

## Vitamin D insufficiency, plasma cytokines, and severe asthma exacerbations in school-aged children



Sylvia E. Szentpetery, MD<sup>a,\*</sup>, Yueh-Ying Han, PhD<sup>a,\*</sup>, John M. Brehm, MD, MPH<sup>a,\*</sup>, Edna Acosta-Pérez, PhD<sup>b</sup>, Erick Forno, MD, MPH<sup>a</sup>, Nadia Boutaoui, PhD<sup>a</sup>, Glorisa Canino, PhD<sup>b</sup>, John F. Alcorn, PhD<sup>a</sup>, and Juan C. Celedón, MD, DrPH, FAACAP<sup>a</sup>

### Clinical Implications

- Our findings suggest that vitamin D insufficiency influences the pathogenesis of severe asthma exacerbations through nonatopic mechanisms, such as resistance to inhaled corticosteroids and enhanced susceptibility to viral infections.

### TO THE EDITOR:

Severe asthma exacerbations lead to child distress, missed workdays, and school absences. Such exacerbations account for approximately one-third of the estimated \$56 billion spent per year on asthma care.<sup>1</sup>

In the United States, Puerto Ricans share a disproportionate burden from asthma. We previously showed that vitamin D insufficiency (a plasma 25(OH)D level of <30 ng/mL) is associated with severe asthma exacerbations in Puerto Ricans, with stronger effects in children with nonatopic asthma than in those with atopic asthma.<sup>2</sup>

On the basis of our findings and those of others, we hypothesized that vitamin D insufficiency leads to severe asthma exacerbations through mechanisms other than altered T<sub>H</sub>2 immune responses.<sup>3</sup> To test this hypothesis, we examined vitamin D insufficiency, plasma cytokines, and severe asthma exacerbations in Puerto Rican children.

Also see this article's Online Repository at [www.jaci-inpractice.org](http://www.jaci-inpractice.org). A total of 678 children aged 6 to 14 years were recruited in San Juan using a multistage probability sample design.<sup>2</sup> Only those with data on plasma vitamin D and cytokine levels (n = 578) are included. Study protocol included questionnaires and collection of blood samples. Written parental consent and assent from participating children were obtained, and the study was approved by institutional review boards.

Asthma was defined as physician-diagnosed asthma and 1 or more episode of wheeze in the previous year. A severe asthma exacerbation was defined as 1 or more hospitalization, or 1 or more visit to the emergency department or urgent care for asthma that led to treatment with systemic (oral, intramuscular, or intravenous) corticosteroids, or 1 or more course of systemic steroid for asthma.

IgE to each of 5 allergens (Der p 1, Bla g 2, Fel d 1, Can f 1, Mus m 1) was determined using the UniCAP 100 system (Pharmacia & Upjohn, Kalamazoo, Mich). For each allergen, an IgE level of 0.35 IU/mL or more was considered positive, and

atopy was defined as 1 or more positive IgE to the allergens tested.

Plasma vitamin D (25-hydroxy-vitamin D) was measured using the Waters high-performance liquid chromatography system with tandem mass spectrophotometry (Water Corporation, Milford, Mass). Fourteen cytokines (IL-1 $\beta$ , IL-4, IL-6, IL-10, IL-17A, IL-17F, IL-21, IL-22, IL-23, IL-25, IL-31, IL-33, IFN- $\gamma$ , and TNF- $\alpha$ ) were measured in plasma samples using Bio-Plex Pro Human T<sub>H</sub>17 cytokine panel on the BioPlex HTF system (Bio-Rad Laboratories Inc, Hercules, Calif). Assays were designed on magnetic beads in a capture-sandwich immunoassay format, with samples measured in duplicate and levels presented as pg/mL. Undetectable cytokine levels were assigned a constant (half the lowest limit of detection; see Table E1 in this article's Online Repository at [www.jaci-inpractice.org](http://www.jaci-inpractice.org)). All cytokine levels were log10-transformed for data analysis.

