Title

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Journal

CHEST Journal, 162(1)

ISSN

0012-3692

Authors

Witonsky, Jonathan
Elhawary, Jennifer R
Eng, Celeste
et al.

Publication Date

2022-07-01

DOI

10.1016/j.chest.2021.12.664

Peer reviewed

CHEST

Race- and Ethnicity-Based Spirometry Reference Equations

Are They Accurate for Genetically Admixed Children?

Jonathan Witonsky, MD; Jennifer R. Elhawary, MS; Celeste Eng, BS; José R. Rodríguez-Santana, MD; Luisa N. Borrell, DDS, PhD; and Esteban G. Burchard, MD, MPH

BACKGROUND: Variation in genetic ancestry among genetically admixed racial and ethnic groups may influence the fit of guideline-recommended spirometry reference equations, which rely on self-identified race and ethnicity.

RESEARCH QUESTION: What is the influence of genetic ancestry on the fit of race- and ethnicity-based spirometry reference equations in populations of genetically admixed children?

STUDY DESIGN AND METHODS: Cross-sectional fit of guideline-recommended race- and ethnicity-based spirometry reference equations was evaluated in healthy control participants from case-control studies of asthma. Anthropometry, blood samples, and spirometric measurements were obtained for 599 genetically admixed children 8 to 21 years of age. Genetic ancestry was estimated using genome-wide genotype data. Equation fit, measured as a mean *z* score, was assessed in self-identified African American (n = 275) and Puerto Rican (n = 324) children as well as genetic ancestry-defined strata of each population.

RESULTS: For African American children, African American-derived equations fit for predicting FEV_1 and FVC in those with an African ancestry more than the median (81.4%-100.0%), whereas composite equations for "other/mixed" populations fit for predicting FEV_1 and FVC in those with African ancestry at or less than the median (30.7%-81.3%). For Puerto Rican children with African ancestry at or less than the median (6.4%-21.3%), White-derived equations fit both FEV_1 and FVC, whereas for those with African ancestry more than the median (21.4%-87.5%), White-derived equations fit the FEV_1 and the composite equations fit the FVC.

INTERPRETATION: Guideline-recommended spirometry reference equations yielded biased estimates of lung function in genetically admixed children with high variation of African ancestry. Spirometry could benefit from reference equations that incorporate genetic ancestry, either for more precise application of the current equations or the derivation and use of new equations.

CHEST 2022; 162(1):184-195

KEY WORDS: genetic ancestry; lung function; pediatrics; race and ethnicity; reference equations

FOR EDITORIAL COMMENT, SEE PAGE 11

ABBREVIATIONS: GALA II = Genes-Environments and Admixture in Latino Americans; GLI = Global Lung Function Initiative; LLN = lower limit of normal; NHANES III = Third National Health and Nutrition Examination Survey; SAGE = Study of African Americans, Asthma, Genes, and Environments; $zFEV_1$ = mean *z* score for FEV_1; *z*FVC = mean *z* score for FVC

AFFILIATIONS: From the Department of Pediatrics (J. Witonsky), Department of Medicine (J. R. Elhawary and C. Eng), Department of Medicine and Department of Bioengineering and Therapeutic Sciences (E. G. Burchard), University of California, San Francisco, San Francisco, CA; the Centro de Neumología Pediátrica (J. R. Rodríguez-Santana), San Juan, PR; and the Department of Epidemiology and

Take-home Points

Study Question: What is the influence of genetic ancestry on the fit of race- and ethnicity-based spirometry reference equations in populations of genetically admixed children?

Results: After accounting for intrapopulation variation in African ancestry, guideline-recommended spirometry reference equations yielded biased estimates of lung function in genetically admixed children.

Interpretation: Spirometry could benefit from reference equations that incorporate genetic ancestry, either for more precise application of the current equations or for the derivation and use of new equations.

Great debate has taken place over the use of racial and ethnic classifications in medicine and biomedical research.[1,2] Pulmonary function testing is one of the few clinical applications where self-reported race or ethnicity are used to define a so-called normal range.[3] Multiple factors have been shown to be associated with racial and ethnic differences in lung function, including chest dimensions, the ratio of sitting height to standing height, and altitude.[4-7] Also a significant inverse relationship exists between African genetic ancestry and both FEV_1 and FVC, which are robust to adjustment for covariates related to early life exposures, air pollution, and socioeconomic status.[8,9]

Normative equations of lung function have been developed by testing large populations categorized by self-reported race and ethnicity.[10,11] However, many populations have ancestral contributions from multiple continents (ie, genetically admixed), and as a result, self-identified racial and ethnic categories may not capture an individual's genetic ancestry fully.[12-15] Therefore, using self-reported race and ethnicity may result in misclassifying individuals with respect to the normal range for physiologic lung measures if the latter are more dependent on ancestry.[8]

The most widely used spirometry reference equations, and those recommended in the American Thoracic Society and European Respiratory Society guidelines, were derived by the Global Lung Function Initiative (GLI) in White, African American, North Asian, and South East Asian adults and children 3 to 95 years of age.[11,16] The GLI also offers a composite equation—an average of the four population-specific GLI equations—for use in multiracial or unrepresented populations. Before the widespread availability of the GLI equations, the Third National Health and Nutrition Examination Survey (NHANES III) equations, derived from White, African American, and Mexican American adults and children 8 to 80 years of age, were the recommended spirometric references, with their ongoing use considered appropriate when maintaining continuity is necessary.[10,17] The GLI and NHANES III equations, hereafter referred to as guideline-recommended spirometry reference equations (e-Appendix 1), were derived to detect clinically important differences in normal lung function observed between racial and ethnic groups. Selecting an inappropriate reference equation—or ignoring race or ethnicity by using a one-size-fits-all approach—can cause unintended clinical consequences, including errors and delays in disease detection and medical management, misclassification of disease severity, denial of disability claims, exclusion from employment opportunities, and ineligibility for life-saving treatments such as transplants and other surgeries.[18-23]

Several studies have demonstrated that the guideline-recommended lung function equations are well-fitted to populations similar to those from which they were derived.[24-30] However, to date, none of these studies have evaluated equation fit after considering intrapopulation variation in genetic ancestry, which we

Biostatistics (L. N. Borrell), Graduate School of Public Health and Health Policy, City University of New York, New York, NY.

