Measurements and Main Results: **Vitamin D insufficiency was common in children with (44%) and without (47%) asthma. In multivariate analyses, vitamin D insufficiency was associated with higher odds of greater than or equal to one severe asthma exacerbation in the prior year (odds ratio [OR], 2.6; 95% confidence interval [CI], 1.5–4.9; $P = 0.001$) and atopy, and a lower FEV_1/FVC in cases. After stratification by atopy, the magnitude of the association between vitamin D insufficiency and severe exacerbations was greater in nonatopic (OR, 6.2; 95% CI, 2–21.6; $P = 0.002$) than in atopic (OR, 2; 95% CI, 1–4.1; $P = 0.04$) cases.**

Conclusions: **Vitamin D insufficiency is associated with severe asthma exacerbations in Puerto Rican children, independently of racial ancestry, atopy, or markers of disease severity or control.**

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Vitamin D insufficiency has been associated with asthma exacerbations in childhood, but this finding could be explained by underlying disease severity or racial ancestry.

What This Study Adds to the Field

We show that vitamin D insufficiency is common in children in Puerto Rico. Among Puerto Rican children with asthma, vitamin D insufficiency is associated with severe disease exacerbations, independently of African racial ancestry, time outdoors, atopy, and other markers of disease severity or control.

Keywords: vitamin D; asthma exacerbations; Puerto Ricans; childhood

Vitamin D insufficiency (a serum level of 25(OH)D <30 ng/ml) is common among children in the United States mainland. For example, a nationwide study of 2,759 U.S. children ages 6–11 years found vitamin D insufficiency in approximately 62% of non-Hispanic whites, approximately 86% of Hispanics, and approximately 96% of non-Hispanic blacks (1). Vitamin D insufficiency has been associated with increased asthma morbidity, particularly severe disease exacerbations, in observational studies of children of school age in Costa Rica (2) and North America (3).

Although current evidence from observational (2–5) and experimental studies (6–9) suggests that vitamin D insufficiency leads to severe asthma exacerbations or increased asthma morbidity in childhood (10), no study of vitamin D and asthma morbidity has accounted for objectively assessed racial ancestry and time spent outdoors. This is critical to exclude whether vitamin D insufficiency is associated with severe asthma exacerbations or asthma morbidity through "reverse causation" (e.g., more severe asthma leading to reduced time outdoors and thus decreased exposure to sunlight and reduced vitamin D levels) or underlying racial ancestry (e.g., if African ancestry, which is correlated with melanin content and skin pigmentation, led to both vitamin D insufficiency and increased asthma morbidity).

Few studies of vitamin D and asthma have included unaffected (control) subjects. Examination of vitamin D status and allergy or lung function in children without asthma is important, given inconsistent findings in previous studies (10). Lack of association between vitamin D insufficiency and lung function or atopy in unaffected children would suggest that vitamin D influences severe asthma exacerbations through mechanisms

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TABLE 1. BASELINE CHARACTERISTICS OF PARTICIPATING CHILDREN*

Covariate	Cases (*n* = *287*)	Control Subjects (*n* = *273*)
Age, yr	10.1 (2.6)	10.5 (2.7)
Female sex	116 (40%)	142 (52%)†
Body mass index, *z* score	0.71 (1.17)	0.49 (1.14)‡
At least one parent graduated from high school	235 (82%)	215 (79%)
Household income < $15,000 per yr	193 (69%)	173 (66%)
Private or employer-based health insurance	87 (30%)	95 (35%)
Parental history of asthma	193 (67%)	86 (32%)§
Current exposure to environmental tobacco smoke	131 (46%)	99 (36%)‡
Exposure to *in utero* smoking	33 (12%)	26 (10%)
Premature birth	27 (9%)	15 (6%)
Use of inhaled corticosteroids in the prior year	90 (31%)	
Bla g in house dust, μg/g‖	0.34 (0.73)	0.29 (0.68)
Der p in house dust, μg/g‖	0.66 (0.51)	0.65 (0.5)
Percentage of African ancestry	25 (12)	25 (12)
Serum vitamin D level, ng/ml	32 (8)	31 (8)‡
Serum vitamin D level <30 ng/ml	127 (44%)	128 (47%)
Virtually always outdoors	78 (27%)	68 (25%)
High vitamin D intake in diet	199 (70%)	147 (54%)§
Played on a sports team in the prior year	139 (72%)	125 (64%)
Prebronchodilator FEV_1, ml¶	1,896 (670)	2,043 (749)‡
Prebronchodilator FEV_1/FVC	81 (9)	84 (9)‡
Total IgE, IU/ml‖	2.5 (0.7)	2.2 (0.7)§
Positive IgE to cockroach (Bla g) ⩾0.35 IU/ml	114 (40%)	74 (27%)†
Positive IgE to dust mite (Der p) ⩾0.35 IU/ml	183 (64%)	121 (45%)§
⩾1 positive allergen-specific IgE	195 (69%)	134 (50%)§
⩾1 severe asthma exacerbation in the prior year	153 (53%)	
⩾1 hospitalization for asthma in the prior year	60 (21%)	
⩾1 Emergency department/urgent care visit requiring steroids in the prior year	80 (28%)	
Intravenous or oral steroids for asthma in the prior year	130 (45%)	