Bivariate analyses were conducted using 2-sample *t* tests or chi-square tests. Linear regression was used for the multivariable analysis of vitamin D or vitamin D insufficiency and cytokine levels, which was conducted in all subjects, and separately in cases and control subjects. Logistic regression was used for the multivariable analysis of vitamin D insufficiency and severe asthma exacerbations, which was stratified *a priori* by atopic versus nonatopic asthma. All multivariable models were adjusted for age, sex, household income (<\$15,000 or  $\geq$ \$15,000 per year, the median household income for Puerto Rico in 2008-2009), and second-hand smoke exposure. Models for severe exacerbations were additionally adjusted for body mass index *z* score,<sup>4</sup> use of inhaled corticosteroids (ICSs) in the previous 6 months, and 4 principal components (PCs) for the 14 cytokines tested. Principal-component analysis was used to reduce the dimensionality of the 14 cytokines to a smaller set of uncorrelated independent components. PCs were extracted using varimax rotation, with the factor selection based on an eigenvalue cutoff of 1.0. SAS version 9.3 (SAS Institute, Inc, Cary, NC) was used for all analyses.

The main characteristics of study participants are summarized in Table E2 in this article's Online Repository at [www.jaci-inpractice.org](http://www.jaci-inpractice.org). Compared with children without asthma (n = 283), those with asthma (n = 295) were more likely to be male and to be currently exposed to second-hand smoke, and had a slightly higher vitamin D level. There were no significant differences in age, household income, type of health insurance, or vitamin D insufficiency between cases and control subjects.

Table I presents the results of the multivariable analysis of the relation between plasma vitamin D and log10-transformed cytokine levels. After a Bonferroni correction for 14 tests (cytokines), vitamin D was found to be significantly and positively associated with IL-10 in all subjects (*P* < .0036). Among all subjects, vitamin D was positively associated with IL-21, IL-25, and IL-31 at *P* < .01. Similar results with regard to the magnitude and direction of the observed associations were obtained in the analysis stratified by case-control status, but there was no significant result (likely due to smaller sample size). Similar findings were also obtained in an analysis of vitamin D insufficiency and cytokine levels (see Table E3 in this article's Online Repository at [www.jaci-inpractice.org](http://www.jaci-inpractice.org)).

**TABLE I.** Multivariable analysis of the relation between plasma vitamin D level (predictor) and log10-transformed cytokine levels (outcomes), in all subjects and by case/control status

| Cytokine      | Coefficient ( $\beta$ ) |                    |                 |
|---------------|-------------------------|--------------------|-----------------|
|               | All subjects (N = 578)  | Controls (n = 283) | Cases (n = 295) |
| IL-1b         | 0.008                   | 0.007              | 0.009           |
| IL-4          | 0.010                   | 0.013              | 0.008           |
| IL-6          | 0.006                   | 0.008              | 0.005           |
| IL-10         | 0.018*                  | 0.018              | 0.018           |
| IL-17A        | -0.004                  | -0.007             | -0.001          |
| IL-17F        | -0.001                  | 0.015              | -0.012          |
| IL-21         | 0.012†                  | 0.010              | 0.014           |
| IL-22         | 0.012                   | 0.005              | 0.016           |
| IL-23         | 0.006                   | 0.002              | 0.009           |
| IL-25         | 0.006†                  | 0.007              | 0.005           |
| IL-31         | 0.014†                  | 0.019              | 0.010           |
| IL-33         | 0.005                   | 0.006              | 0.004           |
| IFN- $\gamma$ | 0.006                   | 0.015              | -0.001          |
| TNF- $\alpha$ | 0.003                   | 0.003              | 0.003           |

Multivariable linear models adjusted for age, sex, household income, current exposure to second-hand smoke, and (in all subjects) case/control status.

\* $P < .0036$  (0.05/14 tests).

† $P < .01$ .