Drs Borrell and Burchard contributed equally to this manuscript.

FUNDING/SUPPORT: This work was supported in part by the Sandler Family Foundation, the American Asthma Foundation, the RWJF Amos Medical Faculty Development Program, Harry Wm. and Diana V. Hind Distinguished Professor in Pharmaceutical Sciences II, the National Institutes of Health, National Heart, Lung, and Blood Institute [Grants R01HL117004, 1X01HL134589, R01HL128439, R01HL135156, and R01HL141992], the National Institute of Environmental Health Sciences [Grants R01ES015794 and R21ES24844], the National Institute on Minority Health and Health Disparities [Grants R01MD010443 and R56MD013312], the National Human Genome Research Institute [Grant U01HG009080], the National Institute of General Medical Sciences [Grant T32GM007546], the National Center for Advancing Translational Sciences [UCSF-CTSI Grant Number TL1TR001871], and the UCSF Pediatric Allergy, Immunology, and Bone Marrow Transplantation Division.

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CORRESPONDENCE TO: Jonathan Witonsky, MD; email: jonathan.witonsky@ucsf.edu

DOI: https://doi.org/10.1016/j.chest.2021.12.664

demonstrated is associated with lung function in African American and Latino individuals.[8,9] In this study, we evaluated the guideline-recommended White, African American, Mexican American, and composite reference equations for two independent genetically admixed populations of African American and Puerto Rican children. Specifically, we assessed lung function equation fit in each population as well as the genetic ancestry-defined strata of each population (ie, subpopulations).

Study Design and Methods

Study Populations

Spirometric predictions and genetic ancestry proportions were calculated using clinical and genetic data from healthy control participants among two case-control studies of asthma conducted between 2006 and 2014: the Genes-Environments and Admixture in Latino Americans (GALA II) study and the Study of African Americans, Asthma, Genes, and Environments (SAGE).[9,31] Participants were 8 to 21 years of age at the time of recruitment and were healthy with no history of coughing, wheezing, or shortness of breath in the 2 years before enrollment. Parents and grandparents of study participants self-identified as Puerto Rican in GALA II or African American in SAGE. GALA II participants were recruited from five urban study centers throughout the United States (Chicago, Bronx, Houston, San Francisco Bay Area, and Puerto Rico), and SAGE participants were recruited from the San Francisco Bay Area. All participants provided written consent to being in the study. Consent was obtained from all participants 18 years of age and older and from parents or legal guardians of minor participants. Institutional review board committee names and project approval numbers are provided as a data supplement.

In total, 3,226 healthy children without asthma were enrolled in the two studies (2,538 in GALA II and 688 in SAGE). Spirometric measurements and genome-wide genetic data were available for 606 children whose parents or grandparents self-identified as Puerto Rican (n = 326) or African American (n = 280). After excluding irregular spirometric measurements (e-Appendix 1), 599 children remained: 275 African American children and 324 Puerto Rican children.

Assessments

Spirometric measurements were collected from all participants with testing performed in accordance with American Thoracic Society recommendations (e-Appendix 1).[32] Genotyping was performed using the Affymetrix Axiom LAT1 array (World Array 4; Affymetrix), which includes 817,810 single nucleotide polymorphisms. This array was designed to capture genetic variation in populations of African descent such as African American and Latino individuals.[33] The genetic ancestry of each study participant was determined using an unsupervised analysis in the ADMIXTURE software package, as described elsewhere.[9]

We examined the distribution of genetic ancestry in each population and used the African ancestry median to classify African American (median, 81.3%; range, 30.7%-100%) and Puerto Rican (median, 21.3%; range, 6.4%-87.5%) populations into two groups: more than the median and at median or less. Reference equation fit was assessed in the African American population (n = 275) and the African American subpopulations with African ancestry more than the population median (n = 137) and at the population median or less (n = 138). Similarly, equation fit was assessed in the Puerto Rican population (n = 324) and the Puerto Rican subpopulations with African ancestry more than the population median (n = 162) and at the population median or less (n = 162).

Statistical Analyses

Demographic characteristics were compared within each subpopulation using the Student t test and Wilcoxon rank-sum test for normally and nonnormally distributed continuous variables, respectively, and the χ^2 statistic for dichotomous variables.

