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African Ancestry and Lung Function in Puerto Rican Children

John M. Brehm, MD, MPH¹, Edna Acosta-Pérez, PhD², Lambertus Klei, PhD³, Kathryn Roeder, PhD⁴, Michael Barmada, PhD⁵, Nadia Boutaoui, PhD¹, Erick Forno, MD, MPH⁶, Michelle Cloutier, MD⁷, Soma Datta, MS⁸, Roxanne Kelly, MBA⁸, Kathryn Paul, BS⁸, Jody Sylvia, MS⁸, Deanna Calvert, BS⁹, Sherell Thornton-Thompson, CCRP⁹, Dorothy Wakefield, MS⁷, Augusto A. Litonjua, MD, MPH⁸, María Alvarez, MD², Angel Colón-Semidey, MD², Glorisa Canino, PhD², and Juan C. Celedón, MD, DrPH^{1,*}

¹Division of Pediatric Pulmonary Medicine, Allergy and Immunology, Dept. of Pediatrics, Children's Hospital of Pittsburgh of UPMC, University of Pittsburgh School of Medicine, Pittsburgh, PA

²Behavioral Sciences Research Institute, University of Puerto Rico, San Juan, Puerto Rico

³Department of Psychiatry, University of Pittsburgh School of Medicine

⁴Department of Statistics, Carnegie Mellon University, Pittsburgh, PA

⁵Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh

⁶Division of Pediatric Pulmonology, Dept. of Pediatrics, University of Miami, Miami, FL

⁷Department of Pediatrics, University of Connecticut Health Center, Farmington, CT

⁸Channing Laboratory, Dept. of Medicine, Brigham and Women's Hospital, Boston, MA

⁹Connecticut Children's Medical Center, Hartford, CT

Abstract

Background—Puerto Ricans and African Americans share a significant proportion of African ancestry. Recent findings suggest that African ancestry influences lung function in African American adults.

Objective—To examine whether a greater proportion of African ancestry is associated with lower FEV₁ and FVC in Puerto Rican children, independently of socioeconomic status (SES), healthcare access or key environmental/lifestyle (EL) factors.

Methods—Cross-sectional case-control study of 943 Puerto Rican children ages 6 to 14 years with (n=520) and without (n=423) asthma (defined as physician-diagnosed asthma and wheeze in the prior year) living in Hartford (CT, n=383) and San Juan (PR, n=560). We estimated the percentage of African racial ancestry in study participants using genome-wide genotypic data. We tested whether African ancestry is associated with FEV₁ and FVC using linear regression. Multivariate models were adjusted for indicators of SES and healthcare, and selected EL exposures.

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*Corresponding author: Juan C. Celedón, M.D., Dr.P.H., Division of Pediatric Pulmonary Medicine, Allergy and Immunology, Dept. of Pediatrics, Children's Hospital of Pittsburgh of UPMC, 4401 Penn Avenue, Pittsburgh, PA 15224, Phone: 412.692.8429; Fax: 412.692.7636, juan.celedon@chp.edu.

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Results—After adjustment for household income and other covariates, each 20% increment in African ancestry was significantly associated with lower pre-bronchodilator(BD) FEV₁ (–105 ml, 95% confidence interval [CI] = –159 ml to –51 ml, P <0.001) and FVC (–133 ml, 95% CI –197 ml to –69 ml, P <0.001), and post-BD FEV₁ (–152 ml, 95% CI=–210 ml to –94 ml, P <0.001) and FVC (–145 ml, 95% CI= –211 to –79 ml, P <0.001) in children with asthma. Similar but weaker associations were found for pre- and post-BD FEV₁ (change for each 20% increment in African ancestry= –78 ml, 95% CI= –131 to –25 ml, P=0.004), and for post-BD FVC among children without asthma.

Conclusions—Genetic and/or EL factors correlated with African ancestry may influence childhood lung function in Puerto Ricans.

Keywords

ancestry; FEV₁; FVC; Puerto Ricans; childhood

INTRODUCTION

Childhood asthma is a major public health problem in the United States, particularly among certain ethnic minority groups and the economically disadvantaged^{1,2}. Members of two ethnic groups (Puerto Ricans and African Americans) have, on average, markedly higher asthma morbidity^{1,2} and African racial ancestry^{3,4} than Mexican Americans or whites.

Percentage of African ancestry was recently shown to be linearly and inversely associated with two measures of lung function (pre-bronchodilator FEV₁ and FVC) in African American adults, whose mean African ancestry was 72.8%³. Because African ancestry is correlated with low socioeconomic status (SES), inadequate access to health care and certain environmental exposures in African Americans, this finding could be explained by any or all of these factors. Whether African ancestry is associated with lung function in children or in members of other ethnic groups with lower average proportion of African ancestry (e.g., Puerto Ricans) is also unknown.