**TABLE II.** Vitamin D insufficiency (plasma 25(OH)D <30 ng/mL) and severe disease exacerbations in the previous year, by atopic vs nonatopic asthma

| Asthma type                   | Vitamin D insufficiency, Odds ratio (95% CI) |                 |                 |
|-------------------------------|----------------------------------------------|-----------------|-----------------|
|                               | Model 1                                      | Model 2         | Model 3         |
| Nonatopic asthma (n = 92)     |                                              |                 |                 |
| ≥1 severe asthma exacerbation | 5.0 (1.6-15.3)*                              | 5.6 (1.8-17.7)* | 5.8 (1.8-18.4)* |
| ≥1 hospitalization for asthma | 5.2 (1.5-17.4)*                              | 5.2 (1.5-18.1)* | 6.2 (1.7-22.5)* |
| Atopic asthma (n = 202)       |                                              |                 |                 |
| ≥1 severe asthma exacerbation | 1.9 (0.9-3.7)                                | 2.2 (1.1-4.4)†  | 1.9 (0.9-3.7)   |
| ≥1 hospitalization for asthma | 2.1 (0.9-4.7)                                | 2.2 (0.9-5.0)   | 2.0 (0.9-4.4)   |

BMI, Body mass index.

All models adjusted for age, sex, household income, BMI z score, and use of ICSs. Model 2 additionally adjusted for plasma levels of IL-10, IL-21, IL-25, and IL-31. Model 3 additionally adjusted for 4 PCs from cytokine analysis.

\* $P < .01$ .

† $P < 0.05$ .

Table II presents the multivariable analysis of vitamin D insufficiency and severe asthma exacerbations or hospitalizations for asthma, by atopic status. After adjusting for age, sex, household income, BMI z score, and use of ICSs, vitamin D insufficiency was significantly associated with 5-fold increased odds of 1 or more severe exacerbation among children with nonatopic asthma (model 1). Similar findings were obtained after the multivariable models were additionally adjusted for levels of all plasma cytokines that were associated with vitamin D at  $P < .01$  in all subjects (IL-10, IL-21, IL-25 and IL-31; model 2) or for the 4 cytokine-derived PCs (model 3). Vitamin D insufficiency was similarly associated with increased odds of 1 or more hospitalization among children with nonatopic asthma (both models). In contrast to our results for nonatopic asthma, vitamin D insufficiency was less strongly and mostly nonsignificantly associated with severe asthma exacerbations or hospitalizations among children with atopic asthma.

Our findings further support detrimental effects of vitamin D insufficiency on severe asthma exacerbations through non-atopic mechanisms, because our results were largely unchanged after adjustment for plasma cytokines. For example, vitamin D may protect against viral or bacterial infections by strengthening epithelial barriers,<sup>5</sup> or by increasing the expression of antimicrobial peptides such as cathelicidin.<sup>6,7</sup> Alternatively, vitamin D may enhance response to ICSs,<sup>8</sup> as suggested by our finding of a positive association between vitamin D and IL-10 among all participants in the present study.

Our study is cross-sectional, and we cannot thus examine temporal relationships. Although we measured IgEs to 5 common allergens in Puerto Rico, we may have underestimated sensitization to other allergens. Moreover, we cannot exclude confounding by unmeasured variables, such as air pollution. Nonetheless, our findings suggest that vitamin D insufficiency leads to severe asthma exacerbations in children through

nonatopic mechanisms, including resistance to ICSs and enhanced susceptibility to viral infections.

<sup>a</sup>Division of Pediatric Pulmonary Medicine, Allergy, and Immunology, Children's Hospital of Pittsburgh of UPMC, University of Pittsburgh, Pittsburgh, Pa

<sup>b</sup>Behavioral Sciences Research Institute, University of Puerto Rico, San Juan, Puerto Rico  
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Corresponding author: Juan C. Celedón, MD, DrPH, FAACAP, Division of Pediatric Pulmonary Medicine, Allergy and Immunology, Children's Hospital of Pittsburgh of UPMC, 4401 Penn Ave, Pittsburgh, PA 15224. E-mail: [juan.celedon@chp.edu](mailto:juan.celedon@chp.edu).

\*These authors contributed equally to this work.