Cross-sectional fit of the equations was determined by calculating FEV_1 and FVC z scores.[24,34,35] We excluded the ratio of FEV_1 to FVC from the analysis because the ratio is independent of race and ethnicity.[11,25,36,37] The z-score means for FEV_1 and FVC were calculated using the GLI White, African American, and composite equations and the NHANES III White, African American, and Mexican American equations. Because all participants were healthy with assumed normal lung function, an equation was deemed "best fit" when the mean z score was closest to 0 (perfect fit being a mean z score of 0), and an equation was deemed to have sufficient fit if the mean z score was between −0.5 and 0.5 using two one-sided t tests for equivalence.[24,34,35] Assessment of significance testing for two one-sided t tests was performed using 95% CIs and P values.[38] An equation was deemed appropriate if it provided sufficient fit for both FEV_1 and FVC. A sensitivity analysis was performed assessing fit using genetic ancestry tertile, quartile, and quintile distributions. To assess for misclassification, the proportion of participants with observed spirometry data less than the lower limit of normal (LLN), which corresponds to the 5th percentile ($z = -1.65$) of predicted values, also was evaluated for FEV_1 and FVC. Data analyses were conducted using R version 4.1.2 software (R Foundation for Statistical Computing).[39]

Results

Table 1 summarizes general characteristics of participants in each racial and ethnic group. Sex, age, height, and weight distributions were comparable across each subpopulation. Statistically significant differences were found in lung function, as measured by the mean z score for FEV_1 ($zFEV_1$) and mean z score for FVC (zFVC), with Puerto Rican subpopulations with African ancestry at or less than the median ($zFEV_1$, 0.69; zFVC, 0.55) having higher means than their peers with African ancestry at more than the median ($zFEV_1$, 0.43; zFVC, 0.23). Lung function also was higher for African American subpopulations with African ancestry at or less than the median ($zFEV_1$, 0.41; zFVC, 0.56) compared with African American subpopulations with African ancestry at more than the median ($zFEV_1$, 0.29; zFVC, 0.43), but this difference was not statistically significant.

TABLE 1] Distribution of Selected Characteristics for Participants in GALA II and SAGE Based on Race or Ethnicity and Genetic Ancestry Categories, 2006-2014

Variable	African American Children			Puerto Rican Children		
		African Ancestry (Median, 81.3%)			African Ancestry (Median, 21.3%)	
	All (N = 275)	At or Less Than the Median (n = 138)	More Than the Median (n = 137)	All (n = 324)	At or Less Than the Median (n = 162)	More Than the Median (n = 162)
Boys, %	41	43	39	45	43	48
Age, y	16.2 ± 3.8	15.9 ± 3.9	16.5 ± 3.7	14.0 ± 3.0	14.2 ± 3.2	13.8 ± 2.7
Height, cm	162.3 ± 13.6	161.4 ± 13.6	163.1 ± 13.6	158.6 ± 11.6	157.9 ± 12.7	159.4 ± 10.3
Weight, kg	67.3 ± 24.4	66.1 ± 22.7	68.4 ± 26.1	57.8 ± 18.8	56.6 ± 16.9	59.0 ± 20.5
Ancestry, %						
African	79.5 ± 9.6	73.2 ± 9.6	85.9 ± 3.2[a]	22.7 ± 10.5	15.1 ± 3.3	30.2 ± 9.8[a]
Native	—[b]	—[b]	—[b]	10.2 ± 3.1	10.6 ± 3.5	9.8 ± 2.7[a]
$zFEV_1$	0.35 ± 0.95	0.41 ± 0.97	0.29 ± 0.94	0.56 ± 1.21	0.69 ± 1.14	0.43 ± 1.28[a]
zFVC	0.49 ± 0.99	0.56 ± 0.97	0.43 ± 1.01	0.39 ± 1.27	0.55 ± 1.15	0.23 ± 1.36[a]

Data are presented as mean (SD), unless otherwise specified. Spirometry *z*-scores based on Global Lung Function Initiative 2012 equations, using the African American-derived equation for African American children and the composite equation for Puerto Rican children.[11] GALA II = Genes-Environments and Admixture in Latino Americans; SAGE = Study of African Americans, Asthma, Genes, and Environments; $zFEV_1$ = *z* score for FEV_1; zFVC = *z* score for FVC.

[a]Differences between subpopulations: $P < .05$ (Student *t* test and Wilcoxon rank-sum test).

[b]Native American ancestry was not determined for African American individuals.

Genetic Ancestry Proportions

When comparing subpopulations according to the median distribution, the means ± SDs of African ancestry were 73.2 ± 9.6% and 85.9 ± 3.2% for African American children at or less than the median and at more than the median, respectively (Table 1). These values were 15.1 ± 3.3% and 30.2 ± 9.8%, respectively, among Puerto Rican children. Figure 1 shows the European, African, and Native American genetic admixture in the African American and Puerto Rican populations.

Fit to African American Children

Tables 2 and 3 provide equations fit to spirometric measurements in the study population and subpopulations of African American children based on genetic ancestry distribution. The NHANES III equations derived from African American individuals, and the GLI composite equations were appropriate for African American children at the population level, providing sufficient fit for predicting FEV_1 and FVC. Among African American children, the mean *z* scores were 0.17 (95% CI, 0.06-0.28) for predicting FEV_1 and 0.34 (95% CI, 0.23-0.45) for predicting FVC using the NHANES III African American equations, whereas they were −0.32 (95% CI, −0.43 to −0.20) for predicting FEV_1 and −0.14 (95% CI, −0.27 to −0.02) for predicting FVC using the GLI composite equations. The GLI composite equations resulted in the least misclassification for both FEV_1 and FVC, with 5.8% and 6.2% less than the LLN, respectively.