We hypothesized that African ancestry is associated with lower FEV₁ and FVC in Puerto Rican children, and that this association is independent of socioeconomic status (SES), healthcare access or environmental/lifestyle factors potentially correlated with racial ancestry and/or lung function. To test this hypothesis, we examined the relation between African ancestry (assessed using genome-wide genotypic data) and lung function measures in a cohort of Puerto Rican children with and without asthma.

METHODS

Subject recruitment

From September of 2003 to July of 2008, children were recruited from 15 public elementary/middle schools in Hartford that enrolled a significant proportion (42%–94%) of Puerto Rican children. Informational flyers with a study description were distributed to all parents of children in grades K to 8 in participating schools by mail (n=10,881) or in person during Open House and other school activities (n=885). Parents of 640 children completed a screening questionnaire. Of these 640 children, 585 (91.4%) were eligible for inclusion; parents of 449 (76.7%) of these 585 children agreed to participate. There were no significant differences in age, gender, or area of residence between eligible children who did (n=449) and did not (n=136) agree to participate. Of the 425 children in whom blood samples were collected, 416 (98%) had sufficient DNA for genotyping and were included in the initial analysis.

From March of 2009 to June of 2010, children in San Juan were chosen from randomly selected households, using a scheme similar to that of a prior study.⁵ In brief, households in the Standard Metropolitan Area of San Juan were selected by a multistage probability sample design.⁵ Primary sampling units (PSUs) were randomly selected neighborhood clusters based on the 2000 U.S. census, and secondary sampling units were randomly selected households within each individual PSU. A household was eligible if 1 resident was a child 6 to 14 years old. In households with more than one eligible child, a maximum of five children were randomly selected. Within each housing unit selected, children were enumerated and one child per eligible household was selected for screening. In households with multiple eligible children, one child was randomly selected by using Kish tables. On the basis of the sampling design, a total of 7,073 households were selected, and 6,401 (90.5%) were contacted. Of these 6,401 households, 1,111 had 1 child within the age range of the study who met other inclusion criteria (see below). In an effort to reach our target sample size (~700 children), we attempted to enroll a random sample (n=783) of these 1,111 children. Parents of 106 of these 783 eligible households refused to participate or could not be reached. There were no significant differences in age, gender, or area of residence between eligible children who did (n=677 [86.5%]) and did not (n=106 [13.5%]) agree to participate. Blood samples were collected in 592 (87.3%) of these 677 children; 583 (98.5%) of these 592 children had sufficient DNA for genotyping and were included in the initial analyses.

At both study sites, the main recruitment tool was a screening questionnaire given to parents of children ages 6 to 14 years to obtain information about the child's respiratory health and Puerto Rican ancestry. We selected as cases children who had physician-diagnosed asthma, wheeze in the prior year, and four Puerto Rican grandparents. We selected as controls children who had no physician-diagnosed asthma, no wheeze in the prior year and four Puerto Rican grandparents.

Study Procedures

Study participants completed a protocol (see Online Supplement) that included questionnaires, spirometry, and collection of blood (for DNA extraction, and measurements of total and allergen-specific IgE in serum and 25-hydroxy-vitamin D [hereafter referred to as vitamin D] in plasma) and dust (for measurement of dust mite and cockroach allergens) samples. 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 Connecticut Children's Medical Center (Hartford, CT), the University of Puerto Rico (San Juan, Puerto Rico), Brigham and Women's Hospital (Boston, MA), and the University of Pittsburgh (Pittsburgh, PA).

Spirometry was conducted with an EasyOne (NDD Medical Technologies, Andover, MA) spirometer following American Thoracic Society recommendations⁶. The best FEV₁ and FVC were selected for data analysis. 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.

Genotyping and Estimation of Racial Ancestry

Genotyping of ~2.5 million markers was conducted in DNA from study subjects using the HumanOmni2.5 BeadChip (Illumina, Inc., San Diego, CA). We removed single-nucleotide polymorphisms (SNPs) that were not in Hardy-Weinberg equilibrium ($P < 10^{-6}$) in control subjects, had minor allele frequency lower than 1% or a failure rate greater than 2%. Ancestry was estimated using the Local Ancestry in admixed Populations (LAMP) method and software.^{7,8} The analysis was restricted to SNPs that were present in all three ancestral

populations and that were not in tight linkage disequilibrium (using the software default of $r^2 > 0.1$), leaving a final sample of 85,059 SNPs. The algorithm uses ancestral proportions from prior studies (in this case, Tang et. al.⁹) and data from reference panels to estimate ancestral proportions for racially admixed populations. Puerto Ricans are an admixture of European, African, and Native Americans populations. To approximate this admixture, we used reference panels from HapMap¹⁰ for Europeans (CEU [Utah residents from Western and Central Europe] and TSI [Tuscans]) and Africans (YRI [Yorubans from West Africa]), and from the Human Genome Diversity Project (HGDP) for Native Americans.¹¹ For visualization of ancestry in Figure 1, principal components were calculated for the study subjects and the three ancestral proportions using EIGENSTRAT¹² on the common SNPs in the study population and the three ancestral populations. Additional information on the study methods is available in the Online Supplement.

