Serum Vitamin D Levels and Severity of Childhood Asthma in Costa Rica

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Online Supplement

Methods

Study Population

Children included in the study were index cases for a family–based genetic study of asthma in Costa Rica. Subject recruitment and study procedures have been described in detail elsewhere (1, 2). From February of 2001 to December of 2006, short questionnaires were sent to the parents of 13,125 children ages 6 to 14 years who were enrolled in 113 schools in Costa Rica. Of the 7,282 children whose parents returned screening questionnaires, 2,714 had asthma (defined as physician–diagnosed asthma and either at least two respiratory symptoms or asthma attacks in the previous year). Of these 2,714 children, 616 (22.7%) unrelated children had high probability of having at least 6 great–grandparents born in the Central Valley of Costa Rica and were willing to participate in our study. The former criterion was required to increase the likelihood that children would be descendants of the founder population of the Central Valley (3). There was no significant difference in sex or grade in school between children who did and did not agree to participate in the study.

Written consent was obtained from the parents of participating children, from whom assent was also obtained if ≥8 years old. The study was approved by the Institutional Review Boards of the Hospital Nacional de Niños (San José, Costa Rica) and Brigham and Women's Hospital (Boston, MA).

PROCEDURES

Study participants completed a protocol that included a questionnaire, spirometry, allergy skin testing, measurement of serum total and allergen-specific IgE, peripheral blood eosinophil count, 25–hydroxyvitamin D, and (on a separate visit) assessment of

airway responsiveness to methacholine. In addition, a stool specimen was examined for ova and parasites in the first 137 study participants (because helminths were not found in any instance, stool samples were not collected for the remaining study subjects).

Questionnaires

Parents of each participating child completed slightly modified versions of questionnaires used in the Collaborative Study on the Genetics of Asthma (4) and the International Study of Asthma and Allergies in Childhood (ISAAC) (5). These questionnaires were used to obtain information of demographics, participants' general and respiratory health, healthcare utilization, and various environmental exposures.

Pulmonary Function Testing

Spirometry was conducted following American Thoracic Society recommendations (6) using a Survey Tach Spirometer (Warren E. Collins, Braintree, MA). Subjects were instructed to avoid use of short–acting bronchodilators for at least four hours before testing. Spirometric maneuvers were performed with subjects seated and wearing a noseclip. The best FEV_1 and FVC were selected for data analysis of FEV_1 and FEV_1/FVC. After completing baseline spirometry, subjects were given 200 μg (2 puffs) of an albuterol metered–dose inhaler using a spacer, and spirometry was repeated after 15 minutes.

Methacholine challenge testing

After completion of baseline spirometry, subjects whose FEV_1 was at least 65% of predicted underwent methacholine challenge testing using a slightly modified version of the Chatham protocol (7). The study protocol consisted of five breaths of saline solution followed by one breath of a 1 mg/ml methacholine solution, one and four breaths of a 5

mg/ml methacholine solution, and one breath of a 25 mg/ml methacholine solution. All inhalations, which were taken from a DeVilbiss 646 nebulizer (Sunrise Medical, Carlsbad, CA), lasted 6 seconds and were followed by 2 seconds of breath holding. After each inhalation level, spirometry was performed at 180, 210, and 240 seconds. The test was terminated if the FEV_1 declined by at least 20% from the best FEV_1 value after inhalation of saline solution.

Serum Total and Allergen–specific IgE, and Peripheral Blood Eosinophil Count

Serum total and allergen–specific IgE levels were determined by the UniCAP 250 system (Pharmacia & Upjohn, Kalamazoo, MI), with samples measured in duplicate. Total serum IgE levels were transformed to a $\log_{10}$ scale for data analysis. Serum was assayed for IgE to each of three allergens: cockroach (*Blatella germanica* [*Bla g* 1]); dust mite (*Dermatophagoides pteronyssinus* [*Der p* 1]); and *Ascaris lumbricoides*. Peripheral blood eosinophil count was measured by Coulter–counter techniques and then transformed to a $\log_{10}$ scale for data analysis.