When using the African genetic ancestry median to classify African American children, only the GLI composite equations were appropriate for predicting both FEV_1 and FVC with mean *z* scores of −0.25 (95% CI, −0.42 to −0.09) for FEV_1 (5.8% less than the LLN) and −0.08 (95% CI, −0.25 to 0.10) for FVC (5.8% less than the LLN) for African American children classified at or less than the median. Despite fitting for FEV_1 predictions (0.24; 95% CI, 0.08-0.39), the NHANES III equations derived from African Americans did not statistically fit for predicting FVC (0.41; 95% CI, 0.26-0.56) in African American children classified at or less than the median. In contrast, for African American children with African ancestry at more than the median, the NHANES III equations derived from African American individuals were appropriate for predicting FEV_1 (0.11; 95% CI, −0.04 to 0.26) and FVC (0.27;

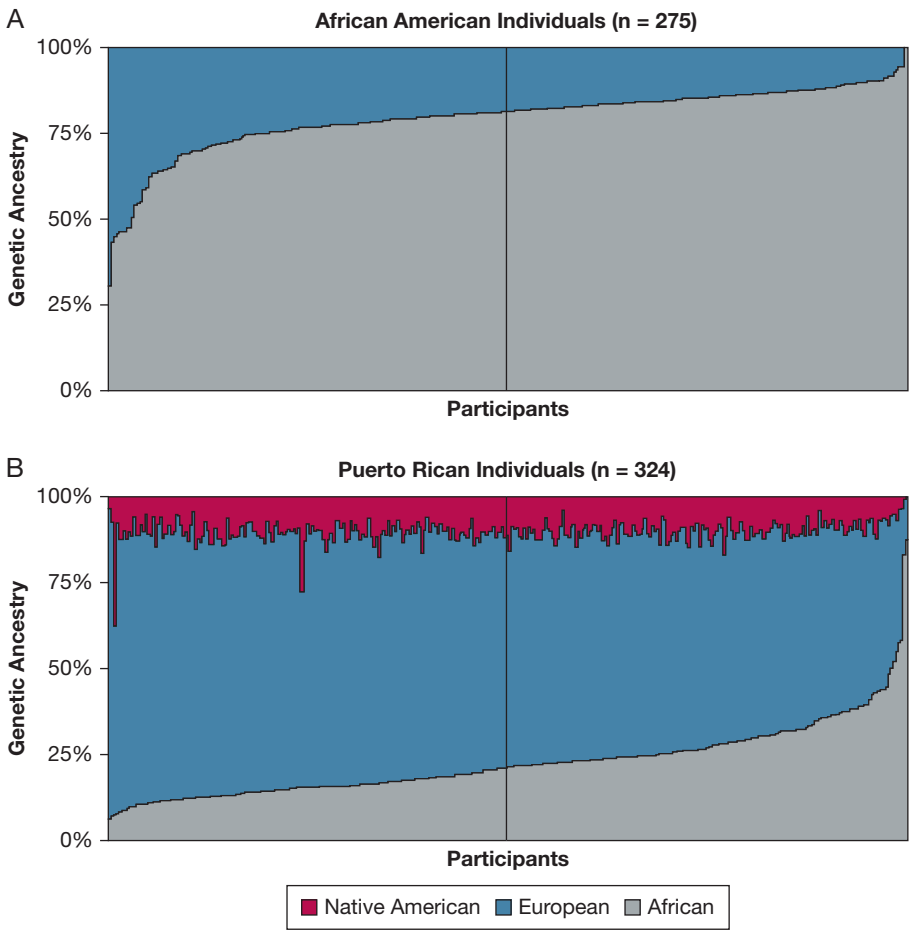

Figure 1 – *A, B, Histograms showing the Proportions of European, African, and Native American genetic admixture in African American (Study of African Americans, Asthma, Genes, and Environments) (A) and Puerto Rican (Genes-Environments and Admixture in Latino Americans) (B) children.*

95% CI, 0.10-0.43). GLI equations derived from African American individuals fit for predicting FEV_1 (0.29; 95% CI, 0.13-0.44), but statistically underestimated FVC (0.43; 95% CI, 0.26-0.60) in African American children with African ancestry at more than the median. The GLI composite equations resulted in the least misclassification for both FEV_1 and FVC in all subpopulations. Figure 2A provides the fit for the NHANES III and GLI equations using other distribution cut points of African ancestry in addition to the median. The figure underscores the poor fit of the equations in African American children when using other African ancestry cut points.

Fit to Puerto Rican Children

At the population level, the GLI equations derived from White individuals were appropriate for Puerto Rican children with mean *z* scores of –0.06 (95% CI, –0.19 to 0.06) for predicting FEV_1 (6.8% less than the LLN) and –0.33 (95% CI, –0.46 to –0.21) for predicting FVC (11.1% less than the LLN) (Tables 4, 5). For Puerto Rican children with African ancestry at or less than the median and at more than the median, the GLI and NHANES III equations derived from White individuals fit for predicting FEV_1. The White-derived equations fit for predicting only FVC among Puerto Rican children with African ancestry at or less than the median. The GLI composite equation fit for predicting FVC in the subpopulation of Puerto Rican children with African ancestry at more than the median. However, the GLI composite equations underestimated FEV_1 predictions among those with African ancestry at or less than the median (Fig 2B). In Puerto Rican children with African ancestry at or less than the median, both GLI and NHANES III equations derived from White individuals were appropriate for predicting FEV_1 and FVC.