*Values are the number (%) for binary variables or mean (SD) for continuous variables.
† Comparison between cases and control subjects: $P < 0.01$.
‡ Comparison between cases and control subjects: $P < 0.05$.
§ Comparison between cases and control subjects: $P < 0.001$.
‖ Allergen levels and total IgE were transformed to a logarithmic (log10) scale.
¶ FEV_1 presented as absolute values because of lack of predicted values for Puerto Ricans.

other than alterations in lung structure or regulation of allergic immune responses, including antiviral properties or enhanced steroid responsiveness (10).

Even though Puerto Ricans share a disproportionate burden of childhood asthma in the United States (11), no study has examined vitamin D status and asthma or asthma morbidity in this ethnic group. Most Puerto Ricans are racially admixed, with various proportions of European, African, and Native American ancestry (12). We hypothesized that vitamin D insufficiency is common and associated with severe asthma exacerbations in Puerto Rican children, and that this association is independent of African racial ancestry, time spent outdoors, atopy, and reported dietary intake of vitamin D. To test this hypothesis, we examined the relationship between vitamin D insufficiency and severe asthma exacerbations in 560 Puerto Rican children with (n = 287) and without (n = 273) asthma living in San Juan, Puerto Rico.

METHODS

See the online supplement for a more detailed description of the study protocol and procedures.

Subject Recruitment

From March 2009 to June 2010, children in San Juan were chosen from randomly selected households, using a scheme similar to that of a prior study (13). In brief, households in the Standard Metropolitan Area of San Juan were selected by a multistage probability sample design. Primary sampling units were randomly selected neighborhood clusters based on the 2000 US censuses, and secondary sampling units were randomly selected households within each individual primary sampling units. A household was eligible if greater than or equal to one resident was a child 6–14 years old. In households with more than one eligible child, a maximum of five children were randomly selected. Within each housing unit selected, children were enumerated and one child per eligible household was selected for screening. In households with multiple eligible children, one child was randomly selected by using Kish tables. On the basis of the sampling design, a total of 7,073 households were selected, and 6,401 (90.5%) were contacted. Of these 6,401 households, 1,111 had greater than or equal to one child within the age range of the study who met other inclusion criteria (*see below*). To reach our target sample size (~700 children), we enrolled a random sample (n = 783) of these 1,111 children. Parents of 105 (13.4%) of these 783 eligible households refused to participate or could not be reached. 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. Blood samples were collected in 592 (87.3%) of these 678 children; 583 (98.5%) of these 592 children had sufficient DNA for genotyping and were included in the analysis. The main recruitment tool was a screening questionnaire given to parents of children ages 6–14 years to obtain information about the child's respiratory health and Puerto Rican ancestry. We selected as cases children who had physician-diagnosed asthma, wheeze in the prior year, and four Puerto Rican grandparents. We selected as control subjects children who had no physician-diagnosed asthma, no wheeze in the prior year, and four Puerto Rican grandparents.

