

Letter to the Editor

Mendelian randomization analysis demonstrates that low vitamin D is unlikely causative for pediatric asthma*To the Editor:*

Asthma is the most common chronic childhood disease, affecting approximately 8% of children in the United States. Multiple studies show strong associations between low serum 25-hydroxyvitamin D (25(OH)D) concentration and childhood asthma incidence and severity.¹⁻³ Vitamin D deficiency is highly prevalent in atopic pediatric patients, and vitamin D has been hypothesized to contribute to childhood asthma through regulation of airway reactivity, sensitivity to corticosteroids, or modulation of immune function.⁴ Vitamin D supplementation studies, however, have failed to reveal a benefit for vitamin D in preventing or treating asthma.^{5,6} Recently, Mendelian randomization has been used to help dissect direction and extent of causality identified in association studies between modifiable risk factors and disease.

Our overarching goal was to use Mendelian randomization to examine the hypothesis that low 25(OH)D concentration contributes significantly to asthma prevalence or exacerbations. First, we performed cross-sectional analyses to confirm the inverse association between serum 25(OH)D concentration and asthma in our cohort. We then performed a genome-wide association study (GWAS) to confirm that previously identified genetic determinants of vitamin D status were similarly determinative of 25(OH)D concentration in our cohort. Finally, we used the genetic determinants confirmed in our GWAS to perform our Mendelian randomization.

The Children's Hospital of Philadelphia Center for Applied Genomics biorepository consisted of 56,835 subjects; 12,842 were used in the Asthma Study Cohort. Asthma severity was inferred from the electronic medical record (see Figs E1 and E2 in this article's Online Repository at www.jacionline.org) per the National Asthma Education and Prevention Program guidelines.⁷ Our cross-sectional analysis was done using Stata 13.1. Our

Mendelian randomization was performed using R (<http://www.r-project.org/>). Our cross-sectional and Mendelian randomization analyses had greater than 90% power to detect an odds ratio of 1.73 and 1.4, respectively (see Tables E1 and E2 in this article's Online Repository at www.jacionline.org).⁸

Our cross-sectional analysis was performed on 642 individuals from the Asthma Study Cohort who had serum 25(OH)D concentration evaluated as part of routine clinical care (296 patients and 348 controls). Similar to previous reports,⁹ more than half of all subjects (60.6%) in our cohort were vitamin D insufficient (25(OH)D concentration < 30 ng/mL), 27.8% were vitamin D deficient (<20 ng/mL), and 6.2% were severely vitamin D deficient (<10 ng/mL). Unadjusted serum 25(OH)D concentrations were lower in patients with asthma than in controls (25.8 ng/mL vs 28.1 ng/mL; $P = .008$). In multivariate logistic regression, a 10-ng/mL increase in 25(OH)D concentration was associated with an 18% decreased odds of asthma ($P = .02$; Fig 1, A).

Of asthma cases, 74 subjects (26%) were treated for at least 1 severe exacerbation. Serum 25(OH)D concentration was inversely associated with the frequency of systemic steroids such that for every 10-ng/mL increase in serum 25(OH)D concentration, there was a 34% decrease in the frequency of systemic glucocorticoids for severe asthma exacerbations ($P < .002$; Fig 1, B). Our cohort reproduced similar associations between 25(OH)D concentration and asthma reported previously.¹⁻³

We performed a GWAS on 1388 white subjects in the Center for Applied Genomics vitamin D cohort (Table I) to confirm that loci previously associated with serum 25(OH)D concentration were associated with serum 25(OH)D concentration in our cohort; our GWAS confirmed significant associations between serum 25(OH)D concentration and single-nucleotide polymorphisms (SNPs) near 2 genes (*GC* and *CYP2R1*). Consistent with previous reports in the literature, we found that the gene variants identified that affect serum 25(OH)D concentration are responsible for approximately 10% of the population variation in 25(OH)D concentration (eg, 10-15 ng/mL or 25-37 nmol/L); having variants that positively affect 25(OH)D concentration as