Allergy Skin Testing

Skin testing was performed according to the ISAAC protocol. In addition to histamine and saline controls, the following antigens were applied to the volar surface of the forearm: Dermatophagoides pteronyssinus, Dermatophagoides farinae, Blatella germanica, Periplaneta americana, cat dander, dog dander, mixed grass pollen, mixed tree pollen, and Alternaria tenuis. A test was considered positive if the maximum diameter of the wheal was 3 mm after subtraction of the maximum diameter of the negative control.

Serum 25–Hydroxyvitamin D_3 (25(OH)D)

Serum levels of 25–hydroxyvitamin D (hereafter referred to as vitamin D) are considered as the best circulating biomarker of vitamin D metabolic status and reflect contributions from all sources of vitamin D (i.e. diet and sun exposure) (8, 9). A single measurement of serum vitamin D was performed on all subjects using a radioimmunoassay method in Dr. Bruce Hollis' laboratory at the University of South Carolina (10, 11). Vitamin D levels were transformed to a $\log_{10}$ scale for data analysis. In descriptive analyses, we also categorized vitamin D levels into deficient (<20 ng/ml), insufficient (≥20 and <30 ng/ml), and sufficient (≥30ng/ml) based on previous recommendations (12-14).

Measurement of Der p Allergen in House Dust Samples

Dust samples were collected with a vacuum clear (model 6735; Douglas Quikut; Walnut Ridge, AR) from the following five areas of the child's household: the upper mattress surface of the child's bed; an upholstered chair or sofa in the family room or the living room; and floor samples from the child's bedroom, the family room or living room, and the kitchen. *Der p* was measured in the composite dust sample collected from the five areas of the home by monoclonal antibody enzyme–linked immunosorbent assays (15).

Statistical Analysis

The following variables were examined as possible confounders of the relationship between vitamin D levels and the outcomes of interest: age, gender, serum total and allergen–specific IgE, eosinophil count, FEV_1, FEV_1/FVC, absolute bronchodilator response (ml), parental report of mold/mildew in the home, use of anti–inflammatory medications in the previous year, parental history of asthma, parental

history of eczema, parental history of hay fever, and *Der p* allergen levels. BMI was considered as a gender–specific z–score (calculated based on the US CDC 2000 growth charts) (16).

A descriptive analysis of univariate predictors was performed using quartiles of vitamin D. P values were calculated using the Cochran-Armitage test for trend for binary predictors, and linear regression for continuous variables. We examined the relation between $\log_{10}$ vitamin D and the following continuous outcomes using linear regression: total IgE, eosinophil count, log–transformed dose–response slope to methacholine, baseline FEV_1, and bronchodilator responsiveness (ml). We examined the relation between $\log_{10}$ vitamin D and the following binary outcomes using logistic regression: use of anti–inflammatory medications (inhaled corticosteroids or leukotriene inhibitors) in the previous year, any hospitalization within the past year, any visit to urgent care or the emergency department within the past year, and $PD_{20} \leq 8.58$ μmol of methacholine. Finally, we used a generalized linear model with a negative binomial distribution and a log link function to examine the number (count) of hospitalizations over the past year. Negative binomial regression is similar to Poisson regression for analyzing discrete count data. Negative binomial regression requires fewer assumptions than Poisson regression, and generally results in more conservative P values (17). Negative binomial regression also performs better than Poisson regression when the data are overdispersed, as is the case in this dataset.

A stepwise approach was used to build all multivariate models. All of the final models included vitamin D level, age, gender, BMI z–score, and parental education (as a surrogate for socioeconomic status). Other variables also remained in the final models if

they were significant at $P < 0.05$ or if they satisfied a change in estimate criterion (≥10%) in the parameter estimate (e.g., odds ratio). All analyses were performed using SAS version 9.1 and JMP 7 (both from SAS Institute, Cary, NC).

RESULTS

Predictors of Vitamin D levels

In bivariate analyses of potential predictors of vitamin D levels, only age was statistically significant (β –.007, p = .009), while male gender was nearly significant (β .02, p = .05). BMI z–score and parental education (as a surrogate for socioeconomic status) were not significantly associated with vitamin D levels. However, BMI and socioeconomic status have been previously associated with both asthma and vitamin D levels (18-21), and thus they were included in all multivariate models.