Study Procedures

Participants completed a protocol that included questionnaires; spirometry; and collection of blood (for DNA extraction, and measurements of total and allergen-specific IgE in serum and 25-hydroxy-vitamin D

TABLE 2. VITAMIN D, SELECTED COVARIATES, SEVERE ASTHMA EXACERBATIONS, AND MEASURES OF ALLERGY AND LUNG FUNCTION IN PUERTO RICAN CHILDREN*

	Cases			Control Subjects		
Covariates	Vitamin D <30 ng ml	Vitamin D ≥30 ng ml	*P* for Difference†	Vitamin D <30 ng ml	Vitamin D ≥30 ng ml	*P* for Difference†
Age, yr	11 (3)	9.5 (2.5)	<0.001	11 (2)	9.7 (2.7)	<0.001
Female sex	64 (50%)	52 (33%)	0.002	78 (61%)	64 (44%)	0.006
Body mass index, *z* score	0.89 (1.08)	0.56 (1.23)	0.02	0.6 (1.13)	0.4 (1.14)	0.1
At least one parent graduated from high school	104 (82%)	131 (82%)	1	106 (83%)	109 (75%)	0.1
Household income <$15,000 per yr	83 (66%)	110 (71%)	0.5	74 (59%)	99 (73%)	0.01
Private or employer-based health insurance	42 (33%)	45 (28%)	0.4	50 (39%)	45 (31%)	0.2
Parental history of asthma	82 (65%)	111 (70%)	0.3	43 (34%)	43 (30%)	0.5
Current exposure to environmental tobacco smoke	53 (42%)	78 (49%)	0.2	47 (37%)	52 (36%)	0.9
Use of inhaled corticosteroids in the prior 6 mo	44 (35%)	46 (29%)	0.3			
Bla g in house dust, μg/g‡	0.37 (0.76)	0.32 (0.7)	0.6	0.2 (0.63)	0.37 (0.71)	0.04
Der p in house dust, μg/g‡	0.78 (0.47)	0.58 (0.52)	0.001	0.63 (0.52)	0.67 (0.49)	0.5
Percent African ancestry	26 (11)	25 (12)	0.6	25 (13)	24 (12)	0.3
Virtually always outdoors	28 (22%)	50 (31%)	0.08	29 (23%)	39 (27%)	0.4
Vitamin D drawn in the summer	39 (31%)	54 (34%)	0.6	58 (45%)	60 (41%)	0.5
High vitamin D intake in diet	80 (63%)	119 (75%)	0.03	59 (46%)	88 (61%)	0.02
Prebronchodilator FEV_1, ml§	2,030 (685)	1,787 (638)	0.003	2,225 (727)	1,877 (733)	0.0001
Prebronchodilator FEV_1/FVC	80 (10)	82 (9)	0.1	84 (8)	83 (9)	0.2
Total IgE, IU/ml‡	2.5 (0.7)	2.4 (0.7)	0.3	2.2 (0.7)	2.2 (0.7)	0.9
≥1 positive allergen-specific IgE to allergens	97 (76%)	98 (62%)	0.01	64 (50%)	70 (49%)	0.8
≥1 severe asthma exacerbation in the prior year	80 (63%)	73 (46%)	0.003			
≥1 hospitalization for asthma in the prior year	35 (28%)	25 (16%)	0.01			
≥1 Emergency department/urgent care visit requiring steroids in the prior year	43 (34%)	37 (23%)	0.04			
Intravenous or oral steroids for asthma in the prior year	66 (52%)	64 (40%)	0.04			

*Values are the number (%) for binary variables or mean (SD) for continuous variables.
† *P* for difference between insufficient and sufficient vitamin D levels, *t* test for continuous variables, chi-square for binary variables.
‡ Allergen levels and total IgE were transformed to a logarithmic (log10) scale.
§ FEV_1 presented as absolute values because of lack of predicted values for Puerto Ricans.

