A Genome-Wide Association Study of Post-bronchodilator Lung Function in Children with Asthma

To the Editor:

Three meta-analyses of genome-wide association studies (GWAS) in healthy subjects have revealed multiple loci for FEV_1 and FEV_1/FVC (1–3), but only one study included even a small proportion of children (~7%) (3). A separate GWAS of lung function in children and young adults (including subjects with and without respiratory diseases) did not yield significant results but replicated prior findings in adults (4), and a study in Hutterites identified variants in the 15q23 locus related to lung function (5). The only GWAS of lung function in subjects with asthma (aged 12 years and older, mostly adults) did not show novel results but replicated findings in healthy subjects (6). No prior GWAS has examined post-bronchodilator (post-BD) FEV_1 and FEV_1/FVC, which may best reflect true lung function (and not solely disease severity) in subjects with asthma.

Even though Puerto Ricans share a disproportionate burden from asthma in the United States (7), there has been no GWAS of lung function in this ethnic group. GWAS of minority populations at risk are important from a public health perspective and can identify novel susceptibility variants for well-characterized phenotypes (8). Moreover, loci identified in patients without asthma likely influence lung function in patients with asthma (9), but unidentified variants may also influence lung function in subjects with asthma (10). We conducted a GWAS of understudied lung function phenotypes (post-BD FEV_1 and FEV_1/FVC) in a unique high-risk population (Puerto Rican children with asthma) and attempted to replicate our top findings in independent cohorts. We also examined whether previous findings for GWAS of pre-BD lung function were replicated for post-BD lung function.

From September 2003 to June 2010, 618 children with asthma (physician-diagnosed asthma and one or more episode of wheeze in the previous year) were recruited in Hartford, Connecticut (n = 267), and San Juan, Puerto Rico (n = 351), as described elsewhere (11). At both study sites, eligibility criteria included age 6–14 years and having four Puerto Rican grandparents. All participants completed a protocol that included questionnaires, blood sample collection, and spirometry (conducted with an EasyOne spirometer; NDD Medical Technologies, Andover, MA), following American Thoracic Society recommendations for children (12). After baseline spirometry, subjects were given 200 μg of an albuterol metered-dose inhaler, and spirometry was repeated after 15 minutes. The best FEV_1 and FVC from each test were selected for analysis. Of the 618 participants, 560 had blood samples and sufficient DNA for genome-wide genotyping, conducted using the HumanOmni2.5 BeadChip platform (Illumina Inc., San Diego, CA), as previously described (11). After excluding subjects with low marker call rate (n = 31), sex mismatch (n = 6), or missing post-BD phenotypes (n = 76), 447 subjects remained in the analysis. 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).

Replication was attempted in three cohorts of children with asthma: the Genetics of Asthma in Costa Rica Study (13), the Childhood Asthma Management Program (14), and the Genes-environments and Admixture in Latino Americans study (15). The Genotype-Tissue Expression Biorepository (16) was then used to assess the association between our top-replicated single nucleotide polymorphisms (SNPs) and expression of nearby genes in human tissues. Bronchial epithelial brushings were obtained from healthy nonsmokers: three males and one female (median age, 27 yr; range, 24–40 yr). Expression data were generated using Illumina TrueSeq RNA sequencing. RNAseq data were mapped to the human genome (build 37.2), and relative expression level was assessed by fragments per kilobase of transcript per million mapped reads (17).

For the GWAS, we built linear regression models under an additive genetic model, adjusting for age, sex, principal components, and height. The top 20 SNPs were then tested using the same genetic model in the replication cohorts. The results for the replication cohorts were combined in a meta-analysis with weights proportional to the inverse variance of the beta.

