Folate Deficiency, Atopy, and Severe Asthma Exacerbations in Puerto Rican Children

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Abstract

Background: Little is known about folate and atopy or severe asthma exacerbations. We examined whether folate deficiency is associated with number of positive skin tests to allergens or severe asthma exacerbations in a high-risk population and further assessed whether such association is explained or modified by vitamin D status.

Methods: Cross-sectional study of 582 children aged 6 to 14 years with (n = 304) and without (n = 278) asthma in San Juan, Puerto Rico. Folate deficiency was defined as plasma folate less than or equal to 20 ng/ml. Our outcomes were the number of positive skin tests to allergens (range, 0–15) in all children and (in children with asthma) one or more severe exacerbations in the previous year. Logistic and negative binomial regression models were used for the multivariate analysis. All multivariate models were adjusted for age, sex, household income, residential proximity to a major road, and (for atopy) case/control status; those for severe exacerbations were also adjusted for use of inhaled

corticosteroids and vitamin D insufficiency (a plasma 25[OH] D < 30 ng/ml).

Measurements and Main Results: In a multivariate analysis, folate deficiency was significantly associated with an increased degree of atopy and 2.2 times increased odds of at least one severe asthma exacerbation (95% confidence interval for odds ratio, 1.1–4.6). Compared with children who had normal levels of both folate and vitamin D, those with both folate deficiency and vitamin D insufficiency had nearly eightfold increased odds of one or more severe asthma exacerbation (95% confidence interval for adjusted odds ratio, 2.7–21.6).

Conclusions: Folate deficiency is associated with increased degree of atopy and severe asthma exacerbations in school-aged Puerto Ricans. Vitamin D insufficiency may further increase detrimental effects of folate deficiency on severe asthma exacerbations.

Keywords: asthma; children; folate; Puerto Ricans

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After a rise in prevalence over the preceding decades, nearly 26 million people had active asthma in the United States in 2010 (1). Changes in dietary habits or supplementation of nutrients may partly explain the current burden of asthma in

industrialized countries such as the United States (2).

Folate, a cofactor in the transfer of one-carbon moieties, aids in the formation of S-adenosylmethionine, a key molecule in DNA methylation. Because DNA

methylation can affect gene expression, there has been considerable interest in the role of folate in the pathogenesis of asthma (3). Such interest was further enhanced by a murine model in which high maternal dietary intake of methyl donors

(including but not limited to folate) during pregnancy enhanced allergic airway inflammation and Th2 immune responses in the offspring (4). However, ecological evidence does not support a strong effect of folate supplementation during pregnancy on asthma in humans, as the rise in asthma prevalence preceded the widespread introduction of grain fortification and folate supplementation to prevent neural tube defects in the United States (5). Consistent with this ecological observation, two recent independent reviews of evidence from observational studies concluded that prenatal folate supplementation has no major effects on the development of childhood asthma (3, 6).

Even though multiple studies have examined the relationship between prenatal use of folate and new-onset asthma, very few studies have examined whether folate status is associated with disease morbidity or disease severity in subjects with established asthma (3, 7). This question is relevant, given results of a recent metaanalysis of 11 observational studies, which showed that high dietary intake of vegetables (a source of folate) is associated with 11% reduced risk of wheeze in children and adults (8). Moreover, folate deficiency or a low folate level has been associated with increased risks of wheeze, atopy, or an elevated total IgE in cross-sectional studies of children and adults (9, 10).

Puerto Rican children have not only a high prevalence and morbidity from asthma (11) but also a low dietary intake of vegetables (8). As of 2008, they also had a relatively low intake of folate, despite a 14-year-old island-wide campaign to increase such intake to ameliorate the incidence of neural tube defects (12).

We hypothesized that folate deficiency is significantly associated with severe asthma exacerbations in Puerto Rican children with asthma and with an increased degree of atopy in Puerto Rican children with and without asthma. Because we previously reported that vitamin D insufficiency is associated with severe asthma exacerbations in Puerto Rican children (13), we further hypothesized that vitamin D insufficiency would potentiate an association between folate deficiency and asthma exacerbations or atopy in these children. To examine these hypotheses, we examined the relation between plasma folate level and degree of atopy or severe asthma exacerbations among children living in the metropolitan area of San Juan, Puerto Rico.

Methods

Subject Recruitment

From March of 2009 to June of 2010, children in San Juan (Puerto Rico) were chosen from randomly selected households using a multistage probability sampling design, as previously described (14, 15). Primary sampling units 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.