Statistical analysis

Our primary outcomes were FEV₁ and FVC measured before (pre-bronchodilator [BD]) and after (post-BD) administration of inhaled albuterol. Secondary outcomes included other measures of lung function, allergy and asthma severity or control, as follows: FEV₁/FVC, bronchodilator responsiveness ($[(\text{post-BD FEV}_1 - \text{baseline FEV}_1) / (\text{baseline FEV}_1 \times 100)]$), total IgE, a positive IgE to dust mite, a positive IgE to cockroach, and ≥ 1 severe asthma exacerbation (≥ 1 visit to the emergency department (ED) or urgent care, or ≥ 1 hospitalization for asthma) in the prior year.

All analyses were conducted separately in children with and without asthma, first for each study site and then for the combined cohort. Because of their potential correlation with African ancestry and/or lung function, the following covariates were examined in bivariate analyses: parental history of asthma, household income ($<$ vs. $\geq$ \$15,000 [near the median income for households in Puerto Rico in 2008–9¹³], private or employer-based health insurance vs. others, use of inhaled corticosteroids [ICS] in the previous 6 months, prematurity,^{14,15} current exposure to environmental tobacco smoke (ETS),¹⁶ *in utero* ETS exposure, body mass index (BMI)¹⁷ as a z-score [based on 2000 CDC growth charts¹⁸], indoor exposure to dust mite (Der p 1)¹⁹ and cockroach (Bla g 2) allergens,²⁰ plasma vitamin D,²¹ and (for the analyses of the combined cohort) study site (Hartford vs. San Juan). Given prior results for African ancestry and lung function in African American adults,³ we examined linear trends for the relation between quintiles of African ancestry and the covariates/outcomes of interest using linear regression in bivariate analyses. Linear regression was then used for the multivariate analysis. A stepwise approach was used to build all multivariate models. All of the final models included African ancestry, age, sex, height, height², BMI, household income, ICS use and (for the combined cohort) study site. Other variables 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). LAMP version 2.3 (<http://lamp.icsi.berkeley.edu/lamp/>) was used for ancestry estimation and SAS version 9.2 (SAS Institute, Cary, NC) for all other analyses.

RESULTS

Subjects' characteristics and estimation of racial ancestry

After excluding subjects with low marker call rate, 383 (92%) of the 416 participants from Hartford and 560 (96.1%) of the 583 participants from San Juan remained in this analysis, comprising 943 children with (cases, $n=520$) and without (control subjects, $n=423$) asthma. A comparison of children who were and were not included in this analysis (based on having blood samples and genotypic data) is shown in **eTable 1**. In San Juan, cases or control subjects included in the current analysis were significantly more likely to have a lower

household income than those not included; there was no significant difference in lung function measures or in our secondary outcomes between the two groups. In Hartford, cases included in this analysis had a lower household income and were less likely to have used ICS in the prior year or to have been born premature than those not included. There was no significant difference in lung function measures or in our secondary outcomes between the two groups. Compared to control subjects not included in our analysis in Hartford, those included were more likely to be boys; there was no significant difference in lung function measures between the two groups.

We performed a principal-components analysis, which clusters participants based on similar racial ancestry (Figure 1).¹⁰ The Puerto Rican children in our study primarily fall along an axis between the European and African cohorts in HapMap, suggesting that they have mainly admixed European and African ancestry, with a smaller component of Native American ancestry. The mean estimated ancestral proportions for study participants are shown in **eTable 2**.

The main characteristics of study participants are summarized in Table 1. Compared to control subjects (at each study site and in the combined cohort), cases were significantly more likely to have parental history of asthma, higher BMI and total IgE, and to have a positive IgE to cockroach and a lower pre-bronchodilator FEV₁/FVC. Compared to control subjects in San Juan and in the combined cohort, cases were significantly more likely to have lower post- bronchodilator FEV₁/FVC, and to be currently exposed to ETS. Consistent with previous findings in Puerto Rican children and adults⁴, there was no significant difference in African ancestry between cases and control subjects.

Bivariate analysis

We then tested whether African ancestry is associated with the covariates or outcomes of interest in children with asthma (cases) at each study site (**eTable 3**) and the combined cohort (Table 2). In the bivariate analyses of all cases, percentage of African ancestry was significantly associated with lower household income, not having private or employer-based health insurance, lower (pre- or post-BD) FEV₁ and FVC, a higher level of Der p (dust mite) in house dust, a higher total IgE, and having a positive IgE to cockroach (Table 2). There was no significant association between African ancestry and pre-BD FEV₁/FVC, bronchodilator response, or ≥ 1 severe asthma exacerbation in the previous year.