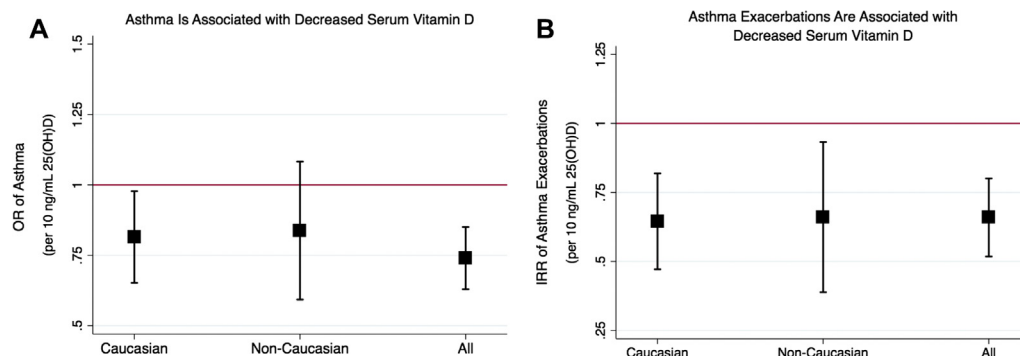


FIG 1. A, Multivariate logistic regression model stratified by race comparing the odd ratio (OR) of asthma cases. **B**, Multivariate logistic regression model stratified by race comparing the OR of severe asthma exacerbations. Multivariate Poisson regression with robust variance stratified by race comparing the incidence rate ratio (IRR) of severe asthma exacerbations for cases with at least 1 severe exacerbation. OR/IRR is for a 10-ng/mL change in serum 25(OH)D concentration. All models are adjusted for age, sex, body mass index Z score, and month. Error bars indicate the 95% CI.

TABLE I. Associations of SNPs and vitamin D association testing

Dependent variable	Gene	Adjusted*			Nonadjusted†		
		Beta	95% CI	P value	Beta	95% CI	P value
rs2282679	GC	6.51×10^{-1}	4.32×10^{-1} to 8.70×10^{-1}	5.79×10^{-9}	4.60×10^{-1}	2.83×10^{-1} to 6.36×10^{-1}	5.16×10^{-4}
rs17467825	GC	6.55×10^{-1}	4.34×10^{-1} to 8.75×10^{-1}	5.73×10^{-9}	4.66×10^{-1}	2.89×10^{-1} to 6.44×10^{-1}	2.32×10^{-10}
rs1155563	GC	5.50×10^{-1}	3.38×10^{-1} to 7.62×10^{-1}	3.54×10^{-7}	3.84×10^{-1}	2.11×10^{-1} to 5.57×10^{-1}	4.09×10^{-10}
rs7041	GC	4.91×10^{-1}	2.87×10^{-1} to 6.95×10^{-1}	2.40×10^{-6}	5.05×10^{-1}	3.38×10^{-1} to 6.72×10^{-1}	1.11×10^{-8}
rs10741657	CYP2R1	3.41×10^{-1}	1.03×10^{-1} to 5.79×10^{-1}	4.99×10^{-3}	3.29×10^{-1}	1.33×10^{-1} to 5.26×10^{-1}	3.04×10^{-4}
rs10766192	CYP2R1	3.73×10^{-1}	1.62×10^{-1} to 5.83×10^{-1}	5.26×10^{-4}	2.03×10^{-1}	3.22×10^{-2} to 3.73×10^{-1}	8.94×10^{-7}
rs10500804	CYP2R1	3.54×10^{-1}	1.46×10^{-1} to 5.62×10^{-1}	8.68×10^{-4}	2.00×10^{-1}	3.10×10^{-2} to 3.69×10^{-1}	7.28×10^{-2}
rs7944926	DHCR7/ NADSYN1	2.35×10^{-1}	-2.08×10^{-1} to 2.55×10^{-1}	8.42×10^{-1}	1.07×10^{-1}	-7.27×10^{-2} to 2.87×10^{-1}	1.99×10^{-11}
Score (rs2282679, rs10741657)	GC, CYP2R1	3.09×10^{-1}	2.13×10^{-1} to 4.06×10^{-1}	3.42×10^{-10}	2.38×10^{-1}	1.60×10^{-1} to 3.15×10^{-1}	1.82×10^{-9}

*Multivariate linear regression model adjusted for age, sex, body mass index Z score, and season.