A total of 7,073 households were selected, and 6,401 (90.5%) were contacted; 1,111 of these 6,401 households had at least one child who met inclusion criteria (i.e., aged 6 to 14 years, four Puerto Rican grandparents, and residence in the same dwelling for at least 1 year). Of these 1,111 households, 438 (39.4%) had at least one eligible child 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, defined as having neither physician-diagnosed asthma nor wheeze in the prior year) was randomly selected from the remaining 673 households.

To reach a target sample size of ~700 children (which would give us $\geq 90\%$ power to detect an odds ratio ≥ 2 for exposures with a prevalence $\geq 25\%$), we attempted to enroll a random sample ($n = 783$) of these 1,111 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 (including 351 cases and 327 control subjects), 582 (85.8%) had serum folate measurements and are thus included in the current analysis.

Study Procedures

Study participants completed a protocol that included administration of questionnaires, spirometry, allergy skin testing, and collection of blood samples. One of the child's parents (usually [for ~93% of subjects] the mother) completed a questionnaire that was slightly modified from one used in the Collaborative Study of

the Genetics of Asthma (16). This questionnaire was used to obtain information about the child's general and respiratory health, sociodemographic characteristics, family history, current exposure to environmental tobacco smoke (ETS), and early-life exposure to ETS (in utero or before 2 years of age).

Height and weight were measured to the nearest centimeter and pound, respectively. Spirometry was conducted with an EasyOne spirometer (NDD Medical Technologies, Andover, MA). All participants had to be free of respiratory illnesses for 4 or more weeks, and they were also instructed to avoid (when possible) the use of inhaled short- and long-acting bronchodilators for at least 4 and at least 12 hours before testing, respectively. Forced expiratory maneuvers were judged to be acceptable if they met or exceeded American Thoracic Society criteria modified for children (17). The best FEV₁ and FVC were selected for data analyses.

Skin test reactivity to aeroallergens, histamine, and saline diluent was assessed using a multitest device (Lincoln Diagnostics, Decatur, IL) on the skin of the forearm (in a site free of eczema). The aeroallergens tested included dust mite mix (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), *Blomia tropicalis*, dust mix, German cockroach (*Blattella germanica*), mouse pelt, dog dander, cat dander, mold mix, *Alternaria tenuis*, mixed tree pollen, mixed grass pollen, mugwort sage, *Penicillium*, weed mix, and ragweed (Alk-Abello, Round Rock, TX). A skin test result was considered positive if the maximum wheal diameter exceeded the diluent wheal diameter by at least 3 mm.

Plasma folate was measured using the UniCel DxI 800 device (Beckman Coulter, Brea, CA), which was adjusted with a six-point Beckman Coulter calibrator. After plasma treatment to release folate from binding proteins, a competitive-binding receptor assay was used. Folate complexes were bound to the solid phase in a reaction vessel, with the subsequent addition of chemiluminescent substrate to assess folate concentration. Folate measurements were reported as ranging from 0 ng/ml to 20 ng/ml (the upper limit of detection) or as greater than 20 ng/ml.

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, PR), Brigham and Women's Hospital (Boston, MA), and the University of Pittsburgh (Pittsburgh, PA).

Statistical Analysis

Plasma folate level was categorized as deficient (≤ 20 ng/ml, heretofore referred to as folate deficiency) or normal (> 20 ng/ml) (18). Our primary outcomes of interest were the degree of atopy (defined as the sum of positive skin tests to the allergens tested [i.e., 0–15]) in all participants, and

(in cases only) having had one or more severe disease exacerbations (defined as having received at least one course, lasting at least 3 days, of systemic corticosteroids for asthma [19]) in the previous year.

Bivariate analyses were conducted using two-tailed *t* tests for pairs of categorical and continuous variables and Fisher exact tests for categorical variables. For the multivariate analysis, we used negative binomial regression to analyze the degree of atopy (given the distribution of the number of skin tests) and logistic regression to analyze one or more severe

asthma exacerbations in the previous year. Because of their potential correlation with plasma folate and atopy or severe asthma exacerbations, the following covariates were examined in bivariate analyses: age, sex, parental education (at least one parent completed high school vs. none), household income ($<$ vs. $\geq$ \$15,000 [near the median income for households in Puerto Rico in 2008–2009]) (20), body mass index as *z*-score (based on CDC 2000 growth charts [21]), parental (paternal or maternal) history of asthma, current exposure to ETS, FEV₁ and FEV₁/FVC, vitamin D insufficiency