Multivariate analysis

Table 3 shows the results of the multivariate analysis of African ancestry and FEV₁ and FVC in cases at each study site and for the combined cohort. There was a significant inverse association between percentage of African ancestry and pre- and post-BD FEV₁ and FVC in San Juan and in the combined cohort. In Hartford, African ancestry was inversely associated with FEV₁ and FVC, but this association was only statistically significant for post-BD FEV₁. We found no significant modification of the effect of African ancestry on any lung function measure by any covariate.

Because of high collinearity between household income and type of health insurance, we did not include both variables in the same models. Replacing household income with insurance type or changing the threshold for household income from \$15,000 to \$30,000 yielded similar findings (**eTable 4**). There was no significant association between African ancestry and total/cockroach-specific IgE or FEV₁/FVC after adjustment for household income and other covariates.

We then assessed whether African ancestry is associated with FEV₁ and/or FVC in control subjects (Table 4). In the multivariate analysis for San Juan and the combined cohort, percentage of African ancestry was inversely associated with lung function measures; for the combined cohort, this association was significant for all outcomes except pre-BD FVC. In this analysis, the results in Hartford were non-statistically significant but in the same direction as in San Juan.

DISCUSSION

We found that African ancestry is significantly associated with lower (pre- and post-BD) FEV₁ and FVC in Puerto Rican children with asthma, and with (pre- and post-BD) FEV₁ and post-BD FVC in Puerto Rican children without asthma. Our assessment of African ancestry is robust and likely very precise, as we obtained estimates of African ancestry that are nearly identical to those from our primary method (LAMP) when we used a Bayesian method (STRUCTURE²²) that does not take *a priori* ancestral proportions into account ([r for results from LAMP and STRUCTURE=0.99, P <0.0001).

Kumar et al reported a linear inverse association between African ancestry and pre-BD FEV₁ and FVC in a cohort of 777 African American adults without asthma, which was then replicated in two cohorts including 1,392 African American adults without asthma³. Our results extend those findings to children (with and without asthma) who have markedly lower average proportion of African ancestry (Puerto Ricans) than African Americans³. Although we do not have an adequate comparison group for our results in children with asthma (cases), our estimate of the effect of African ancestry on pre-BD FEV₁ or pre-BD FVC in children without asthma (control subjects) is similar to that reported for the two replication cohorts of African Americans included in a previous study³. Unlike the prior analysis in African Americans³, ours accounted for factors potentially correlated with African ancestry and lung function or asthma morbidity in subjects of African descent in the U.S., including indicators of SES and healthcare, ETS exposure, prematurity, allergen exposure, and plasma vitamin D level.

The estimated effect of African ancestry on FEV₁ or FVC was larger in cases than in controls in our study, and this difference was not explained by variables commonly associated with increased morbidity among children with asthma (see above). Although this may represent true modification of the effect of ancestry by asthma, we cannot exclude the possibility that African ancestry is associated with non-adherence with prescribed medications among Puerto Rican children, independently of indicators of SES or type of health insurance. Of note, however, we obtained similar results when the analysis was restricted to cases using ICS (data not shown).

Differential entry of subjects because of reasons related to both the exposure and outcome of interest (selection bias) and chance are unlikely explanations for our findings. Firstly, if children with asthma were more likely to enter the study because of both high proportion of African ancestry and increased disease severity, African ancestry would have been associated with at least one of our secondary outcomes (e.g., severe asthma exacerbations or total/allergen-specific IgE), which was not the case. Secondly, although only a small proportion of parents invited to participate in Hartford completed a screening questionnaire, the participation rate among eligible children in Hartford and San Juan was 76.5% and 86.5%, which is high for a study of an ethnic minority group. Even though our results were more significant in San Juan than in Hartford, they were of similar magnitude and in the same direction across study sites, despite differences in sample size, average percentage of African ancestry, and recruitment approach. Fourthly (and most importantly), our results are consistent with those of a prior study of 2,962 African American adults³. Because our study

was hypothesis-driven and our primary outcomes were not truly independent (e.g., pre- and post-BD FEV₁ are correlated), we did not adjust for multiple testing. However, our significant results in cases ($10^{-7} < P < 10^{-4}$ for all lung function measures) or controls ($P = 0.02$ for all lung function measures except pre-BD FVC) would remain so after applying a Bonferroni correction (e.g., $P < 0.025$ [0.05/2 primary outcomes] in all instances). Among cases, our results for lung function would remain significant even after a conservative adjustment for testing all primary and secondary outcomes ($P < 0.006$ [0.05/8] in all instances).

We recognize additional limitations to our findings, including potential misclassification of certain covariates (e.g., prematurity, early-life ETS) in our cross-sectional study (e.g., the observed association between reported ICS use and increased risk of disease exacerbations is likely due to prescription patterns for children with greater asthma severity). In addition, we are unable to distinguish separate effects of European or Native American ancestry because of the modest proportion of Native American ancestry (12.4%) in study participants.