†Univariate linear regression model.

TABLE II. Associations of asthma indices and vitamin D genetic risk score

Clinical phenotype	Beta	95% CI	P value
Asthma cases*	-3.51×10^{-4}	-4.36×10^{-3} to 4.32×10^{-3}	.85
Asthma severity†	2.15×10^{-2}	-8.77×10^{-3} to 5.31×10^{-2}	.16
Severe exacerbations (no exacerbations vs any)*	-8.33×10^{-3}	-1.02×10^{-1} to 8.51×10^{-2}	.86
Severe exacerbations frequency‡	-2.14×10^{-2}	-5.06×10^{-2} to 8.60×10^{-3}	.17

*Univariate logistic regression.

†Univariate ordinal logistic regression.

‡Univariate zero-inflated Poisson regression.

compared with those that negatively affect 25(OH)D concentration is the equivalent of a lifetime of supplementation with 1000 to 2000 IU of cholecalciferol. We used the SNP rs10741657 located near the *CYP2R1* gene on chromosome 11p15 and the SNP rs2282679 located in the vitamin D-binding protein (*GC*) gene on chromosome 4p12 to generate a vitamin D propensity score. Scores generated with these SNPs were highly correlated with serum 25(OH)D concentration (odds ratio, 1.92; 95% CI, 1.54-2.39; $P = 2.52 \times 10^{-15}$). We used these scores to perform a Mendelian randomization. In contrast to our cross-sectional analysis, genetic risk scores in our Mendelian randomization analysis were not associated with cases of asthma ($\beta = -0.0000351$; $P = .85$; Table II). We also found no associations between vitamin D genetic propensity scores and severe asthma exacerbations ($\beta = -0.00833$; $P = .86$; Table II).

Increasing serum 25(OH)D concentration was associated with decreased odds of asthma and was further associated with decreased frequency of severe asthma exacerbations in our cross-sectional analysis. This result supports the idea that our cohort is substantially similar to previously evaluated cohorts. In our well-powered Mendelian randomization analysis, however, the genetic vitamin D propensity score was not associated with asthma or severe asthma exacerbations. Recent studies that show no benefit of vitamin D supplementation for preventing or treating asthma^{5,6} are consistent with the Mendelian randomization results we present here. These findings lend support to the idea that low 25(OH)D concentration may not cause asthma, but that instead asthma predisposes to low 25(OH)D concentration.

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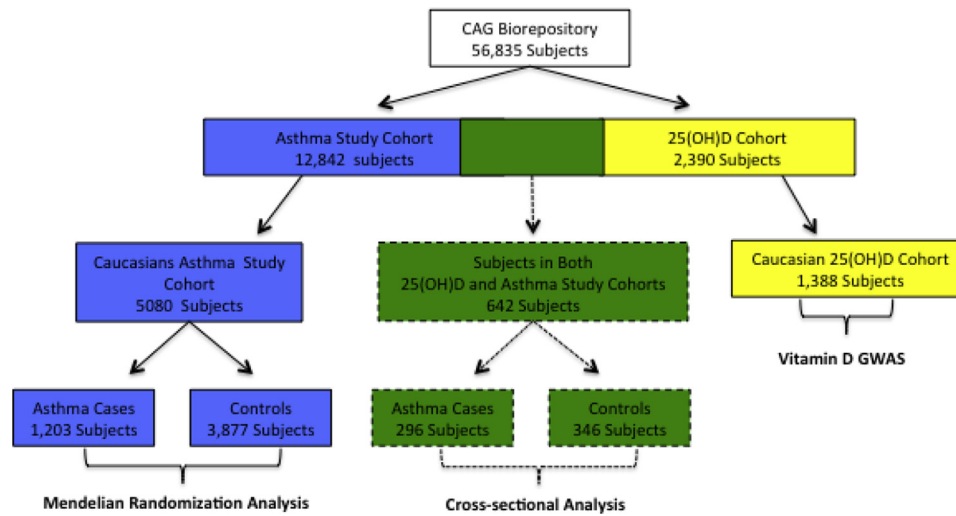