Table 1. Main characteristics of participating children

Characteristics	Children with Asthma (<i>n</i> = 304)	Children without Asthma (<i>n</i> = 278)	All Children (<i>n</i> = 582)
Demographics and other covariates			
Age, mean (SD), yr	10.0 (2.6)	10.5 (2.8)*	10.3 (2.7)
Female sex	127 (42)	144 (52)*	271 (47)
BMI, <i>z</i> -score, mean (SD)	0.67 (1.18)	0.51 (1.11)	0.59 (1.15)
Current exposure to environmental tobacco smoke	139 (46)	98 (35) [†]	237 (41)
Household income $\geq$ \$15,000/yr	95 (32)	90 (34)	185 (33)
Parental history of asthma	205 (68)	86 (31) [†]	291 (50)
Residential proximity to a major road, mean (SD), m	334 (269)	380 (301)	356 (285)
Atopy			
Skin test reactivity to allergens			
At least one positive skin test	230 (80)	178 (72)*	408 (77)
Total no. of positive skin tests, mean (SD)	4.4 (4.1)	3.6 (3.9)*	4.1 (4.0)
Skin test reactivity to			
<i>Alternaria</i>	61 (21)	55 (22)	116 (22)
<i>Blomia tropicalis</i>	162 (58)	110 (46) [†]	272 (52)
Cat	97 (34)	78 (32)	175 (33)
Cockroach	100 (35)	62 (26)*	162 (31)
Dog	74 (26)	49 (20)	123 (23)
Dust mix	104 (36)	58 (24) [†]	162 (31)
Dust mite mix	149 (52)	102 (42)*	251 (47)
Grass mix	48 (18)	32 (15)	80 (17)
Mold mix	31 (11)	32 (13)	63 (12)
Mouse	64 (22)	46 (19)	110 (21)
Mugwort/sage	39 (15)	27 (13)	66 (14)
<i>Penicillium</i>	62 (22)	42 (17)	104 (20)
Ragweed	120 (42)	87 (35)	207 (39)
Tree mix	77 (27)	66 (27)	143 (27)
Weed mix	72 (25)	51 (21)	123 (23)
Vitamin D/folate status			
Vitamin D insufficiency [‡]	134 (44)	129 (46)	263 (45)
Folate deficiency [§]	63 (21)	65 (23)	128 (22)
Asthma severity or control			
FEV ₁ , % predicted, mean (SD)	87 (15)	91 (15) [†]	89 (15)
FEV ₁ /FVC, mean (SD)	0.81 (0.091)	0.84 (0.089) [†]	0.82 (0.091)
≥ 1 Severe asthma exacerbation in the prior year	162 (53)		

Definition of abbreviation: BMI = body mass index.

Values shown as *n* (%) unless otherwise noted. Asthma status was determined by physician diagnosis of asthma.

**P* < 0.05 for comparison of cases and control subjects.

[†]*P* < 0.01 for comparison of cases and control subjects.

[‡]Plasma 25(OH)D < 30 ng/ml.

[§]Plasma folate < 20 ng/ml.

^{||}A severe asthma exacerbation was defined as a hospitalization or emergency department visit requiring treatment with systemic corticosteroids or a course of systemic corticosteroids to treat asthma lasting at least 3 days.

(a plasma 25[OH]D level < vs. ≥ 30 ng/ml) (13), distance from the child's residence to a major roadway (22), and use of inhaled corticosteroids (ICS) in the previous 6 months.

A stepwise approach was used to build the multivariate models. All final models included age, sex, household income, residential proximity to a major road, and (for degree of atopy, including all participants) case/control status. Other covariates (*see above*) remained in the final models if they were significant at $P < 0.05$ or if they satisfied a change in estimate criterion ($\geq 10\%$) in the parameter estimate (β coefficient). All first-order interactions between folate and other covariates were evaluated in the final models.

Given prior findings for vitamin D insufficiency (13) and results from our current analysis of folate deficiency of severe asthma exacerbations, we then created a composite score, as follows: 0 if the child had neither vitamin D insufficiency nor folate deficiency, 1 if the child had either vitamin D insufficiency or folate deficiency, and 2 if the child had both vitamin D insufficiency and folate deficiency. We then examined the relation between this composite score and severe asthma exacerbations. SAS version 9.3 (SAS Institute, Cary, NC) was used for all analyses.

Results

Table 1 shows the main characteristics of study participants. Compared with control subjects, cases were slightly younger and more likely to be boys, to be exposed to ETS, to have a parental history of asthma, to have at least one positive skin test to allergens, and to have a greater degree of atopy (a higher number of positive skin tests) but lower spirometric measures of lung function (FEV_1 and FEV_1/FVC). There were no significant differences in household income, residential proximity to a major road, folate deficiency, or vitamin D insufficiency between cases and control subjects.