Our results for lung function in cases differ from those of Salari et al., who reported no association between African ancestry and pre-BD FEV₁ in 181 Puerto Rican subjects (8 to 40 years old) with asthma²³. Differences between that study and ours include sample size, richness of genome-wide genotyping, and the age range of participants. Our negative findings for African ancestry and asthma *per se* are consistent with those of a prior case-control study of 291 Puerto Rican subjects ages 8 to 40 years⁴. In that study, there was a significant interaction between African ancestry and SES on asthma, which was not replicated in the current analysis (data not shown). In contrast to our results, a case-control study of 733 Afro-Caribbeans from Cartagena (Colombia) found an association between African ancestry and asthma and a higher total IgE. However, that study only accounted for area of residence as a surrogate marker of SES and EL factors.²⁴

In summary, our findings suggest that African ancestry is associated with lower FEV₁ and FVC in Puerto Rican children, independently of SES and healthcare access, ETS and allergen exposure, and vitamin D level. Genetic variants predominantly found in West Africans and/or early-life environmental/lifestyle factors that may be correlated with African ancestry but were unmeasured in this study (e.g., maternal nutrition) may influence lung development and growth during childhood in Puerto Ricans. Future analyses combining local admixture mapping with genome-wide association studies should help identify polymorphisms or haplotypes associated with lung function in Puerto Ricans and other populations of African descent.

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Abbreviations

FEV₁	forced expiratory volume in 1 second
FVC	forced vital capacity

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Key Messages

- African ancestry is associated with reduced FEV₁ and FVC in Puerto Rican children, independently of indicators of socioeconomic status, healthcare access and key environmental/lifestyle exposures.
- Genetic variants and/or early-life environmental/lifestyle factors correlated with African ancestry may influence lung development and growth during childhood in Puerto Ricans.

Figure 1. Principal component plot of study subjects (Puerto Ricans) compared to Hapmap populations representing European and African ancestry, and Human Genome Diversity Project populations representing Native American ancestry.

Table 1

Baseline characteristics of participating children¹

Covariate	San Juan		Hartford		Combined cohort	
	Cases	Controls ²	Cases	Controls ²	Cases	Controls ²
	N=287	N=273	N=233	N=150	N=520	N=423
Age (years)	10.1(2.6)	10.5(2.7)	9.8(2.8)	9.5(2.6)	10(2.7)	10.1(2.7)
Female gender	116(40%)	142(52%) ^{**}	119(51%)	73(49%)	235(45%)	215(51%)
BMI (z-score)	0.7(1.2)	0.5(1.1) [*]	1(1.3)	0.7(1.3) [*]	0.8(1.3)	0.6(1.2) ^{**}
At least one parent graduated from high school	235(82%)	215(79%)	154(66%)	115(77%) [*]	389(75%)	330(78%)
Household income < \$15,000/year	193(69%)	173(66%)	132(63%)	87(63%)	325(66%)	260(65%)
Private or employer-based health insurance	87(30%)	95(35%)	50(23%)	34(24%)	137(27%)	129(31%)
Parental history of asthma	193(67%)	86(32%) ^{***}	153(66%)	62(41%) ^{***}	346(67%)	148(35%) ^{***}
Current exposure to environmental tobacco smoke	131(46%)	99(36%) [*]	109(47%)	60(40%)	240(46%)	159(38%) ^{**}
Exposure to in utero smoking	33(12%)	26(10%)	45(19%)	26(17%)	78(16%)	52(13%)
Premature birth	27(9%)	15(6%)	12(5%)	7(5%)	39(8%)	22(5%)
Use of inhaled corticosteroids in the prior 6 months	90(31%)		81(35%)		171(33%)	
Plasma vitamin D level (ng/ml)	32.5(8.2)	31(7.6) [*]	24.4(7.1)	25.4(7.3)	28.9(8.7)	29(7.9)
Der p in house dust (μg/g) ³	0.7(0.5)	0.7(0.5)	-0.5(0.5)	-0.5(0.6)	0.2(0.8)	0.3(0.8) [*]
Bla g in house dust (μg/g) ³	0.3(0.7)	0.3(0.7)	0.2(0.7)	0.2(0.7)	0.3(0.7)	0.2(0.7)
Pre-bronchodilator FEV ₁ (ml) ⁴	1896(670)	2043(749) [*]	1913(685)	1956(687)	1904(676)	2012(728) [*]
Post-bronchodilator FEV ₁ (ml) ⁴	2031(723)	2125(760)	2027(730)	2051(703)	2029(725)	2100(741)
Pre-bronchodilator FVC (ml) ⁴	2346(799)	2458(890)	2331(837)	2338(839)	2339(816)	2415(873)
Post-bronchodilator FVC (ml) ⁴	2423(838)	2479(884)	2389(835)	2374(811)	2408(836)	2444(860)
Pre-bronchodilator FEV ₁ /FVC	0.81(0.09)	0.84(0.09) ^{**}	0.82(0.08)	0.84(0.09) [*]	0.82(0.09)	0.84(0.09) ^{***}
Post-bronchodilator FEV ₁ /FVC	0.84(0.09)	0.86(0.08) ^{**}	0.85(0.09)	0.87(0.06)	0.84(0.09)	0.86(0.07) ^{**}
Bronchodilator response to albuterol as percent of FEV ₁	4.9(11.2)	4(7.9)	4.7(9.7)	2.3(8.4) [*]	4.8(10.6)	3.4(8.1) [*]
Total IgE (IU/ml) ³	2.5(0.7)	2.2(0.7) ^{***}	2(0.7)	1.8(0.6) ^{**}	2.3(0.7)	2.1(0.7) ^{***}
IgE to cockroach (Bla g) 0.35 IU/ml	114(40%)	74(27%) ^{**}	69(32%)	28(20%) ^{**}	183(37%)	102(25%) ^{***}