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## REFERENCES

1. Centers for Disease Control and Prevention. Asthma in the U.S. Atlanta, GA: Centers for Disease Control and Prevention; 2011:1-4.
2. Brehm JM, Acosta-Perez E, Klei L, Roeder K, Barmada M, Boutaoui N, et al. Vitamin D insufficiency and severe asthma exacerbations in Puerto Rican children. *Am J Respir Crit Care Med* 2012;186:140-6.
3. Paul G, Brehm JM, Alcorn JF, Holguin F, Aujla SJ, Celedon JC. Vitamin D and asthma. *Am J Respir Crit Care Med* 2012;185:124-32.
4. Kuczmarski RJ, Ogden CL, Grummer-Strawn LM, Flegal KM, Guo SS, Wei R, et al. CDC growth charts: United States. *Adv Data* 2000;314:1-27.
5. Schwalfenberg GK. A review of the critical role of vitamin D in the functioning of the immune system and the clinical implications of vitamin D deficiency. *Mol Nutr Food Res* 2011;55:96-108.
6. White JH. Vitamin D as an inducer of cathelicidin antimicrobial peptide expression: past, present and future. *J Steroid Biochem Mol Biol* 2010;121:234-8.
7. Hansdottir S, Monick MM, Hinde SL, Lova N, Look DC, Hunninghake GW. Respiratory epithelial cells convert inactive vitamin D to its active form: potential effects on host defense. *J Immunol* 2008;181:7090-9.
8. Brehm JM, Schuermann B, Fuhlbrigge AL, Hollis BW, Strunk RC, Zeiger RS, et al. Serum vitamin D levels and severe asthma exacerbations in the Childhood Asthma Management Program study. *J Allergy Clin Immunol* 2010;126:52-58.e5.

## METHODS

## Subject recruitment

Details on subject recruitment and study design have been previously described. In brief, from March 2009 to June 2010, children were recruited from randomly selected households in San Juan, using a multistage probabilistic sampling design. A household was eligible if 1 or more resident was a child aged 6 to 14 years who had 4 Puerto Rican grandparents and had lived in the household for at least 1 year. Of 1111 eligible households, 438 (~39%) had 1 or more child with asthma (defined as physician-diagnosed asthma and at least 1 episode of wheeze in the previous year). From those 438 households, 1 child with asthma was selected (at random, if there was more than 1 such child). Similarly, 1 child without asthma was randomly selected from the remaining 673 households. In an effort to reach a target sample size of approximately 700 children (which would give us  $\geq 90\%$  power to detect an odds ratio of  $\geq 2$  for exposures with a prevalence of  $\geq 25\%$ ), we attempted to enroll a random sample ( $n = 783$ ) of these 1111 children. Parents of 105 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 ( $n = 678$  [86.6%]) and did not ( $n = 105$  [13.4%]) agree to participate. Of the 678 study participants, 578 (85.3%) had blood samples collected and available plasma measurements of cytokines and vitamin D, and are thus included in the current analysis.

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) and the University of Pittsburgh (Pittsburgh, Pa).

Principal-component analysis was conducted using SAS 9.3 version "Proc Factor" procedure specified METHOD=PRIN to request that the principal axis (principal factors) method be used for the initial extraction, specified PRIORS=ONE to perform a principal-component analysis, specified ROTATE=VARIMAX to request a varimax rotation, and specified MINEIGEN=1 to allow the factor selection or component to be retained on the basis of an eigenvalue cutoff of 1.0. All 14 cytokines were included in the initial principal-component analysis analysis that resulted in 4 factors.

TABLE E1. LLD of assays used for cytokine measurements

| Cytokine      | LLD (pg/mL) | All subjects (n = 578) | Controls (n = 283) |             | Cases (n = 295) |             |
|---------------|-------------|------------------------|--------------------|-------------|-----------------|-------------|
|               |             | Median* (pg/mL)        | Median* (pg/mL)    | Number <LLD | Median* (pg/mL) | Number <LLD |
| IL-1 $\beta$  | 0.02        | 0.78                   | 0.82               | 6           | 0.73            | 15          |
| IL-4          | 0.52        | 8.52                   | 8.10               | 114         | 8.79            | 116         |
| IL-6          | 0.67        | 7.86                   | 7.92               | 24          | 7.86            | 37          |
| IL-10         | 0.3         | 7.94                   | 9.14               | 79          | 7.83            | 98          |
| IL-17A        | 0.49        | 8.24                   | 9.69               | 70          | 7.19            | 80          |
| IL-17F        | 0.8         | 32.01                  | 21.65              | 115         | 37.42           | 97          |
| IL-21         | 2.13        | 1.07                   | 1.07               | 167         | 1.07            | 190         |
| IL-22         | 0.03        | 4.60                   | 6.24               | 76          | 2.94            | 113         |
| IL-23         | 1.55        | 0.78                   | 0.78               | 256         | 0.78            | 258         |
| IL-25         | 0.07        | 4.20                   | 4.21               | 2           | 4.19            | 1           |
| IL-31         | 0.49        | 42.57                  | 42.39              | 43          | 42.57           | 59          |
| IL-33         | 0.58        | 59.34                  | 63.50              | 11          | 56.20           | 30          |
| IFN- $\gamma$ | 0.43        | 13.45                  | 11.36              | 98          | 18.22           | 95          |
| TNF- $\alpha$ | 0.07        | 6.58                   | 6.58               | 1           | 6.63            | 0           |