[hereafter referred to as vitamin D] in plasma) and dust (for measurement of dust mite and cockroach allergens) samples. Plasma vitamin D was measured using the Waters high-performance liquid chromatography system with tandem mass spectrophotometry (Waters Corporation, Milford, MA). Vitamin D intake was estimated using a food frequency questionnaire (14). Time spent outdoors during weekends and holidays, usual time spent outdoors during daily activities, and sunscreen use were assessed using a validated questionnaire (15). Subjects were genotyped using the HumanOmni2.5 BeadChip (Illumina, Inc., San Diego, CA). After removing single-nucleotide polymorphisms that were not in Hardy–Weinberg equilibrium ($P < 10^{-6}$) in control subjects, that had minor allele frequency lower than 1% or a failure rate greater than 2%, or that were in linkage disequilibrium ($r^2 \geq 0.10$) with other single-nucleotide polymorphisms, there were 85,059 single-nucleotide polymorphisms from which to estimate ancestry by the Local Ancestry in adMixed Populations method (16) using estimated ancestral proportions for Puerto Ricans (17) (*see* Table E1 in the online supplement) and data from external reference panels. Given the racial admixture patterns of Puerto Ricans, we used reference panels from HapMap (18) for Europeans and West Africans, and from the Human Genome Diversity Project for Native Americans (19) for ancestry estimation.

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

Statistical Analysis

Our primary outcome was severe asthma exacerbations in the previous year, defined as per a recent consensus statement as at least one hospitalization or visit to the emergency department or urgent care for asthma that led to treatment with systemic (oral, intramuscular, or intravenous) corticosteroids, or at least one course of systemic steroids for asthma (20). Secondary outcomes included FEV_1, FEV_1/FVC, serum total IgE, and having at least one positive IgE to five common allergens.

All analyses were conducted separately in cases and control subjects. Because of their potential correlation with vitamin D insufficiency and the outcomes of interest, the following covariates were examined in bivariate analyses: percentage of African ancestry; time spent outdoors; vitamin D intake through supplements or diet (classified as high or low based on intake of foods rich in vitamin D or vitamin supplements); season of collection of blood sample for vitamin D measurement; maternal and paternal history of asthma; household income (less than vs. greater than or equal to $15,000 [near the median income for households in Puerto Rico in 2008–2009) (21); use of inhaled corticosteroids (ICS) in the previous 6 months; prematurity (22); current exposure to environmental tobacco smoke (ETS) (23); *in utero* ETS exposure; body mass index (24) as a *z* score (based on 2000 Centers for Disease Control and Prevention growth charts) (25); and indoor exposure to dust mite (Der p 1) (26) and cockroach (Bla g 2) allergens (27). Logistic or linear regression was used for the multivariate analysis. All multivariate models included vitamin D insufficiency, African ancestry, time spent outdoors, age, sex, household income, dietary vitamin D intake, and ICS use; the analysis of FEV_1 was additionally adjusted for height

and height-squared. Additional covariates (*see above*) were included in the initial multivariate models if they were associated with the outcome at α less than 0.25 in bivariate analyses, and then subjected to removal in a step-wise fashion. At each step of model building, the variable with the largest P value was excluded; the procedure stopped when all variables not forced into the model had P values less than 0.05. We then tested for interaction between vitamin D insufficiency and the covariates included in the final models.

Local Ancestry in adMixed Populations version 2.3 (http://lamp.icsi.berkeley.edu/lamp/) was used for ancestry estimation and SAS version 9.2 (SAS Institute, Cary, NC) for all other analyses.

RESULTS

After excluding subjects with low marker call rate, missing genotyping, or missing vitamin D level, 287 (82%) of the 351 cases and 273 (83%) of the 327 control subjects remained in this analysis. Cases included in this analysis were more likely to have a household income less than $15,000 per year than those not included (69% vs. 51%; $P < 0.05$); there were no other significant differences (e.g., in lung function measures, total or allergen-specific IgE, or severe asthma exacerbations) between cases who were and were not included ($P > 0.20$ in all instances). Control subjects included in this analysis were also more likely to have a household income less than $15,000 than those not included (66% vs. 46%; $P < 0.05$); there were no other significant differences (e.g., in lung function measures or total or allergen-specific IgE) between those who were and were not included ($P > 0.20$ in all instances).

The main characteristics of study participants are summarized in Table 1. Compared with control subjects, cases had significantly higher (albeit slightly) mean plasma vitamin D levels, higher vitamin D intake through diet or supplements, and a higher total IgE. Cases were also significantly more likely to have at least one positive IgE to allergens and to have a lower FEV_1 or FEV_1/FVC than control subjects. There were no significant differences in age, household income, parental education, type of health insurance, or prevalence of vitamin D insufficiency between cases and control subjects.