Covariate	San Juan		Hartford		Combined cohort	
	Cases	Controls ²	Cases	Controls ²	Cases	Controls ²
IgE to dust mite (Der p)	N=287 183(64%)	N=273 121(45%)*	N=233 81(42%)*	N=150 45(33%)*	N=520 264(55%)*	N=423 166(41%)*
1 severe asthma exacerbation in the prior year ⁵	201(70%)		114(49%)		315(61%)	
Percentage of African ancestry	25.2(11.7)	24.7(12.5)	21.9(7.7)	21.6(9.9)	23.7(10.3)	23.6(11.7)

¹ Values are the No.(%) for binary variables or mean(standard deviation) for continuous variables

² Comparison between cases and controls at each study site and the combined cohort:

* P <0.05,

** P<0.01,

*** P<0.001

³ Allergen levels and total IgE were transformed to a logarithmic (log10) scale

⁴ FEV₁ and FVC presented as absolute values because of lack of predicted values for Puerto Ricans

⁵ 1 visit to the emergency department or urgent care requiring steroids, or hospitalization, or IV or oral steroids for asthma in the prior year.

Table 2

African ancestry, selected covariates and lung function measures in Puerto Rican children with asthma[/]

Covariates	Quintiles of African Ancestry					P for trend
	Q1 (0–14.9%)	Q2 (15.0–19.3%)	Q3 (19.4–24.2%)	Q4 (24.3–31.9%)	Q5 (32–80.8%)	
Age (years)	10(3)	10(3)	9.8(2.6)	9.7(2.6)	10(3)	0.4
Female gender	44(47%)	44(41%)	51(47%)	47(44%)	49(46%)	0.9
BMI (z-score)	0.8(1.16)	0.94(1.12)	0.92(1.34)	0.91(1.31)	0.65(1.43)	0.4
At least one parent graduated from high school	69(74%)	86(80%)	79(73%)	76(72%)	79(75%)	0.5
Household income < \$15,000/year	51(58%)	57(58%)	69(70%)	67(66%)	81(79%)	0.001
Private or employer-based health insurance	35(39%)	34(32%)	25(24%)	24(23%)	19(18%)	0.0003
Parental history of asthma	60(65%)	74(70%)	71(66%)	75(71%)	66(62%)	0.8
Current exposure to environmental tobacco smoke	40(43%)	42(40%)	52(48%)	58(55%)	48(45%)	0.2
Exposure to in utero smoking	10(12%)	15(15%)	19(18%)	20(19%)	14(14%)	0.5
Premature birth	6(7%)	9(9%)	10(9%)	7(7%)	7(7%)	0.8
Use of inhaled corticosteroids in the prior 6 months	36(39%)	35(33%)	37(34%)	35(33%)	28(26%)	0.1
Plasma vitamin D level (ng/ml)	29(9)	29(8)	28(9)	29(9)	30(8)	0.4
Der p in house dust ($\mu\text{g/g}$) ²	0.14(0.78)	-0.01(0.73)	0.2(0.82)	0.09(0.79)	0.4(0.69)	0.009
Bla g in house dust ($\mu\text{g/g}$) ²	0.26(0.77)	0.22(0.73)	0.21(0.71)	0.35(0.83)	0.23(0.65)	0.8
Pre-bronchodilator FEV ₁ (ml) ³	1989(694)	1965(769)	1918(665)	1838(679)	1819(555)	0.03
Post-bronchodilator FEV ₁ (ml) ³	2162(760)	2136(815)	2033(694)	1935(706)	1895(613)	0.002
Pre-bronchodilator FVC (ml) ³	2404(879)	2438(917)	2356(827)	2287(787)	2216(643)	0.04
Post-bronchodilator FVC (ml) ³	2518(869)	2526(958)	2405(797)	2320(774)	2281(754)	0.01
Pre-bronchodilator FEV ₁ /FVC	0.83(0.07)	0.81(0.09)	0.82(0.08)	0.8(0.09)	0.82(0.09)	0.4
Post-bronchodilator FEV ₁ /FVC	0.86(0.08)	0.85(0.08)	0.85(0.08)	0.83(0.1)	0.84(0.1)	0.04
Bronchodilator response as percent of baseline FEV ₁	5.4(10.6)	5.7(9.8)	4.7(9)	4.7(13.1)	3.7(9.8)	0.2
Total IgE (IU/ml) ²	2.2(0.7)	2.3(0.7)	2.3(0.7)	2.3(0.7)	2.4(0.7)	0.03
IgE to cockroach (Bla g) 0.35 IU/ml	26(30%)	35(35%)	38(37%)	36(35%)	48(46%)	0.04
IgE to dust mite (Der p) 0.35 IU/ml	42(52%)	55(57%)	50(50%)	56(57%)	61(60%)	0.35
1 severe asthma exacerbation in the prior year ⁴	44(47%)	51(48%)	48(44%)	42(40%)	63(59%)	0.28