Table 2 displays the results of bivariate analyses of folate deficiency and covariates of interest. Compared with children with normal folate levels, children with folate deficiency were significantly older, more likely to have a household income above the median level, and more likely to have a

greater number of positive skin tests and vitamin D insufficiency. In addition, there was a non-statistically significant trend for an association between folate deficiency and at least one severe asthma exacerbation in the previous year. Folate deficiency was not significantly associated with sex, body mass index, current exposure to ETS, parental history of asthma, residential proximity to a major road, spirometric measures of lung function (FEV_1 and FEV_1/FVC), or use of inhaled corticosteroids in the previous 6 months.

Table 3 shows the results of the analysis of folate deficiency and degree of atopy in all study participants. In both the bivariate and multivariable analyses, folate deficiency was significantly associated with an increased degree of atopy. After adjustment for case/control status and other covariates, folate deficiency was associated with having ~ 1.25 times (calculated by exponentiating the estimated

coefficient or β , as follows: $e^{0.22}$) more positive skin tests to allergens. For example, a child who would have (on average) four positive skin tests with a normal folate level would instead have (on average) approximately five positive skin tests if he/she had folate deficiency.

Table 4 shows the results of the analysis of folate and severe asthma exacerbations, in cases only. In a multivariate analysis adjusting for age, sex, household income, use of inhaled corticosteroids, vitamin D insufficiency, and residential proximity to a major road (Model 1), there was a non-statistically significant trend for an association between folate deficiency and 1.9 times increased odds of at least one severe asthma exacerbation in the prior year ($P = 0.06$). After additional adjustment for indicators of asthma severity (FEV_1/FVC and atopy, Model 2), folate deficiency was significantly associated with 2.2 times increased odds of

Table 2. Main characteristics of study participants, by folate deficiency (plasma folate ≤ 20 ng/ml)

Characteristics	Folate Deficiency (n = 128)	Normal Folate Level (n = 454)
Demographics and other covariates		
Age, mean (SD), yr	11.4 (2.7)	9.9 (2.6)*
Female sex	67 (52)	244 (54)
BMI, z-score, mean (SD)	0.6 (1.1)	0.6 (1.2)
Current exposure to environmental tobacco smoke	54 (42)	183 (40)
Household income $\geq$ \$15,000/yr	56 (44)	129 (30)*
Parental history of asthma	68 (54)	223 (49)
Residential proximity to a major road, mean (SD), m	372 (298)	351 (282)
Atopy		
≥ 1 Positive skin test to allergens	99 (83)	309 (75)
No. positive skin tests to allergens, mean (SD)	4.9 (4.2)	3.8 (3.9)*
Vitamin D status		
Vitamin D insufficiency†	68 (53)	195 (43)†
Asthma severity or control		
FEV_1 , % predicted, mean (SD)	89 (15)	89 (16)
FEV_1/FVC	0.83 (0.097)	0.82 (0.089)
Use of inhaled corticosteroids in the previous 6 months§	24 (19)	71 (16)
≥ 1 Severe asthma exacerbation in the prior year§	39 (62)	123 (51)

Definition of abbreviation: BMI = body mass index.

Values shown as n (%) unless otherwise noted. Percentages expressed as a proportion of subjects with available data.

* $P < 0.01$ for comparison of children with and without folate deficiency.

†Plasma 25(OH)D < 30 ng/ml.

‡ $P < 0.05$ for comparison of children with and without folate deficiency.

§In children with asthma (cases). A severe asthma exacerbation was defined as a hospitalization or emergency department visit requiring treatment with systemic corticosteroids or a course of systemic corticosteroids to treat asthma lasting at least 3 days.

Table 3. Analysis of folate deficiency and degree of atopy among children with and without asthma (n = 584)

Variable	No. Positive Skin Tests to Allergens Coefficient (95% CI), P Value	
	Unadjusted	Adjusted*
Folate deficiency	0.26 (0.08 to 0.44), <0.01	0.22 (0.03 to 0.41), 0.02
Household income $\geq$ \$15,000/yr		−0.05 (−0.23 to 0.13), 0.6
Residential proximity to a major road, per every 100 m		0.03 (−0.0015 to 0.05), 0.06
Asthma status, case vs. control		0.21 (0.04 to 0.39), 0.02

Definition of abbreviation: CI = confidence interval.

Folate deficiency: plasma folate $\leq$ 20 ng/ml.