Discussion

Pulmonary function testing is one of the few applications in clinical medicine where self-identified race or ethnicity is used to predict normative values. Our findings are consistent with previous observations that

TABLE 2] Fit of Spirometry Equation FEV_1 Predictions to Spirometric Measurements in African American Children, 2006-2014

Variable	All (n = 275)	African Ancestry	
		At or Less Than the Median, 30.7%-81.3% (n = 138)	More than the Median, 81.4%-100.0% (n = 137)
NHANES III			
White			
$zFEV_1$	−0.95 (−1.07 to −0.84)	−0.88 (−1.04 to −0.73)	−1.03 (−1.19 to −0.86)
< Fifth percentile	24.0	23.9	24.1
African American			
$zFEV_1$	0.17 (0.06 to 0.28)[a,b]	0.24 (0.08 to 0.39)[a]	0.11 (−0.04 to 0.26)[a,b]
< Fifth percentile	2.5	2.2	2.9
Mexican American			
$zFEV_1$	−1.19 (−1.30 to −1.07)	−1.12 (−1.27 to −0.96)	−1.26 (−1.42 to −1.09)
< Fifth percentile	31.6	28.3	35.0
GLI			
White			
$zFEV_1$	−0.88 (−0.99 to −0.77)	−0.82 (−0.97 to −0.67)	−0.94 (−1.09 to −0.79)
< Fifth percentile	18.2	18.8	17.5
African American			
$zFEV_1$	0.35 (0.24 to 0.46)[a]	0.41 (0.25 to 0.58)	0.29 (0.13 to 0.44)[a]
< Fifth percentile	1.8	1.4	2.2
Composite			
$zFEV_1$	−0.32 (−0.43 to −0.20)[a,b]	−0.25 (−0.42 to −0.09)[a,b]	−0.38 (−0.54 to −0.22)
< Fifth percentile	5.8	5.8	5.8

Data are presented as mean *z* score (95% CI) or percentage below the fifth percentile. GLI = Global Lung Function Initiative; NHANES III = Third National Health and Nutrition Examination Survey; $zFEV_1$ = *z* score for FEV_1.
[a]Equation provides sufficient fit: mean *z* score < |0.5| ($P < .05$) with 95% CIs inside interval (−0.5, 0.5).[24,34,35]
[b]Equation appropriate for population or subpopulation (provides sufficient fit for both FEV_1 and FVC).

race- or ethnicity-based equations can misestimate lung function after considering intrapopulation variation in African ancestry.[8] The influence of genetic ancestry on equation fit is especially relevant to African American and Puerto Rican populations in which especially wide distributions exist in the proportions of African ancestry.[40] Consequently, the use of race- and ethnicity-based spirometry equations may exacerbate inequities in respiratory health, which disproportionately affect the very populations for whom the equations are the least precise.[41] These populations are most prone to biased lung function predictions when equations derived from African American individuals underestimate lung function predictions in African American children and equations derived from White individuals overestimate lung function predictions in Puerto Rican children. Underestimated lung function predictions result in inflated *z* scores and decreased spirometric sensitivity, which can lead to underdiagnosis or delays in disease detection. Overestimated lung function predictions result in deflated *z* scores and decreased spirometric specificity, which can lead to excessive diagnostic workup and unnecessary treatment. This is of critical concern given that African American and Latino/Hispanic children collectively make up approximately 40% of all children in the United States.[42]

Previous studies have validated the use of the GLI equations derived from African American populations for use in children with presumed African ancestry, including Black school-aged children in the United Kingdom and school-aged children in sub-Saharan Africa.[25,28,30] Although the NHANES III equations derived from African American individuals fit African American children in this study, the GLI equations derived from African American individuals underestimated FVC predictions. The underestimated FVC potentially is attributable to sampling variability, given the relatively small study population. The African American children in our study also could have higher

TABLE 3] Fit of Spirometry Equation FVC Predictions to Spirometric Measurements in African American Children, 2006-2014

Variable	All (N = 275)	African Ancestry	
		At or Less Than the Median, 30.7%-81.3% (n = 138)	More Than the Median, 81.4%-100.0% (n = 137)
NHANES III			
White			
*z*FVC	−0.85 (−0.97 to −0.74)	−0.79 (−0.94 to −0.63)	−0.92 (−1.09 to −0.75)
< Fifth percentile	19.3	14.5	24.1
African American			
*z*FVC	0.34 (0.23 to 0.45)[a,b]	0.41 (0.26 to 0.56)	0.27 (0.10 to 0.43)[a,b]
< Fifth percentile	1.8	0.7	2.9
Mexican American			
*z*FVC	−1.00 (−1.11 to −0.88)	−0.93 (−1.09 to −0.77)	−1.06 (−1.23 to −0.90)
< Fifth percentile	24.0	21.0	27.0
GLI			
White			
*z*FVC	−0.79 (−0.91 to −0.68)	−0.74 (−0.89 to −0.58)	−0.85 (−1.01 to −0.69)
< Fifth percentile	17.8	14.5	21.2
African American			
*z*FVC	0.49 (0.37 to 0.61)	0.56 (0.39 to 0.72)	0.43 (0.26 to 0.60)
< Fifth percentile	2.2	1.4	2.9
Composite			
*z*FVC	−0.14 (−0.27 to −0.02)[a,b]	−0.08 (−0.25–0.10)[a,b]	−0.21 (−0.39 to −0.02)[a]
< Fifth percentile	6.2	5.8	6.6

Data are presented as mean *z* score (95% CI) or percentage below the fifth percentile. GLI = Global Lung Function Initiative; NHANES III = Third National Health and Nutrition Examination Survey; *z*FVC = *z* score for FVC.
[a]Equation provides sufficient fit: mean *z* score < |0.5| ($P < .05$) with 95% CIs inside interval (−0.5, 0.5).[24,34,35]
[b]Equation appropriate for population or subpopulation (provides sufficient fit for both FEV_1 and FVC).