[/]Values are the No.(%) for binary variables or mean(standard deviation) for continuous variables.

² Allergen levels and total IgE were transformed to a logarithmic (log 10) scale

³ FEV₁ and FVC presented as absolute values because of lack of predicted values for Puerto Ricans

⁴ 1 visit to the emergency department or urgent care requiring steroids, or hospitalization, or IV or oral steroids for asthma in the prior year.

Table 3Multivariate analysis of percentage of African ancestry and measures of lung function in cases¹**A) San Juan, PR**

Predictors	Pre-bronchodilator FEV ₁ (ml)	Pre-bronchodilator FVC (ml)	Post-bronchodilator FEV ₁ (ml)	Post-bronchodilator FVC (ml)
<u>Unadjusted</u>				
Each 20% increment in African ancestry	-97(-234-39) (0.2)	-129(-291-34) (0.1)	-163(-312--14) (0.03)	-154(-328-20) (0.08)
<u>Multivariate model</u>				
Each 20% increment in African ancestry	-117(-179--54) (<0.001)	-145(-217--73) (<0.001)	-165(-233--97) (<0.001)	-155(-233--77) (<0.001)
Household income < \$15,000/year	-46(-128-37) (0.3)	-69(-164-26) (0.2)	-86(-174-3) (0.06)	-55(-157-47) (0.3)
Use of inhaled corticosteroids in the prior year	-59(-142-24) (0.2)	-25(-121-71) (0.6)	-95(-184--5) (0.04)	-95(-198-8) (0.07)
BMI (z-score)	72(39-104) (<0.001)	100(63-138) (<0.001)	90(54-125) (<0.001)	115(74-156) (<0.001)

B) Hartford, CT

Predictors	Pre-bronchodilator FEV ₁ (ml)	Pre-bronchodilator FVC (ml)	Post-bronchodilator FEV ₁ (ml)	Post-bronchodilator FVC (ml)
<u>Unadjusted</u>				
Each 20% increment in African ancestry	-114(-346-117) (0.3)	-152(-434-130) (0.3)	-200(-457-57) (0.1)	-238(-532-57) (0.1)
<u>Multivariate model</u>				
Each 20% increment in African ancestry	-92(-210-26) (0.1)	-94(-225-37) (0.2)	-136(-257--14) (0.03)	-119(-249-11) (0.07)
Household income < \$15,000/year	-85(-174-5) (0.07)	-122(-230--15) (0.03)	-73(-168-23) (0.1)	-79(-189-31) (0.2)
Use of inhaled corticosteroids in the prior year	-44(-143-55) (0.4)	-28(-141-86) (0.6)	-60(-162-41) (0.2)	-73(-185-39) (0.2)
BMI (z-score)	48(11-84) (0.01)	103(60-146) (<0.001)	69(30-108) (<0.001)	110(66-154) (<0.001)
Der p in house dust (μg/g) ³	-79(-170-13) (0.09)		-86(-178-6) (0.07)	

C) Combined cohort

Predictors	Pre-bronchodilator FEV ₁ (ml)	Pre-bronchodilator FVC (ml)	Post-bronchodilator FEV ₁ (ml)	Post-bronchodilator FVC (ml)
<u>Unadjusted</u>				
Each 20% increment in African ancestry	-102(-218-15) (0.09)	-129(-269-12) (0.07)	-169(-297--40) (0.01)	-167(-315--19) (0.03)
<u>Multivariate model</u>				