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Table E1. Vitamin D levels and Continuous Measures of Allergy, Asthma Morbidity, and Asthma Severity in Costa Rican Children

	Beta coefficient (95% CI) (P value)		
		Adjusted[1]	
Outcomes	**Unadjusted**	**Model 1**	**Model 2**
Total IgE (IU/ml)[2]	**-.43 (-.82 – -.04) (.03)**	**-.47 (-.86 – -.08) (.02)**	**-.44(-.86 – -.03) (.04)**
Dust Mite specific IgE	**-1.20 (-2.11– -.29) (.01)**	**-1.11 (-2.04 – -.18) (.02)**	-.95 (-1.95 – .03) (.06)
Size of skin test reaction to dust mite	**-.17 (-.32 – -.04) (.01)**	**-.16 (-.30 – -.02) (.03)**	-.10 (-.27 – .05) (.18)
Eosinophil count (cells/m^3)[3]	**-.23 (-.44– -.02) (.03)**	**-.28 (-.49 – -.07) (.009)**	-.22 (-.46 – .008) (.06)
Baseline FEV_1	**-.34 (-.63 – -.05) (.02)**	-.08 (-.28 – .13) (.47)	.02 (-.23 – .26) (.89)
Dose–response slope to methacholine (μmol)	-.26 (-.61 – .07) (.12)	-.32 (-.67 – .02) (.064)	-.27 (-.61 – .08) (.13)
Absolute response to bronchodilator (ml)	**-107 (-190 – -23) (.01)**	**-109 (-191 – -27) (.009)**	**-103 (-197 – -8) (.03)**

[1] Models 1 and 2 were adjusted for age, sex, BMI z–score, and parental education for all outcomes. All models for anti–inflammatory medication use, bronchodilator responsiveness and airway hyperreactivity were additionally adjusted for pre–bronchodilator FEV_1. For model 2, the dose–response slope to methacholine was additionally adjusted for use of anti–inflammatory medications. The remaining variables were adjusted for both anti–inflammatory medication use and airway hyperreactivity.

[2] Outcome was transformed to a $\log_{10}$ scale.

[3] Outcome was transformed to a $\log_{10}$ scale. Model also adjusted for paternal history of hay fever and eczema.

Table E2. Vitamin D Levels and Categorical Measures of Allergy, Asthma Morbidity, and Asthma Severity in Costa Rican Children

	Odds ratio (95% CI) (P value)		
		Adjusted[1]	
Outcomes	**Unadjusted**	**Model 1**	**Model 2**
Total Eosinophil count > 500 cells/m^3	.37 (.11– 1.21) (.10)	**.26 (.07–.90) (.03)**	.28 (.06–1.27) (.10)
Total IgE > 100 IU/ml	**.20 (.05–.81) (.02)**	**.17 (.04–.74) (.02)**	**.08 (.01–.55) (.01)**
Anti–inflammatory medications[2]	.32 (.10 – 1.09) (.07)	**.21 (.06 – .75) (.02)**	.37 (.09 – 1.58) (.18)
Inhaled steroid	.20 (.06 – .68) (.01)	**.14 (.04 – .50) (.004)**	**.21 (.05 – .91) (.04)**
Airway response to ≤8.58 μmol of methacholine	.17 (.03 – 1.06) (.06)	**.16(.03 – 1.0) (.05)**	**.17 (.02 – 1.0) (.05)**
Hospitalizations for asthma in the previous year	**.06 (.004 – .74) (.03)**	**.05 (.004 – .71) (.03)**	**.054 (.003 – .97) (.05)**

Emergency department or urgent care visits for asthma in the previous year	1.87 (.10 – 36.9 (.70)	1.95 (.09 – 40.20) (.67)	.65 (.02 – 25.7) (.82)

[1] Models 1 and 2 were adjusted for age, sex, BMI z–score, and parental education for all outcomes. Airway hyperresponsiveness was additionally adjusted for FEV_1 in all models. For model 2, airway hyperreactivity variables were additionally adjusted for use of anti–inflammatory medications. The anti–inflammatory medication use outcome was additionally adjusted for airway hyperreactivity. The remaining variables were adjusted for both anti–inflammatory medication use and airway hyperreactivity.

[2] Defined as use of inhaled corticosteroids or leukotriene inhibitors.