We then tested whether vitamin D is associated with the covariates or outcomes of interest in bivariate analyses (Table 2). In cases and control subjects, children with vitamin D insufficiency were more likely to be older and female, and to have a higher vitamin D intake and higher FEV_1 than those without vitamin D insufficiency. Compared with cases with vitamin D sufficiency, those with vitamin D insufficiency were significantly more likely to have had at least one hospitalization for asthma in the prior year, to have had at least one emergency department or urgent care visit requiring systemic steroids for asthma in the prior year, to have received at least one course of systemic steroids for asthma in the prior year, and to have at least one positive specific IgE to allergens. There was no significant association between vitamin D insufficiency and total IgE in cases or control subjects. Vitamin D insufficiency was not significantly associated with FEV_1/FVC or having at least one positive IgE to allergens in control subjects.

Table 3 shows the results of the unadjusted and adjusted analyses of vitamin D insufficiency and severe asthma exacerbations in cases. After adjustment for African ancestry, time spent outdoors, dietary vitamin D intake, and other covariates, vitamin D insufficiency was significantly associated with 2.6 times higher odds of at least one severe asthma exacerbation in the prior year. In adjusted analyses of cases, vitamin D insufficiency was significantly associated with lower FEV_1/FVC or having at least one positive IgE to allergens but not with FEV_1 (Table 3) or total IgE (data not shown). Percentage of African ancestry was significantly and independently associated with lower FEV_1 but not with FEV_1/FVC in cases.

To further assess whether reverse causation explains the observed association between vitamin D insufficiency and severe asthma exacerbations, we repeated the analysis in cases after additional adjustment for FEV_1/FVC and having at least one positive IgE to allergens, obtaining very similar results (Table 4). However, there was significant modification of the effect of vitamin D insufficiency on severe asthma exacerbations by atopic status (P for interaction term < 0.01). We thus repeated the analysis after stratification by atopy (Table 5). In this analysis, the magnitude of the association between vitamin D insufficiency and severe asthma exacerbations was greater in nonatopic cases (odds ratio [OR], 6.2; 95% confidence interval [CI], 2–21.6; $P = 0.002$) than in atopic cases (OR, 2; 95% CI, 1–4.1; $P = 0.04$).

Vitamin D insufficiency was not significantly associated with lung function measures (FEV_1 or FEV_1/FVC), total IgE, or having at least one positive IgE to allergens among Puerto Rican children without asthma (control subjects) (*see* Table E2).

DISCUSSION

To our knowledge, this is the first study of vitamin D insufficiency and asthma in Puerto Ricans. Although study participants live on

TABLE 3. MULTIVARIATE ANALYSIS OF VITAMIN D INSUFFICIENCY AND SEVERE ASTHMA EXACERBATIONS, LUNG FUNCTION MEASURES, AND ATOPY IN PUERTO RICAN CHILDREN WITH ASTHMA*

Predictors	⩾1 Severe Asthma Exacerbation in the Prior Year†	Prebronchodilator FEV_1 (*ml*)‡	Prebronchodilator FEV_1/FVC (%)	⩾1 Positive Allergen-specific IgE
Unadjusted				
Vitamin D level < 30 ng/ml	1.7 (1 to 2.9), 0.04	243 (83 to 403), 0.003	−2 (−4 to 0), 0.1	2 (1.2 to 3.5), 0.009
Multivariate model§				
Vitamin D level < 30 ng/ml	2.6 (1.5 to 4.7), <0.001	−68 (−145 to 10), 0.09	−3 (−5 to 0), 0.02	1.8 (1.1 to 3.3), 0.03
Household income < $15,000 per yr	1.3 (0.7 to 2.4), 0.4	−51 (−134 to 32), 0.2	0 (−2 to 3), 1	0.6 (0.3 to 1.1), 0.09
Use of inhaled corticosteroids in the prior year	3.3 (1.8 to 6.1), <0.001	−53 (−136 to 30), 0.2	−1 (−4 to 1), 0.3	1 (0.5 to 1.8), 0.9
Body mass index (*z* score)		77 (44 to 110), < 0.001		
Each 20% increase in African ancestry	0.9 (0.6 to 1.4), 0.7	−113 (−175 to 51), <0.001)	0 (−2 to 2), 0.7	1.1 (0.7 to 1.8), 0.6
Virtually always outside	0.6 (0.3 to 1.1), 0.09	42 (−41 to 126), 0.3	2 (−1 to 4), 0.2	0.8 (0.4 to 1.4), 0.4
High vitamin D intake (diet or supplements)	1.1 (0.6 to 1.9), 0.8	−27 (−107 to 53), 0.5	−1 (−3 to 2), 0.5	0.7 (0.4 to 1.3), 0.3

