

variability of percent predicted FEF₂₅₋₇₅ showed a relationship with the amount of the albuterol-induced increase in FEF₂₅₋₇₅ and the frequency of greater than 30% improvement in FEF₂₅₋₇₅ in response to albuterol. These findings imply that primary functional abnormality in asthma is not explained simply by specific inflammatory pathways without an involvement of airway smooth muscle controlling airway caliber. In addition, a relationship between the percent predicted FEF₂₅₋₇₅ variability and the albuterol-induced improvement of FEF₂₅₋₇₅ reflects that recurrent airway constriction might be responsible for the high variability of percent predicted FEF₂₅₋₇₅.

Quanjer et al⁹ previously argued that FEF₂₅₋₇₅ did not contribute usefully to clinical decision making. On the contrary, the findings of other previous reports support the clinical importance of the FEF₂₅₋₇₅ measurement in asthma.⁴⁻⁶ In addition, the FEF₂₅₋₇₅ measurements were shown to predict the evolution of asthma and subsequent poor asthma outcomes in the long-term follow-up study.¹⁰ Our findings also support the role of FEF₂₅₋₇₅ in identifying a population of children with suboptimal asthma control because a higher risk of future deterioration in asthma control was associated with the greater amount of the percent predicted FEF₂₅₋₇₅ variability. Moreover, the percent predicted FEF₂₅₋₇₅ variability was observed to be a stronger risk factor for the future control losses than the variabilities of other spirometric indices, supporting that FEF₂₅₋₇₅ seems to be a more sensitive indicator of airway dysfunction than other indices.

In conclusion, the variability of percent predicted FEF₂₅₋₇₅ had a relationship with the albuterol-induced improvement of FEF₂₅₋₇₅, suggesting that the high variability of FEF₂₅₋₇₅ reflects the presence of recurrent reversible airflow constriction. In addition, the variability of percent predicted FEF₂₅₋₇₅ was a better predictor of upcoming asthma control losses than that of other standard spirometric indices. These results indicate that FEF₂₅₋₇₅ is useful in detecting clinically relevant reversible airway constriction in children at risk for deteriorating asthma.

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Vitamin D insufficiency, T_H2 cytokines, and allergy markers in Puerto Rican children with asthma



T_H2 cytokines, including interleukin (IL)-4, IL-5, and IL-13, are central to the development of atopic asthma, driving eosinophilia, bronchial hyperreactivity, and mucus hypersecretion.¹ In recent years, treatment of severe atopic asthma has been expanded to include anti-IL-5 antibodies. In parallel, a growing body of evidence suggests that vitamin D may be beneficial in asthma management,² perhaps through immune regulation.³

In the United States, Puerto Rican (PR) children share a disproportionate burden of asthma. We have reported that vitamin D insufficiency (a plasma 25[OH]D < 30 ng/mL) is associated with severe asthma exacerbations in these children.^{4,5} We first examined the relationship between IL-5 or IL-13 and asthma outcomes, including lung function and allergy markers in PR children. On the basis of our previous findings, we then aimed to test whether vitamin D insufficiency modifies any estimated effect of T_H2 cytokines on asthma outcomes.

A total of 678 children aged 6 to 14 years were recruited in San Juan (PR), using a multistage probability sampling design.⁴ The study was approved by the institutional review boards of the University of Puerto Rico and the University of Pittsburgh, and parental consent

and participants' assent were obtained. The study protocol included questionnaires, spirometry, and blood sample collection. Participants with data on spirometry, plasma cytokines (IL-5 and IL-13), and allergy markers were included in this analysis (n = 523). Asthma was defined as physician-diagnosed asthma and 1 or more episodes of wheeze in the previous year. Control subjects had neither physician-diagnosed asthma nor wheeze in the previous year. Atopy was defined as 1 or more positive (>0.35 kU/L) IgE to any of 5 common allergens (Der p 1, Bla g 2, Fel d 1, Can f 1, Mus m 1), as measured by the UniCAP 100 (Pharmacia & Upjohn, Kalamazoo, Michigan). Plasma IL-5 and IL-13 were measured using Bio-Plex Pro Human cytokine IL-5 and IL-13 sets on the BioPlex HTF system (Bio-Rad, Hercules, California), as per the manufacturer's instructions. We included random duplicates within and between plates, and levels are presented as picograms per milliliter. Undetectable cytokine levels were assigned a constant (half the lowest limit of detection), and IL-5 and IL-13 levels were then log₁₀-transformed for analysis. Plasma vitamin D was measured using Waters high-performance liquid chromatography/mass spectrophotometry (Water Corp. Milford, Massachusetts), and

Table 1

Multivariable Analysis of Vitamin D Insufficiency and Plasma Levels of IL-5 and IL-13 Among Children With Asthma (n = 261)