*Multivariate model adjusted for age and sex, in addition to the variables shown in the table. Folate deficiency was associated with having ~ 1.25 times (calculated by exponentiating the estimated coefficient or β , as follows: $e^{0.22}$) more positive skin tests to allergens. For example, a child who would have (on average) four positive skin tests with a normal folate level would instead have (on average) approximately five positive skin tests if he/she had folate deficiency.

at least one severe asthma exacerbation in the previous year ($P = 0.04$). In the two multivariate models shown in Table 4, vitamin D insufficiency was associated with 2.7 to 2.8 times increased odds of at least one severe asthma exacerbation in the previous year ($P < 0.01$ in both instances).

Given that folate deficiency and vitamin D insufficiency were each associated with severe asthma exacerbations, we next conducted an analysis of the relation between a composite score for both conditions and severe exacerbations (Figure 1 and Table E1 in the online supplement). In this analysis, children with either folate deficiency or vitamin D insufficiency (but not both, a composite score of 1) had 1.8 times significantly higher odds of at least one severe asthma exacerbation in the previous year than those with normal levels of folate and vitamin D (a composite score of 0). Compared with children with normal levels of both folate and vitamin D, those with

both folate deficiency and vitamin D insufficiency (a composite score of 2) had nearly eightfold significantly greater odds of having had at least one severe asthma exacerbation in the previous year.

Discussion

We show that folate deficiency is significantly associated with degree of atopy in Puerto Rican children with and without asthma. Among Puerto Rican children with asthma, we also report that folate deficiency is significantly associated with severe asthma exacerbations.

Some (23) but not all (24) previous studies have reported an inverse association between folate status and atopy or wheezing in childhood (reviewed in detail in Reference 3), with one recent study showing an association between high folate level and incident mouse allergy among new (adult) workers at a mouse facility (25).

To our knowledge, however, this is the first report of an association between folate deficiency and severe exacerbations in children with asthma. Moreover, we show plausible synergistic detrimental effects of folate deficiency and vitamin D insufficiency on severe asthma exacerbations in Puerto Rican children.

Extrapolating results from animal studies to humans is challenging. One murine study showed that gestational intake of high to very high doses of methyl donors (including folic acid, vitamin B12, choline, L-methionine, zinc, and betaine) worsens the severity of inherited allergic airway disease, and that this was mediated by increased DNA methylation of genes known to down-regulate Th2 immune responses, such as Runt-related transcription factor 3 (*Runx3*) (4). Of note, however, no animal model has examined the exclusive effects of prenatal folate on allergic airway disease, particularly at a dose range more comparable to that used for

Table 4. Analysis of folate deficiency and severe disease exacerbations in the previous year among children with asthma (n = 304)

Variable	At Least 1 Severe Asthma Exacerbation in the Previous Year OR (95% CI), P Value		
	Unadjusted	Model 1*	Model 2*
Folate deficiency	1.6 (0.9–2.8), 0.13	1.9 (1.0–3.7), 0.06	2.2 (1.1–4.6), 0.04
Household income $\geq$ \$15,000 per year		0.7 (0.4–1.3), 0.27	0.7 (0.4–1.4), 0.37
Use of inhaled corticosteroids in the prior 6 mo		4.3 (2.3–7.9), <0.01	4.6 (2.3–9.0), <0.01
Vitamin D insufficiency†		2.7 (1.5–4.7), <0.01	2.8 (1.5–5.2), <0.01
Residential proximity to a major road, per every 100 m		1.2 (1.1–1.3), <0.01	1.2 (1.1–1.3), <0.01
FEV ₁ /FVC, %			1.0 (0.9–1.1), 0.21
Degree of atopy, per each positive skin test to allergens			1.0 (0.9–1.0), 0.25

Definition of abbreviation: CI = confidence interval; OR = odds ratio.

*Multivariate models adjusted for age and sex, in addition to the variables shown in the table.

†Folate deficiency: plasma folate $\leq$ 20 ng/ml. Vitamin D insufficiency: plasma 25(OH)D < 30 ng/ml.

Figure 1. Multivariate analysis of nutritional status (folate deficiency/vitamin D insufficiency) and one or more severe asthma exacerbations in the previous year. In this analysis, children with normal folate (>20 ng/ml) and vitamin D (>30 ng/ml) plasma levels received a score of 0, children with either folate deficiency (plasma level ≤ 20 ng/ml) or vitamin D insufficiency (plasma level < 30 ng/ml) received a score of 1, and children with both folate deficiency and vitamin D insufficiency received a score of 2. The multivariate model was adjusted for age, sex, household income, proximity to a major road, and use of inhaled corticosteroids in the previous 6 months. CI = confidence interval.

prenatal supplementation in humans. Some human studies have reported an association between maternal high folate status and early childhood wheezing (26), but others have not found an association between folate status during pregnancy or at birth and asthma (3, 6, 27). Moreover, two systematic reviews and/or metaanalyses of birth cohort studies concluded that there is no evidence of a major effect of folic acid supplementation during pregnancy on asthma (3, 6). Consistent with such assessment, we had negative findings for asthma *per se*.