* Beta (95% confidence interval), P value for FEV_1 and FEV_1/FVC, and odds ratio (95% confidence interval), P value for other outcomes.

† At least one visit to the emergency department/urgent care or at least one hospitalization requiring treatment with systemic corticosteroids for asthma, or at least one course of oral corticosteroids for asthma.

‡ FEV_1 additionally adjusted for height and height squared.

§ All multivariate models additionally adjusted for age and sex.

TABLE 4. MULTIVARIATE ANALYSIS OF VITAMIN D INSUFFICIENCY AND AT LEAST ONE SEVERE ASTHMA EXACERBATION IN THE PREVIOUS YEAR

Predictors	Original Model* Plus FEV_1/FVC Ratio	Plus Atopy
Covariates		
Vitamin D level < 30 ng/ml	2.7 (1.5–4.9) (0.001)	2.6 (1.5–4.9) (0.001)
Household income < $15,000 per yr	1.2 (0.6–2.2) (0.6)	1.2 (0.6–2.2) (0.6)
Use of inhaled corticosteroids in the prior year	3.2 (1.7–6.2) (< 0.001)	3.2 (1.7–6.3) (< 0.001)
Each 20% increase in African ancestry	0.9 (0.6–1.5) (0.8)	0.9 (0.6–1.5) (0.8)
Virtually always outside	0.6 (0.3–1.2) (0.2)	0.6 (0.3–1.2) (0.2)
High vitamin D intake (diet or supplements)	1.2 (0.7–2.2) (0.5)	1.3 (0.7–2.4) (0.4)
Prebronchodilator FEV_1/FVC	10.1 (0.5–217.4) (0.1)	10.2 (0.5–226.4) (0.1)
⩾1 positive allergen-specific IgE		1.3 (0.7–2.4) (0.3)

*Original logistic regression model for the outcome of severe asthma exacerbations presented in Table 3. All models were additionally adjusted for age and sex. Results presented are odds ratio (95% confidence interval); *P* value for severe asthma exacerbations.

a tropical island, vitamin D insufficiency was commonly found in children with (44%) and without (47%) asthma. These findings are similar to those of a recent study of a convenience sample of 98 obese and overweight Puerto Rican adults without asthma, of whom 45% had vitamin D levels less than 30 ng/ml (28). The estimated prevalence of vitamin D insufficiency in children with asthma in Puerto Rico (a Caribbean island) is thus markedly higher than that reported for children with asthma living in Costa Rica (a Central American nation, 28%), and underscores the potential significance of vitamin D insufficiency on asthma morbidity in residents of areas with year-round sun exposure or near the Equator.

This is the first study of vitamin D insufficiency and severe asthma exacerbations to account for time spent outdoors and racial ancestry assessed by genetic markers. After adjustment for these and other covariates, vitamin D insufficiency was significantly associated with increased risk of severe asthma exacerbations in Puerto Rican children. This is consistent with our results in studies of children of school age in Costa Rica (2) and North America (3), in whom we lacked data on time outdoors, dietary intake, or racial ancestry. The magnitude of the observed association between vitamin D insufficiency and severe asthma exacerbations in Puerto Rican children is higher than that reported in a multicenter study of North American (including non-Hispanic white, non-Hispanic black, and Hispanic) children (OR, 1.5; 95% confidence interval, CI, 1.1–2) (3) but lower than that reported for hospitalizations in Costa Rican children (nearly tenfold increased odds for children with serum 25[OH]D <20 ng/ml) (2).