Exposure	Interleukin-5 (IL-5) ^a β (95% confidence interval) ^b	Interleukin-13 (IL-13) ^a β (95% confidence interval) ^b
Vitamin D insufficiency (n = 117)		
Total immunoglobulin E ^a	0.62 (0.12, 1.11)*	0.28 (−0.17, 0.73)
Eosinophils ^a	0.28 (0.07, 0.49)*	0.20 (0.01, 0.40)*
Vitamin D sufficiency (n = 144)		
Total immunoglobulin E ^a	0.24 (−0.15, 0.22)	0.13 (−0.20, 0.47)
Eosinophils ^a	−0.01 (−0.23, 0.21)	0.01 (−0.18, 0.21)

All models were adjusted for age, sex, household income, body mass index z score, and use of inhaled steroids.

^aLog-10 transformed^bFor example, among children with vitamin D insufficiency, each log-10 unit increment in IL-5 level is associated with an increment of 0.62 log-units (4.17 IU/mL) in total plasma IgE, with a 95% confidence interval of 0.12 log-units (1.32 IU/mL) to 1.11 log-units (12.88 IU/mL).

*P < .05

**P < .01.

vitamin D insufficiency was defined as a plasma 25(OH)D less than 30 ng/mL.

Bivariate analyses were performed using 2-sample *t* tests or χ^2 tests. Linear regression was used for the multivariable analysis of IL-5 or IL-13 and the outcomes of interest. The analysis of IL-5 or IL-13 and lung function measures (forced expiratory volume in 1 second [FEV₁], forced vital capacity [FVC], and FEV₁/FVC) or allergy markers (total IgE and eosinophil count) was first conducted in all participants, and then separately by subgroups (asthma cases vs controls, atopic asthma vs nonatopic asthma). We decided a priori to conduct analyses stratified by vitamin D insufficiency. All multivariable models were adjusted for age, sex, household income (<\$15,000 or ≥\$15,000/year), body mass index (BMI) z score, and secondhand smoke exposure; those for lung function were additionally adjusted for height and height squared. All analyses conducted in children with asthma were also adjusted for use of inhaled steroids in the previous 6 months.

Compared with children without asthma (n = 262), those with asthma (n = 261) were more likely to be atopic and currently exposed to secondhand smoke, and to have parental history of asthma, higher BMI, higher total IgE and eosinophils, and lower FEV₁ and FEV₁/FVC (data not shown). In a multivariable analysis (eTable 1), each log10-unit increment in IL-5 or IL-13 was significantly associated with 181 mL to 202 mL lower FEV₁, 4.1% to 5.4% lower FEV₁/FVC, higher total IgE, and higher eosinophil count. Similar results were observed in the subgroup analysis of cases, controls, and subjects with atopic asthma, but not in that of nonatopic asthma (eTable 1).

Vitamin D insufficiency was significantly associated with higher IL-5 and IL-13 in children with asthma, particularly atopic asthma (eTable 1). In a multivariable analysis stratified by vitamin D insufficiency, IL-5 was significantly associated with increased total IgE and eosinophils in children with asthma and vitamin D insufficiency, but not in those with asthma and sufficient vitamin D levels (Table 1). Among children with asthma and vitamin D insufficiency, each log10-unit increment in IL-5 was associated with 0.62 and 0.28 log10-unit increments in total IgE and eosinophils, respectively. In these children, each log10-unit increment in IL-13 was significantly associated with a 0.20 log10-unit increment in eosinophils (Table 1).

Vitamin D insufficiency was not significantly associated with lung function in children with or without asthma. Moreover, no significant difference was seen in the relation between IL-5 or IL-13 and FEV₁ or FEV₁/FVC between children with and without vitamin D insufficiency. Vitamin D insufficiency was recently reported to be significantly associated with lower FEV₁, but not with FEV₁/FVC, in a study of 3,001 US children⁶; thus, our negative results for FEV₁ could be explained by limited statistical power.

Our findings indicate that IL-5 and IL-13 are associated with lower lung function and elevated IgE and eosinophils in PR children, particularly among those with atopic asthma. Moreover, vitamin D insufficiency was shown to modify the estimated effect of IL-5 or IL-13 on total IgE and eosinophils, potentially up-regulating T_H2 responses in children with asthma. In mouse models of allergic airway disease, vitamin D treatment has been shown to suppress T_H2 activity and reduce IL-5 and IL-4 production,⁷ decreasing serum total IgE and pulmonary eosinophilia.⁸ Notably, vitamin D supplementation has been reported to reduce IL-13 responses in patients with cystic fibrosis.⁹ Thus, our results further suggest that vitamin D could be an adjunct therapy for children with asthma and high total IgE or eosinophilia.