A) San Juan, PR

Predictors	Pre-bronchodilator FEV ₁ (ml)	Pre-bronchodilator FVC (ml)	Post-bronchodilator FEV ₁ (ml)	Post-bronchodilator FVC (ml)
Each 20% increment in African ancestry ⁴	-105(-159--51) (<0.001)	-133(-197--69) (<0.001)	-152(-210--94) (<0.001)	-145(-211--79) (<0.001)
Household income < \$15,000/year	-55(-114-4) (0.07)	-91(-161--21) (0.01)	-73(-137--10) (0.02)	-66(-138-7) (0.08)
Use of inhaled corticosteroids in the prior year	-56(-117-5) (0.07)	-32(-104-40) (0.4)	-87(-152--22) (0.009)	-89(-163--15) (0.02)
BMI (z-score)	62(38--86) (<0.001)	101(73--130) (<0.001)	82(56--108) (<0.001)	113(83--142) (<0.001)

¹ Beta coefficient (95% confidence interval (CI)), P value shown for all outcomes.

² All multivariate models additionally adjusted for age, gender, height and height²; model for the combined cohort additionally adjusted for study site.

³ Der p allergen levels log10 transformed

⁴ As an example, the pre-BD FEV₁ of a child with 60% African ancestry would be, on average, 105 ml lower than that of child with 40% African ancestry, and 210 ml lower than that of child with 20% African ancestry.

Table 4Multivariate analysis of African ancestry and measures of lung function in control subjects¹

A) San Juan, PR				
Predictors	Pre-bronchodilator FEV₁ (ml)	Pre-bronchodilator FVC (ml)	Post-bronchodilator FEV₁ (ml)	Post-bronchodilator FVC (ml)
<u>Unadjusted</u>				
Each 20% increment in African ancestry	−87(−232-59) (0.2)	−9(−182-164) (0.9)	−86(−239-67) (0.3)	−58(−236-120) (0.5)
<u>Multivariate models</u>				
Each 20% increment in African ancestry	−92(−155-29) (0.004)	−70(−146-5) (0.07)	−86(−150-22) (0.008)	−80(−157-2) (0.04)
Household income < \$15,000/year	−100(−182-17) (0.02)	−88(−186-11) (0.08)	−113(−195-32) (0.007)	−73(−172-26) (0.1)
BMI (z-score)	5(−32-43) (0.8)	20(−25-64) (0.4)	23(−13-60) (0.2)	58(14-103) (0.01)
B) Hartford, CT				
<i>Predictors</i>	<i>Pre-bronchodilator FEV₁ (ml)</i>	<i>Pre-bronchodilator FVC (ml)</i>	<i>Post-bronchodilator FEV₁ (ml)</i>	<i>Post-bronchodilator FVC (ml)</i>
<u>Unadjusted</u>				
Each 20% increment in African ancestry	−131(−358-95) (0.3)	−141(−417-136) (0.3)	−129(−378-120) (0.3)	−151(−438-137) (0.3)
<u>Multivariate models</u>				
Each 20% increment in African ancestry	−43(−132-47) (0.4)	−43(−169-82) (0.5)	−61(−153-30) (0.2)	−75(−176-26) (0.1)
Household income < \$15,000/year	23(−72-117) (0.6)	−109(−241-23) (0.1)	80(−15-176) (0.1)	38(−68-143) (0.5)
BMI (z-score)	41(4-78) (0.03)	138(86-189) (<0.001)	63(25-100) (0.001)	106(65-148) (<0.001)
C) Combined cohort				
<i>Predictors</i>	<i>Pre-bronchodilator FEV₁ (ml)</i>	<i>Pre-bronchodilator FVC (ml)</i>	<i>Post-bronchodilator FEV₁ (ml)</i>	<i>Post-bronchodilator FVC (ml)</i>
<u>Unadjusted</u>				
Each 20% increment in African ancestry	−87(−208-33) (0.2)	−30(−175-116) (0.7)	−87(−216-42) (0.2)	−68(−218-82) (0.4)
<u>Multivariate model</u>				
Each 20% increment in African ancestry ³	−78(−130-27) (0.003)	−55(−121-10) (0.1)	−78(−131-25) (0.004)	−75(−138-12) (0.02)
Household income < \$15,000/year	−61(−123-1) (0.05)	−95(−173-16) (0.02)	−55(−118-8) (0.08)	−50(−124-24) (0.2)
BMI (z-score)	25(−2-51) (0.07)	72(38-106) (<0.001)	44(17-71) (0.002)	82(50-114) (<0.001)

¹ Beta coefficient (95% confidence interval (CI)), P value shown for all outcomes.

² All multivariate models additionally adjusted for age, gender, height and height²; model for the combined cohort additionally adjusted for study site.

³ As an example, the pre-BD FEV₁ of a child with 60% African ancestry would be, on average, 78 ml lower than that of a child with 40% African ancestry and 156 ml lower than that of a child with 20% African ancestry.