We previously reported no significant association between vitamin D status and FEV_1 or FEV_1/FVC in Costa Rican children with asthma (2). Among Puerto Rican children with asthma (cases), we found that vitamin D insufficiency was significantly associated with FEV_1/FVC (a marker of increased disease severity (29, 30) but not with FEV_1. In these children, the magnitude of the observed association between vitamin D insufficiency and severe asthma exacerbations was greater in those who were nonatopic than in those who were atopic, despite similar reported frequency of ICS use in the two groups (30.3% vs. 31.8%; $P = 0.81$). Taken together with the observed lack of association between vitamin D insufficiency and atopy or lung function in Puerto Rican control subjects, and inconsistent findings for vitamin D and atopy or lung function in previous studies (2, 3, 10), our results suggest that vitamin D insufficiency influences the development of severe asthma exacerbations through mechanisms unrelated to abnormalities in regulation of allergic immune response or lung structure *per se* (e.g., increased susceptibility to viral infections or decreased steroid responsiveness).

In contrast to our findings for vitamin D and as previously reported (31), African ancestry was significantly associated with a lower FEV_1 in participating children, a result consistent with prior observations in African American adults without asthma (32). Of note, however, lack of association between vitamin D insufficiency and FEV_1 was not explained by African ancestry, because we obtained similar results for vitamin D in an analysis unadjusted for racial ancestry.

Our study has considerable strengths, including its multistage probability sampling design; availability of genome-wide genotypic data for estimation of racial ancestry; and data on time outdoors and atopy in a well-characterized cohort of children at high risk for asthma morbidity (Puerto Ricans). We also recognize significant limitations of the current findings. First, Puerto Ricans have a lower average proportion of African ancestry than African Americans (33), and thus our results should not be generalized to that ethnic group. However, Puerto Ricans are the second largest Hispanic subgroup in the United States, and our results are also relevant to other ethnic groups with similar average proportion of African ancestry throughout the Americas (e.g., populations in southern Brazil and Colombia) (34, 35). Second, we lack data on skin pigmentation, which has been associated with African ancestry in Puerto Ricans and other

TABLE 5. VITAMIN D AND SEVERE DISEASE EXACERBATIONS IN CHILDREN WITH ASTHMA, WITH AND WITHOUT ATOPY, IN PUERTO RICO*

Predictors	Subjects with Atopy (*n = 195*)	Subjects without Atopy (*n = 92*)
Multivariate model		
Vitamin D level < 30 ng/ml	2 (1–4.1), 0.04	6.2 (2–21.6), 0.002
Household income < $15,000 per yr	1.3 (0.6–2.7), 0.5	1.5 (0.4–5.2), 0.5
Use of inhaled corticosteroids in the prior 6 mo	2.8 (1.3–6.3), 0.008	4.3 (1.5–13.5), 0.01
Each 20% increase in African ancestry	1 (0.5–1.7), 0.9	0.9 (0.4–2), 0.8
Virtually always outside	0.4 (0.2–0.9), 0.03	1.1 (0.4–3.4), 0.8
High vitamin D intake (diet or supplements)	0.8 (0.4–1.6), 0.5	1.4 (0.4–4.8), 0.6

*Odds ratio (95% confidence interval), *P* value for severe asthma exacerbations. All models are additionally adjusted for age and sex.

racially admixed populations (36, 37). However, we obtained similar results when Native American and African ancestry were combined in secondary analyses of vitamin D and severe asthma exacerbations adjusting for non-European ancestry (data not shown). Third, there is likely misclassification of certain exposures (e.g., prematurity, early life ETS) in our cross-sectional study, and we lacked information on adherence to prescribed ICS (the most common controller medication used by children in Puerto Rico). The observed association between ICS use and increased odds of severe asthma exacerbations is likely caused by prescription patterns in the island of Puerto Rico, where only children with an exacerbation or greater disease severity may receive controller medications because of financial constraints (38).

In summary, our findings suggest that vitamin D insufficiency leads to an increased risk of severe asthma exacerbations in Puerto Rican children living in the island of Puerto Rico, independently of African racial ancestry, atopy, and time spent outdoors. Our results also suggest that vitamin D influences the pathogenesis of severe asthma exacerbations through mechanisms other than regulation of allergic immune responses. Findings from this study should be helpful in designing the clinical trials needed to determine whether (and how) vitamin D supplementation reduces severe asthma exacerbations in children of school age living in different latitudes, including Puerto Ricans.

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