In contrast to the extensive literature on folate status in early life and asthma, little is known about folate status (beyond the perinatal period) and either disease exacerbations in children with established asthma or degree of atopy in children with or without asthma. Our findings for folate deficiency and degree of atopy or severe asthma exacerbations may be due to reduced DNA methylation (and thus increased expression) of genes regulating Th2 or Th17 immune responses. Alternatively, folate deficiency may be a marker of reduced dietary intake of vegetables, which has been associated with increased risk of wheeze and could lead to reduced levels of vitamins with antioxidant or antiviral properties (e.g., vitamins A, C, and E) (8).

In general agreement with our results, a cross-sectional study of 8,083 children and adults (aged 2–85 yr) showed that serum folate was inversely associated with wheeze, total IgE, and atopy (defined as a positive skin test to at least one allergen) (9). Similarly, serum folate was inversely associated with total IgE in a study of 120 adults with asthma (10). In contrast to findings from those cross-sectional studies (9, 10), a multivariate analysis of data from a 1-year prospective study of 144 children with persistent asthma (aged 5–17 yr) showed that serum folate was not significantly associated with symptoms or acute visits for asthma, lung function, fractional exhaled nitric oxide, number of positive skin tests to allergens, or asthma-related hospitalizations; although folate level was significantly but nonlinearly associated with total IgE, such finding is of unclear relevance, given all other negative results. A separate study of 138 children studied between ages 2 and 8 years also reported results opposite of ours, in that a cluster of participants with high folate levels in early childhood had subsequently increased rates of allergic sensitization (28). The discrepancy between our findings and those from other studies (7, 28) may be explained by differences in sample size (and thus statistical power), age and race/ethnicity of

participants, timing of folate measurement, and degree of adjustment for potential confounders.

We noted the cooccurrence of folate deficiency and vitamin D insufficiency, suggesting coexisting poor dietary intake and reduced sun exposure: these two vitamin levels likewise have been noted to be low in a previous study of adolescents with and without asthma (29). A 6-month trial of vitamin D supplementation to prevent treatment failure in 408 adults with symptomatic persistent asthma (on ICS) and vitamin D insufficiency reported no effect on the primary outcome (30). However, there was a 37% reduction in the overall exacerbation rate in the vitamin D arm compared with the placebo arm (95% confidence interval [CI] for hazard ratio, 0.39–1.01; $P = 0.05$). Moreover, subjects who achieved vitamin D sufficiency on the vitamin D arm had overall rates that were 40% and 50% lower for treatment failure (95% CI, 0.4–0.9; $P = 0.03$) and asthma exacerbations (95% CI, 0.3–0.8; $P = 0.01$), respectively. Among school-aged children, vitamin D insufficiency has been associated with severe asthma exacerbations in cross-sectional and longitudinal studies including a total of 1,900 subjects in Costa Rica, Puerto Rico, and North America (13, 31, 32). This link, which is consistent

with our results, may be explained by effects of vitamin D on viral illnesses (and thus viral-induced exacerbations), response to inhaled corticosteroids, or antioxidant effects (22, 33).

We recognize several limitations of our findings. First, ours is a cross-sectional study, and thus we cannot determine a temporal relationship between folate status and severe asthma exacerbations or degree of atopy. However, “reverse causation” (e.g., increased severity of atopy leading to food allergies and reduced intake of folate or vitamin D) is an unlikely explanation for our findings for severe asthma exacerbations, which were essentially unchanged after additional adjustment for atopy or FEV₁/FVC. Second, selection bias or confounding is possible in any observational study such as ours. However, selection bias is unlikely to be a major explanation for our results because of both sampling design and the high rate of participation of eligible

subjects. Similarly, confounding by poverty or socioeconomic status is unlikely, given results from multivariate analyses and the fact that folate deficiency was less common in children with lower household income (a correlate of worse asthma severity or control), perhaps due to consumption of more folate-rich staple foods among poorer Puerto Ricans. Third, we cannot exclude a linear relation between folate level and severe exacerbations or atopy, given that levels above 20 ng/ml were not reported as unique values. However, we used a threshold that is both clinically relevant and commonly used. Fourth, we lack data on ICS dose, an indicator of asthma severity. However, ICS use is infrequent in Puerto Rico (Table 2), and our multivariate analysis of exacerbations was adjusted for lung function and ICS use in the prior 6 months. Finally, our findings may not be generalizable to non-Puerto

Rican children. However, our results may be broadly relevant to underserved populations at high risk for asthma morbidity (e.g., African Americans).