lung function given their lower mean African ancestry when compared with the mean African ancestry in other representative African American cohorts.[43]

After considering intrapopulation variation in African ancestry, equations derived from African American populations were no longer well fitted for predicting lung function in African American children with African ancestry at or less than the population's median. Only the GLI composite equation—an equation derived as the average of all GLI data for use in multiracial or unrepresented populations—was fitted appropriately for this subpopulation. Additionally, using the GLI composite equation for African American children in our study yielded the least misclassification. These findings suggest that inappropriate spirometry reference equations might be used for as many as half of all African American children and further illustrate how intrapopulation variation in African ancestry limits the clinical usefulness of a single reference equation for an entire genetically admixed group.

In our study, the GLI equations derived from White individuals were appropriate for the study population of Puerto Rican children. This was an unexpected finding given that the largest study of reference equation fit for Latino/Hispanic adults found that the GLI equations derived from White individuals overestimated lung function among adults who self-identified as Puerto Rican.[27] The finding also was contrary to the assumption that the GLI composite equation would be most appropriate for Puerto Ricans, a population not represented by the four groups from which the GLI equations were derived.

Corroborating prior studies showing that African ancestry is associated inversely with lung function in Puerto Rican individuals,[9,44] the GLI equations derived from White individuals were not appropriate for Puerto Rican children with African ancestry at more than the population's median. In this subpopulation, no equation was fitted appropriately for predicting both FEV_1 and FVC, suggesting that as many as half of all Puerto Rican

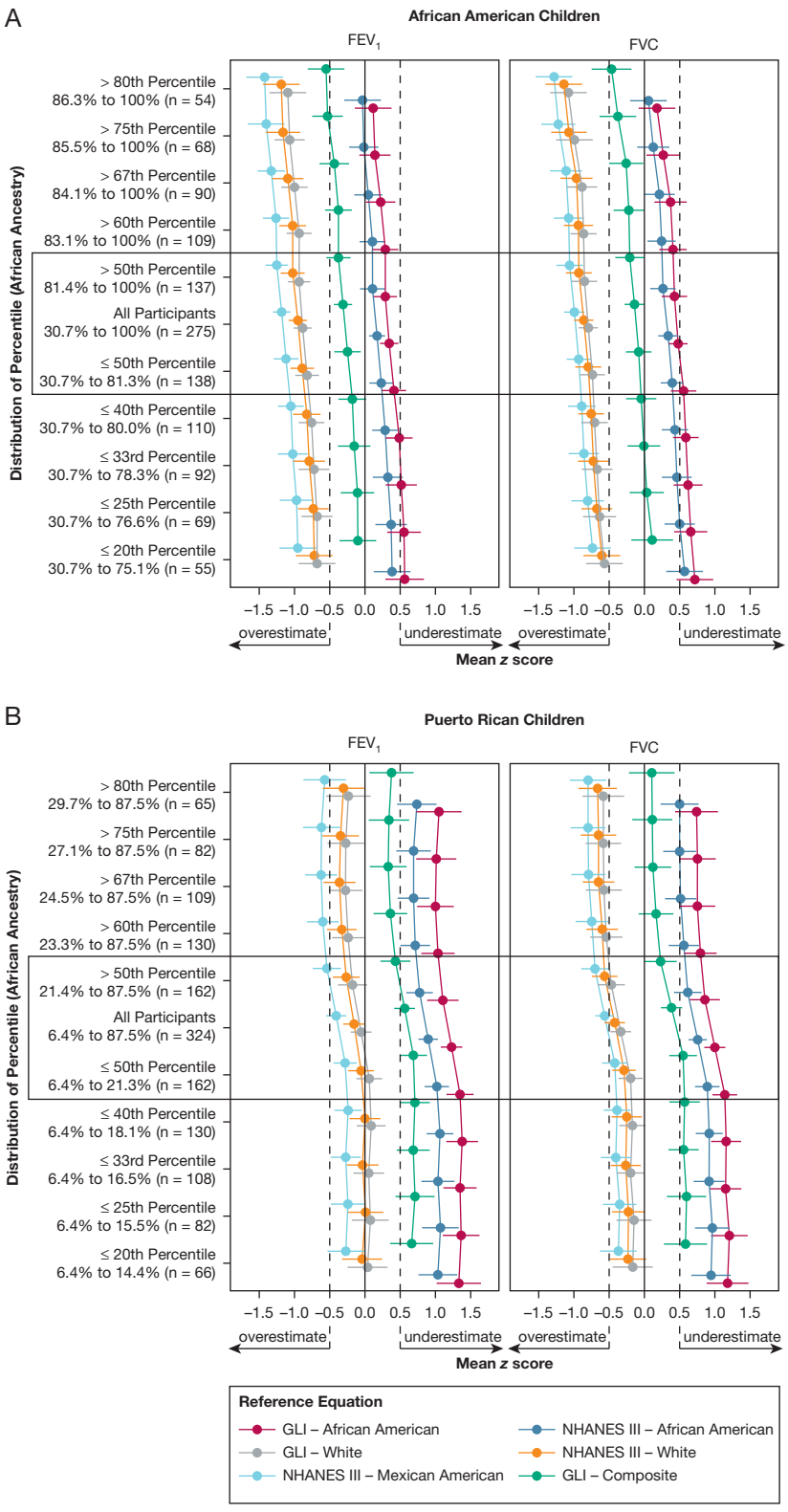

Figure 2 – *A, B, Interval plots showing the fit for the GLI White, African American, and composite equations and the NHANES III White, African American, and Mexican American equations using genetic ancestry median, tertile, quartile, and quintile distributions in African American (Study of African Americans, Asthma, Genes, and Environments) (A) and Puerto Rican (Genes-Environments and Admixture in Latino Americans) (B) children. GLI = Global Lung Function Initiative; NHANES III = Third National Health and Nutrition Examination Survey.*

children are being misclassified for these measures. The lack of an appropriate equation for Puerto Rican children with more African ancestry than the median is especially problematic considering that asthma prevalence and mortality are highest among Puerto Ricans compared with other racial and ethnic