Our study is cross-sectional, and thus we cannot assess temporal relationships. Moreover, we cannot exclude confounding by unmeasured variables (eg, air pollution and adherence to medications), and we did not examine gene-by-vitamin D interactions. In addition, we had limited statistical power to detect modest effects of IL-5 or IL-13 on total IgE or eosinophils in children with asthma and vitamin D sufficiency. Nonetheless, our results suggest that vitamin D insufficiency has detrimental effects on asthma through T_H2 immune mechanisms. Although anti-IL-5 has been shown to be effective in selected subjects with severe asthma,¹⁰ ongoing clinical trials will determine whether vitamin D supplementation is a safe and inexpensive strategy to reduce asthma burden during childhood.

Supplementary Data

Supplementary data associated with this article can be found in the online version at <https://doi.org/10.1016/j.anai.2018.06.004>.

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eTable 1

Multivariate Analysis Between Interleukin-5, Interleukin-13, and Lung Function or Allergy Markers

Outcome	All participants (n = 523)	Controls (n = 262)	Asthma (n = 261) β (95% confidence interval)	Atopic asthma (n = 193)	Non-atopic asthma (n = 51)
Interleukin-5 (IL-5)					
Pre-bronchodilator FEV ₁ (mL) ^a	–181 (–280, –82) [‡]	–197 (–337, –58) [‡]	–172 (–317, –30) [‡]	–179 (–343, –14) [‡]	–64 (–410, 282)
Pre-bronchodilator FVC (mL) ^a	–85 (–204, 33)	–130 (–300, 41)	–37 (–206, 132)	2.9 (–192, 198)	–105 (–490, 279)
Pre-bronchodilator FEV ₁ /FVC (%)	–4.1 (–7.0, –1.2) [‡]	–3.4 (–7.2, 0.5)	–5.3 (–9.7, –0.7) [‡]	–6.2 (–11.6, –0.8) [‡]	–0.2 (–7.3, 6.9)
Total IgE ^b (IU/mL)	0.27 (0.08, 0.46) [‡]	0.20 (–0.05, 0.46)	0.38 (0.09, 0.79) [‡]	0.42 (0.08, 0.77) [‡]	0.12 (–0.49, 0.72)
Eosinophils ^b	0.13 (0.03, 0.23) [‡]	0.12 (–0.02, 0.26)	0.14 (–0.01, 0.29)	0.18 (–0.01, 0.37)	0.07 (–0.22, 0.37)
Vitamin D insufficiency ^c	0.15 (0.02, 0.28) [‡]	0.04 (–0.14, 0.21)	0.29 (0.08, 0.49) [‡]	0.30 (0.06, 0.55) [‡]	–0.02 (–0.40, 0.36)
Interleukin-13 (IL-13)					
Pre-bronchodilator FEV ₁ (mL) ^a	–202 (–290, –114) [‡]	–259 (–394, –123) [‡]	–157 (–271, –42) [‡]	–133 (–259, –7) [‡]	–131 (–506, 244)
Pre-bronchodilator FVC (mL) ^a	–90 (–196, 16)	–156 (–324, 11)	–39 (–174, 97)	19 (–131, 168)	–182 (–600, 235)
Pre-bronchodilator FEV ₁ /FVC (%)	–5.4 (–7.9, –2.8) [‡]	–5.9 (–9.7, –2.1) [‡]	–4.8 (–8.4, –1.3) [‡]	–5.3 (–9.5, –1.2) [‡]	–1.4 (–9.3, 6.5)
Total IgE ^b (IU/mL)	0.20 (0.02, 0.39) [‡]	0.22 (–0.05, 0.49)	0.20 (–0.05, 0.46)	0.29 (0.01, 0.57) [‡]	–0.14 (–0.87, 0.60)
Eosinophils ^b	0.13 (0.03, 0.23) [‡]	0.15 (–0.01, 0.29)	0.12 (–0.01, 0.25)	0.15 (0.01, 0.30) [‡]	0.02 (–0.35, 0.38)
Vitamin D insufficiency ^c	0.15 (0.02, 0.28) [‡]	–0.05 (–0.24, 0.13)	0.33 (0.16, 0.50) [‡]	0.29 (0.10, 0.48) [‡]	0.20 (–0.26, 0.65)

All models were adjusted for age, sex, annual household income, BMI z-score, outdoor physical activity, current exposure to secondhand smoke, and case-control status (for all participants).

FVC, forced vital capacity; FEV₁, forced expiratory volume in 1 second; IgE, immunoglobulin E.

^a Additionally adjusted for height and height squared.

^b Log₁₀ transformed.

^c Defined as a plasma 25(OH)D level <30 ng/mL.

[‡]P < .05;

[‡]P < .01.