LLD, Lowest limit of detection.

\*Median concentration of cytokine levels.

**TABLE E2.** Main characteristics of study participants (n = 578)

| Variables                                           | Controls (n = 283) | Cases (n = 295) |
|-----------------------------------------------------|--------------------|-----------------|
| Age (y)                                             | 10.5 ± 2.7         | 10.1 ± 2.6      |
| Male sex                                            | 136 (48.1)         | 171 (58.0)*     |
| Household income ≤ \$15,000/y                       | 94 (34.8)          | 91 (31.6)       |
| Private or employer-based health insurance          | 100 (35.3)         | 89 (30.2)       |
| BMI, z score                                        | 0.50 ± 1.1         | 0.64 ± 1.2      |
| Current exposure to ETS                             | 102 (36.0)         | 134 (45.7)*     |
| Serum vitamin D level (ng/mL)                       | 30.9 ± 7.5         | 32.4 ± 8.1*     |
| Serum vitamin D level < 30 ng/mL                    | 132 (46.6)         | 131 (44.1)      |
| Use of ICSs in the previous year                    |                    | 92 (31.2)       |
| ≥1 positive allergen-specific IgE                   |                    | 202 (68.7)      |
| ≥1 severe asthma exacerbation in the previous year† |                    | 155 (52.5)      |
| ≥1 hospitalization for asthma in the previous year‡ |                    | 61 (20.7)       |

BMI, Body mass index; ETS, environmental tobacco smoke.

Data are presented as mean ± SD for continuous variables or number (percentage) for binary variables.

\**P* < .05 comparison between case and control subjects.

†n = 109 (70.3%) had atopic asthma and n = 46 (29.7%) had nonatopic asthma.

‡n = 41 (67.2%) had atopic asthma and n = 20 (32.8%) had nonatopic asthma.

**TABLE E3.** Multivariable analysis of the relation between vitamin D insufficiency (predictor) and log10-transformed cytokine levels (outcomes), by case/control status

| Cytokine | Coefficient (β)        |                    |                 |
|----------|------------------------|--------------------|-----------------|
|          | All subjects (N = 578) | Controls (n = 283) | Cases (n = 295) |
| IL-1β    | −0.110*                | −0.088             | −0.130          |
| IL-4     | −0.116                 | −0.033             | −0.179          |
| IL-6     | −0.052                 | −0.050             | −0.047          |
| IL-10    | −0.193*                | −0.156             | −0.204          |
| IL-17A   | 0.067                  | 0.109              | 0.041           |
| IL-17F   | 0.072                  | −0.053             | 0.194           |
| IL-21    | −0.138*                | −0.044             | −0.222*         |
| IL-22    | −0.078                 | 0.073              | −0.195          |
| IL-23    | −0.054                 | 0.061              | −0.152*         |
| IL-25    | −0.054                 | −0.042             | −0.060          |
| IL-31    | −0.123                 | −0.114             | −0.112          |
| IL-33    | −0.008                 | −0.057             | 0.046           |
| IFN-γ    | −0.024                 | −0.070             | 0.012           |
| TNF-α    | −0.001                 | 0.004              | −0.003          |

LLD, Lowest limit of detection.

Multivariable linear models adjusted for age, sex, household income, current exposure to second-hand smoke, and case/control status (in all subjects).

\**P* < .05.

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