The main characteristics of study participants are shown in Table 1. Subjects at both study sites had similar age and lung function, but there was a slightly higher proportion of female subjects in Hartford than in San Juan. The GWAS results for both phenotypes did not show significant evidence of population stratification based on genomic inflation factors (*see* Figure E1 in the online supplement). Eight SNPs were associated with post-BD FEV_1 at $P < 9 \times 10^{-7}$ in Puerto Ricans (Table E1 and Figure E2). Of these eight SNPs, two (rs7946574 and rs2984842) were associated with post-BD FEV_1, at $P < 5 \times 10^{-8}$ (Figure E3). Results for rs7946574 were not significantly replicated, but findings for rs2984842 were replicated in the same direction of effect in all cohorts (combined P for the analysis in replication

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Author Contributions: J.M.B. and J.C.C. conceived of the study, participated in its design and coordination, and helped draft the manuscript. S.M.T., D.C.C.-C., D.H., Q.Y., M.P.-Y., and M.L.S. performed statistical analysis. E.G.B., G.C., E.F., A.A.L., B.A.R, W.C., N.B., E.A.-P., L.A., S.T.W., M.S.-Q., J.K.K., S.E.W., and M.M.C. participated in the design and coordination of the study. All authors read and approved the final manuscript.

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Table 1. Baseline Characteristics of Participating Children

	Puerto Ricans (*N* = *447*)	CAMP (*N* = *568*)	GACRS (*N* = *556*)	GALA II (*N* = *1,858*)
Age, yr, mean (SD)	10 (2.7)	8.9 (2.1)	9.0 (2.0)	12.5 (3.2)
Female sex	225 (46%)	228 (40%)	230 (41%)	835 (44.9%)
FEV_1, L, mean (SD)				
Prebronchodilator	1.9 (0.7)	1.7 (0.5)	1.7 (0.5)	2.4 (0.8)
Post-bronchodilator	2 (0.7)	1.8 (0.5)	1.8 (0.6)	2.6 (0.9)
FEV_1/FVC ratio, %, mean (SD)				
Prebronchodilator	82 (9)	79 (8)	81 (13)	84 (8)
Post-bronchodilator	84 (9)	85 (6)	83 (17)	88 (6)
FVC, L, mean (SD)				
Prebronchodilator	2.3 (0.8)	2.1 (0.6)	2.1 (0.6)	2.9 (1.0)
Post-bronchodilator	2.4 (0.8)	2.2 (0.6)	2.1 (0.7)	3.0 (1.0)
Genotyping platform(s)	Illumina Omni 2.5 M	Illumina 550 + 610k + custom arrays	Illumina 550 + 610 quad + custom arrays	Axiom LAT1 array (World Array 4)
Average (mean) ancestry				
European	Hartford, 63% San Juan, 60%	99.1%	55.7%	53%
African	Hartford, 34% San Juan, 36%	0.3%	0.5%	17%
Native American	Hartford, 3% San Juan, 4%	0.6%	43.8%	30%

Definition of abbreviations: CAMP = Childhood Asthma Management Program; GACRS = Genetics of Asthma in Costa Rica Study; GALA II = Genes-environments and Admixture in Latino Americans.

cohorts = 0.005) (Figure 1). This SNP, an intronic variant of the gene for fibroblast growth factor 14 (*FGF14*), is associated with increased expression of *FGF14* in subcutaneous adipose tissue ($P = 0.01$) and lung ($P = 0.10$) (16). *FGF14* was expressed in bronchial brushes from healthy nonsmokers at a level ~2.5-fold greater than that for the cystic fibrosis transmembrane conductance regulator gene (*CFTR*) and 7-fold greater than that for the gene for hypoxanthine phosphoribosyltransferase 1 (*HPRT1*).

Five SNPs had genome-wide significant ($P < 5 \times 10^{-8}$) associations with post-BD FEV_1/FVC in Puerto Ricans (Table E2, and Figures E2 and E4) but were not replicated in other cohorts.

We then tested whether SNPs identified by GWAS for pre-BD FEV_1 are associated with post-BD FEV_1 in Puerto Rican children. We identified four SNPs (tagging eight published SNPs for pre-BD FEV_1) that were associated with post-BD FEV_1 at $P < 0.05$ in the 4q24 locus (Table E3). This region contains multiple genes, including *GSTCD*, *INTS12*, and *NPNT*.

Our top replicated association for post-BD FEV_1 (for rs2984842) is in *FGF14*, a gene in the fibroblast growth factor family that is most highly expressed in brain tissue; it is also expressed in lung, pituitary, and adipose tissues. Although *FGF14* has not been associated with lung phenotypes, it was highly expressed in bronchial brushes from healthy adults, and other members of the FGF family (with which it shows homology) have been associated with lung development or disease, including *FGF10* (18) and *FGF7* (19). We also replicated previously published findings for pre-BD FEV_1 in the 4q24 locus, using the post-BD FEV_1 phenotype. Lack of replication of some previously identified variants may reflect lack of statistical power because of the small size of our discovery cohort, but it may also be a result of differences in linkage disequilibrium resulting from racial ancestry.