In summary, our findings suggest that folate deficiency, acting alone or in conjunction with vitamin D insufficiency, may lead to severe asthma exacerbations and increased degree of atopy in school-aged children in Puerto Rico. Future prospective studies of serum or plasma folate level (jointly assessed with that of vitamin D and other nutrients), atopy, and severe asthma exacerbations are needed in school-aged Puerto Rican and underserved children. ■

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References

- Akinbami LJ, Moorman JE, Bailey C, Zahran HS, King M, Johnson CA, Liu X. Trends in asthma prevalence, health care use, and mortality in the United States, 2001–2010. *NCHS Data Brief* 2012;94:1–8.
- Han YY, Blatter J, Brehm JM, Forno E, Litonjua AA, Celedón JC. Diet and asthma: vitamins and methyl donors. *Lancet Respir Med* 2013;1: 813–822.
- Blatter J, Han YY, Forno E, Brehm J, Bodnar L, Celedón JC. Folate and asthma. *Am J Respir Crit Care Med* 2013;188:12–17.
- Hollingsworth JW, Maruoka S, Boon K, Garantzios S, Li Z, Tomfohr J, Bailey N, Potts EN, Whitehead G, Brass DM, et al. *In utero* supplementation with methyl donors enhances allergic airway disease in mice. *J Clin Invest* 2008;118:3462–3469.
- Ownby DR. Has mandatory folic acid supplementation of foods increased the risk of asthma and allergic disease? *J Allergy Clin Immunol* 2009;123:1260–1261.
- Crider KS, Cordero AM, Qi YP, Mulinare J, Dowling NF, Berry RJ. Prenatal folic acid and risk of asthma in children: a systematic review and meta-analysis. *Am J Clin Nutr* 2013;98:1272–1281.
- Lin JH, Matsui W, Aloe C, Peng RD, Diette GB, Breyse PN, Matsui EC. Relationships between folate and inflammatory features of asthma. *J Allergy Clin Immunol* 2013;131:918–920.
- Seyedrezazadeh E, Moghaddam MP, Ansarin K, Vafa MR, Sharma S, Kolahdooz F. Fruit and vegetable intake and risk of wheezing and asthma: a systematic review and meta-analysis. *Nutr Rev* 2014;72:411–428.
- Matsui EC, Matsui W. Higher serum folate levels are associated with a lower risk of atopy and wheeze. *J Allergy Clin Immunol* 2009;123: 1253–1259.e2.
- Farres MN, Shahin RY, Melek NA, El-Kabarity RH, Arafa NA. Study of folate status among Egyptian asthmatics. *Intern Med* 2011;50: 205–211.
- Moorman JE, Rudd RA, Johnson CA, King M, Minor P, Bailey C, Scalia MR, Akinbami LJ; Centers for Disease Control and Prevention (CDC). National surveillance for asthma: United States, 1980–2004. *MMWR Surveill Summ* 2007;56:1–54.
- Garcia-Fragoso L, Garcia-Garcia I, Rivera CE. The use of folic acid for the prevention of birth defects in Puerto Rico. *Ethn Dis* 2008;18: S2–168–171.
- Brehm JM, Acosta-Pérez E, Klei L, Roeder K, Barmada M, Boutaoui N, Forno E, Kelly R, Paul K, Sylvia J, et al. Vitamin D insufficiency and severe asthma exacerbations in Puerto Rican children. *Am J Respir Crit Care Med* 2012;186:140–146.
- Bird HR, Canino GJ, Davies M, Duarte CS, Febo V, Ramírez R, Hoven C, Wicks J, Musa G, Loeber R. A study of disruptive behavior disorders in Puerto Rican youth: I. Background, design, and survey methods. *J Am Acad Child Adolesc Psychiatry* 2006;45:1032–1041.
- Brehm JM, Acosta-Pérez E, Klei L, Roeder K, Barmada MM, Boutaoui N, Forno E, Cloutier MM, Datta S, Kelly R, et al. African ancestry and lung function in Puerto Rican children. *J Allergy Clin Immunol* 2012;129:1484–90.e6.
- Blumenthal MN, Banks-Schlegel S, Bleeker ER, Marsh DG, Ober C. Collaborative studies on the genetics of asthma: National Heart, Lung and Blood Institute. *Clin Exp Allergy* 1995;25:29–32.
- American Thoracic Society. Standardization of spirometry, 1994 update. *Am J Respir Crit Care Med* 1995;152:1107–1136.
- Sachdev HP, Shah D. Folate. In: Kliegman R, Stanton B, St. Geme J, Schor N, Behrman R, editors. *Nelson textbook of pediatrics*, 19th ed. Philadelphia: Elsevier/Saunders; 2011. pp. 327–328.
- Reddel HK, Taylor DR, Bateman ED, Boulet LP, Boushey HA, Busse WW, Casale TB, Chaney P, Enright PL, Gibson PG, et al.; American Thoracic Society/European Respiratory Society Task Force on Asthma Control and Exacerbations. An official American Thoracic Society/European Respiratory Society statement. Asthma control and exacerbations: standardizing endpoints for clinical asthma trials and clinical practice. *Am J Respir Crit Care Med* 2009;180: 59–99.
- Coates RJ, Weiss NS, Daling JR, Morris JS, Labbe RF. Serum levels of selenium and retinol and the subsequent risk of cancer. *Am J Epidemiol* 1988;128:515–523.
- Kuczmarski RJ, Ogden CL, Grummer-Strawn LM, Flegal KM, Guo SS, Wei R, Mei Z, Curtin LR, Roche AF, Johnson CL. CDC growth charts: United States. *Adv Data* 2000;314:1–27.
- Rosser F, Brehm JM, Forno E, Acosta-Pérez E, Kurland K, Canino G, Celedón JC. Proximity to a major road, vitamin D insufficiency, and severe asthma exacerbations in Puerto Rican children. *Am J Respir Crit Care Med* 2014;190:1190–1193.
- Matsui EC, Matsui W. Higher serum folate levels are associated with a lower risk of atopy and wheeze. *J Allergy Clin Immunol* 2009;123: 1253–9.e2.