TABLE 4] Fit of Spirometry Equation FEV_1 Predictions to Spirometric Measurements in Puerto Rican Children, 2006-2014

Variable	All (N = 324)	African Ancestry	
		At or Less Than the Median, 6.4%-21.3% (n = 162)	More than the Median, 21.4%-87.5% (n = 162)
NHANES III			
White			
$zFEV_1$	−0.16 (−0.28 to −0.04)[a]	−0.05 (−0.21 to 0.12)[a,b]	−0.27 (−0.46 to −0.09)[a]
< Fifth percentile	7.1	4.9	9.3
African American			
$zFEV_1$	0.90 (0.78 to 1.02)	1.02 (0.86 to 1.18)	0.78 (0.60 to 0.96)
< Fifth percentile	0.3	0.0	0.6
Mexican American			
$zFEV_1$	−0.41 (−0.53 to −0.28)	−0.28 (−0.45 to −0.12)[a]	−0.53 (−0.72 to −0.35)
< Fifth percentile	11.7	9.3	14.2
GLI			
White			
$zFEV_1$	−0.06 (−0.19 to 0.06)[a,b]	0.06 (−0.11 to 0.22)[a,b]	−0.18 (−0.36 to 0.01)[a]
< Fifth percentile	6.8	4.3	9.3
African American			
Mean $zFEV_1$	1.23 (1.10 to 1.36)	1.35 (1.18 to 1.53)	1.10 (0.91 to 1.30)
< Fifth percentile	0.3	0.0	0.6
Composite			
$zFEV_1$	0.56 (0.43 to 0.69)	0.69 (0.51 to 0.86)	0.43 (0.24 to 0.63)
< Fifth percentile	1.5	0.6	2.5

Data are presented as mean (95% CI) or percentage. GLI = Global Lung Function Initiative; NHANES III = Third National Health and Nutrition Examination Survey; $zFEV_1$ = z score for FEV_1.
[a]Equation provides sufficient fit: mean z score < |0.5| ($P < .05$) with 95% CIs inside interval (−0.5, 0.5).[24,34,35]
[b]Equation appropriate for population or subpopulation (provides sufficient fit for both FEV_1 and FVC).

groups.[45,46] Puerto Ricans have wide-ranging proportions of genetic ancestry and self-identify across the spectrum of the US Census racial categories, making the population ideally suited to the ongoing study of race and ethnicity and genetic ancestry as predictors of lung function.[41,47,48]

This study has some important limitations. First, after classifying the populations by their genetic ancestry distribution, the sample sizes were less than what is recommended for spirometry equation validation studies: at least 300 healthy study participants should be included in validation studies because smaller sample sizes can result in differences in z scores of up to 0.5 by chance alone.[49] Consequently, equations could be deemed a poor fit for a subpopulation because our study was underpowered to detect significance when using specified two one-sided t tests criterion. However, to address this limitation, a sensitivity analysis demonstrated how mean z scores tended to trend in the serially stratified subpopulations, revealing the continuous influence of ancestry on equation fit. Second, our findings are not generalizable to adults because the analysis was performed exclusively in children 8 to 21 years of age. Third, although we evaluated the guideline-recommended equations in healthy African American and Puerto Rican children with presumably normal lung function, we recognize the potential for selection or misclassification bias if the effects of social determinants on lung function were artificially minimized.[5,7] Finally, our results were not replicated in other populations of African American and Puerto Rican children, an essential next step in the evaluation of guideline-recommended equations.

Despite these limitations, this is the first study, to our knowledge, assessing the influence of genetic ancestry on the applicability of guideline-recommended race- and ethnicity-based spirometry reference equations for genetically admixed populations. Our study suggested that the use of race- and ethnicity-based spirometry reference equations in genetically admixed populations

TABLE 5] Fit of Spirometry Equation FVC Predictions to Spirometric Measurements in Puerto Rican Children, 2006-2014

Variable	All (N = 324)	African Ancestry	
		At or Less Than the Median, 6.4%-21.3% (n = 162)	More Than the Median, 21.4%-87.5% (n = 162)
NHANES III			
White			
*z*FVC	−0.41 (−0.53 to −0.30)	−0.28 (−0.43 to −0.12)[a,b]	−0.55 (−0.73 to −0.37)
< Fifth percentile	12.0	7.4	16.7
African American			
*z*FVC	0.76 (0.64 to 0.87)	0.89 (0.74 to 1.05)	0.62 (0.44 to 0.80)
< Fifth percentile	0.6	0.0	1.2
Mexican American			
*z*FVC	−0.56 (−0.68 to −0.44)	−0.42 (−0.58 to −0.27)	−0.70 (−0.88 to −0.52)
< Fifth percentile	15.7	10.5	21.0
GLI			
White			
*z*FVC	−0.33 (−0.46 to −0.21)[a,b]	−0.19 (−0.35 to −0.03)[a,b]	−0.48 (−0.66 to −0.29)
< Fifth percentile	11.1	6.8	15.4
African American			
*z*FVC	1.00 (0.87 to 1.13)	1.15 (0.98 to 1.32)	0.86 (0.66 to 1.06)
< Fifth percentile	0.6	0.0	1.2
Composite			
*z*FVC	0.39 (0.25 to 0.53)	0.55 (0.37 to 0.73)	0.23 (0.02 to 0.44)[a]
< Fifth percentile	4.3	1.9	6.8