FIG E1. Center for Applied Genomics study cohorts.

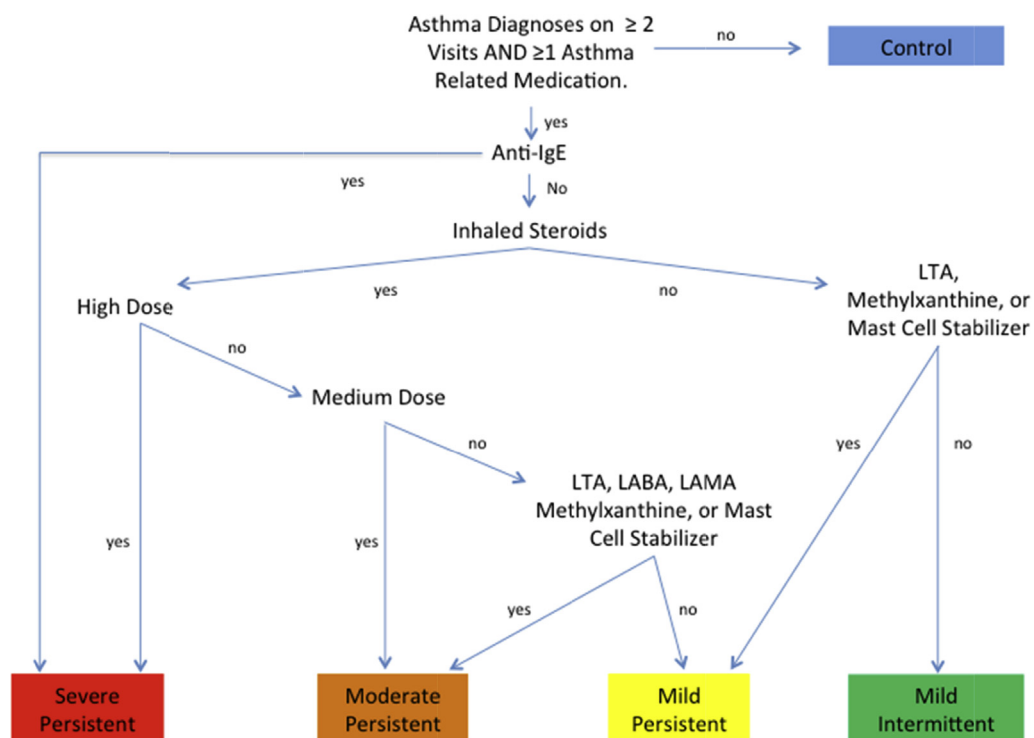


FIG E2. Algorithm for the identification of asthma and asthma severity. *LABA*, Long-acting beta-agonist; *LAMA*, long-acting muscarinic antagonists; *LTA*, leukotriene antagonists.

TABLE E1. Power calculations for cross-sectional analysis

Sample size	642	
Alpha	0.05	
Case/control ratio	0.83	
Vitamin D deficiency prevalence	0.6	
Power	0.8	0.9
Detectable odds ratio	1.60	1.73

TABLE E2. Power calculations for Mendelian randomization

Sample size	5080		
Alpha	0.05		
<i>K</i> (proportion of cases)	0.23		
Variance explained by propensity scores	0.1		
Odds ratio	1.3	1.4	1.5
Noncentrality parameter	7.19	12.37	18.76
Power	0.74	0.94	0.99