- 24 Thuesen BH, Husemoen LL, Ovesen L, Jørgensen T, Fenger M, Gilderson G, Linneberg A. Atopy, asthma, and lung function in relation to folate and vitamin B(12) in adults. *Allergy* 2010;65: 1446–1454.
- 25 Keet CA, Shreffler WG, Peng RD, Matsui W, Matsui EC. Associations between serum folate and vitamin D levels and incident mouse sensitization in adults. *J Allergy Clin Immunol* 2014;133:399–404.
- 26 Håberg SE, London SJ, Stigum H, Nafstad P, Nystad W. Folic acid supplements in pregnancy and early childhood respiratory health. *Arch Dis Child* 2009;94:180–184.
- 27 van der Valk RJ, Kiefte-de Jong JC, Sonnenschein-van der Voort AM, Duijts L, Hafkamp-de Groen E, Moll HA, Tiemeier H, Steegers EA, Hofman A, Jaddoe VW, *et al*. Neonatal folate, homocysteine, vitamin B12 levels and methylenetetrahydrofolate reductase variants in childhood asthma and eczema. *Allergy* 2013;68:788–795.
- 28 Okupa AY, Lemanske RF Jr, Jackson DJ, Evans MD, Wood RA, Matsui EC. Early-life folate levels are associated with incident allergic sensitization. *J Allergy Clin Immunol* 2013;131:226–8. e1, 2.
- 29 Bueso AK, Berntsen S, Mowinckel P, Andersen LF, Lødrup Carlsen KC, Carlsen KH. Dietary intake in adolescents with asthma: potential for improvement. *Pediatr Allergy Immunol* 2011;22:19–24.
- 30 Castro M, King TS, Kunselman SJ, Cabana MD, Denlinger L, Holguin F, Kazani SD, Moore WC, Moy J, Sorkness CA, *et al*; National Heart, Lung, and Blood Institute's AsthmaNet. Effect of vitamin D3 on asthma treatment failures in adults with symptomatic asthma and lower vitamin D levels: the VIDA randomized clinical trial. *JAMA* 2014;311:2083–2091.
- 31 Brehm JM, Celedón JC, Soto-Quiros ME, Avila L, Hunninghake GM, Forno E, Laskey D, Sylvia JS, Hollis BW, Weiss ST, *et al*. Serum vitamin D levels and markers of severity of childhood asthma in Costa Rica. *Am J Respir Crit Care Med* 2009;179:765–771.
- 32 Brehm JM, Schuemann B, Fuhlbrigge AL, Hollis BW, Strunk RC, Zeiger RS, Weiss ST, Litonjua AA; Childhood Asthma Management Program Research Group. Serum vitamin D levels and severe asthma exacerbations in the Childhood Asthma Management Program study. *J Allergy Clin Immunol* 2010;126:52–58 e5.
- 33 Paul G, Brehm JM, Alcorn JF, Holguin F, Aujla SJ, Celedón JC. Vitamin D and asthma. *Am J Respir Crit Care Med* 2012;185:124–132.