Data are presented as mean (95% CI) or percentage. GLI = Global Lung Function Initiative; NHANES III = Third National Health and Nutrition Examination Survey; *z*FVC = *z* score for FVC.
[a]Equation provides sufficient fit: mean *z* score < |0.5| ($P < .05$) with 95% CIs inside interval (−0.5, 0.5).[24,34,35]
[b]Equation appropriate for population or subpopulation (provides sufficient fit for both FEV_1 and FVC).

is limited by intrapopulation variation in genetic ancestry. In addition, our study contributes to the ongoing debate regarding the use of race and ethnicity in clinical algorithms by demonstrating that race and ethnicity alone potentially are insufficient for estimating lung function in a large proportion of African American and Puerto Rican children. We demonstrated that the use of genetic ancestry data instead of race and ethnicity in lung function equations could improve lung function prediction equations for genetically admixed populations. That said, we recognize that incorporating race and ethnicity captures essential epidemiologic information that relates to lung function and overall clinical outcomes not captured by genetic ancestry.[2]

Interpretation

Our study demonstrated that spirometry reference equations, which rely on self-identified race and ethnicity, inconsistently fit homogenous racial and ethnic groups after classifying these populations by their genetic ancestry distribution. Recent scientific advances allow for estimates of genetic ancestry to be measured easily and inexpensively, as exemplified by direct-to-consumer genetic ancestry testing. Spirometry could benefit from reference equations that incorporate genetic ancestry, either for more precise application of the current equations or for the derivation and use of new equations. Meanwhile, the increasing availability of genetic data in the clinical setting can be leveraged to guide selection of the most appropriate spirometry reference equation. Although additional studies are needed to determine the African ancestry cut points at which an individual is most likely to be best fit by a specific race- or ethnicity-based reference equation, our findings suggest that African American children with African ancestry of > 80% are best fit by equations derived from African American populations, whereas those with African ancestry of < 80% are best fit by the GLI composite equation. Puerto Rican children with African ancestry

of < 20% are best fit by equations derived from White populations, whereas no single equation appropriately fits Puerto Rican children with African ancestry of > 20%. Although applying two reference sets may be impractical clinically, Puerto Rican children with African ancestry of > 20% are best fit by equations derived from White populations for predicting FEV_1 and the composite equation for FVC.

In the absence of genetic ancestry data, extra caution must be taken when choosing a spirometry reference equation and interpreting results for racially and ethnically admixed individuals. To account for intrapopulation variation in genetic ancestry, spirometric measurements should be compared with predictions calculated using both the indicated race- and ethnicity-based equation and the GLI composite equation. Racially and ethnically diverse individuals likely are best served if their spirometric measurements are evaluated using multiple equations, with each result taken in the context of clinical symptoms.

Acknowledgments

Author contributions: J. W. contributed to the conception and design of the work, analysis and interpretation of the data, drafting and revision of the manuscript, and, as guarantor of the article, takes responsibility for the content of the manuscript, including the data and analysis. C. E. contributed to the acquisition of data. J. R. E., J. R. R.-S., L. N. B., and E. G. B. contributed to the analysis and interpretation of the data, drafting and revision of the manuscript, and critical review of the manuscript.

Financial/nonfinancial disclosures: None declared.

Other contributions: The authors thank the participants and their families for their contribution, as well as the health care professionals and clinics for their support and participation in the Genes-Environments and Admixture in Latino Americans Study and the Study of African Americans, Asthma, Genes and Environments. In particular, the authors thank the study coordinator Sandra Salazar and the recruiters who obtained the data: Kelley Meade, MD, Adam Davis, MPH, MA, Lisa Caine, Emerita Brigino-Buenaventura, MD, Duanny Alva, MD, Gaby Ayala-Rodriguez, Ulysses Burley, Elizabeth Castellanos, Jaime Colon, Denise DeJesus, Iliana Flexas, Blanca Lopez, Brenda Lopez, MD, Louis Martos, Vivian Medina, Juana Olivo, Mario Peralta, Esther Pomares, MD, Jihan Quraishi, Johanna Rodriguez, Shahdad Saeedi, Dean Soto, Ana Taveras, and Emmanuel Viera. The authors also thank Andrew Zeiger, Oona Risse-Adams, María G. Contreras, Fernando Picazo, MD, Martin Rofael, MD, Angel C. Y. Mak, PhD, Donglei Hu, PhD, Scott Huntsman, MS, Eric Wohlford, MD, PhD, Sam S. Oh, PhD, MPH, Maria Pino-Yanes, PhD, L. Keoki Williams, MD, MPH, Michael A. Lenoir, MD, Noah A. Zaitlen, PhD, Elad Ziv, MD, Thomas Nuckton, MD, Christopher Gignoux, PhD, and Joshua Galanter, MD, for their advice on project conception and analysis.

Additional information: The e-Appendix is available online under "Supplementary Data."

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