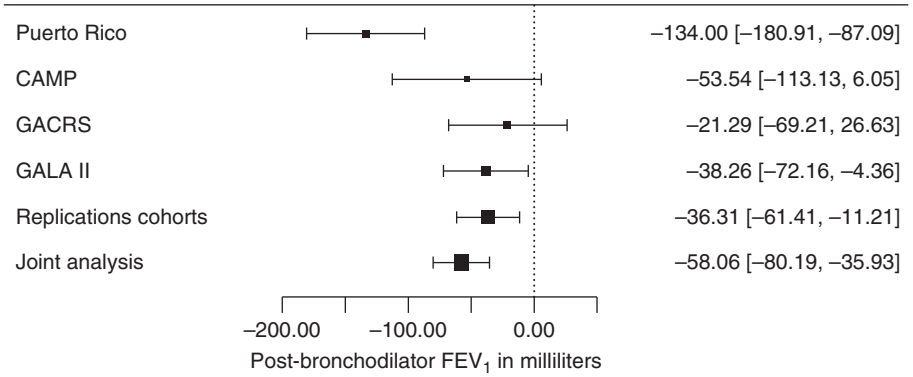

Figure 1. Forest plot of effect sizes on post-bronchodilator FEV_1 (in milliliters) and 95% confidence intervals for rs2984842 in discovery and replication cohorts. CAMP = Childhood Asthma Management Program; GACRS = Genetics of Asthma in Costa Rica Study; GALA II = Genes-environments and Admixture in Latino Americans.

To our knowledge, this is the first GWAS of post-BD lung function and the first GWAS of lung function in Puerto Ricans. We have identified a novel gene associated with post-BD FEV_1 in children with asthma. Further studies are needed to identify functional mechanisms. ■

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Tidal Volume during Assisted Ventilation after Double-Lung Transplantation

To the Editor:

The rate of lung transplants is increasing, and these patients frequently develop respiratory complications necessitating mechanical ventilation (1). When treating these patients, the risks of mechanical ventilation constitute an important concern, and clinicians aim to simultaneously protect the lung while avoiding ventilator-induced diaphragm dysfunction by maintaining spontaneous breathing. Targeting the ideal V_T for each patient is challenging. According to the underlying lung disease of the recipient and his or her previous total lung capacity (TLC), the size of the transplanted lungs may differ from the theoretical TLC based on height. Actual TLC can vary considerably from one transplanted patient to the next and is poorly correlated with height (2). Furthermore, bipulmonary transplanted patients have denervated lungs without vagal afferent fibers. As a consequence, some feedback mechanisms necessary for control of breathing may be lacking, which can result in large V_T, as shown in animals (3) and during exercise in lung-transplanted patients (4).

Neurally adjusted ventilatory assist (NAVA) is a mode of ventilation that delivers support in proportion to diaphragmatic electrical activity, the latter being a direct expression of neural inspiratory activity (5). Over a certain range of assist, the patient is able to fully control his or her V_T, which is not the case with traditional modes of assist. We analyzed V_T in patients ventilated with NAVA after bilateral lung transplantation. We compared these volumes with nontransplanted patients at a similar stage of difficult weaning, using the same approach to titrate NAVA. In addition, we examined the relationship between the patient's V_T under NAVA and their most recent TLC.

Methods

The Ethics Committee of Bordeaux University Hospital approved the study and granted waived consent. The process of determining the donor lung size is explained in the online supplement.

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We systematically applied NAVA when patients failed their first spontaneous breathing trial (SBT), both after lung transplant and for nontransplanted patients (6). After failing the SBT, the NAVA level was titrated to obtain the electrical activity of the diaphragm at ~60% of the highest activity measured during the SBT. On the first day of NAVA ventilation, electrical activity of the diaphragm and V_T data were recorded. A computer equipped with dedicated software (RCR, Maquet Critical Care, Solna, Sweden) was used to record respiratory parameters for 1 hour. Ventilator trends were also recorded for 24 hours, using dedicated NAVA software (SV1.3; Maquet Critical Care). Data are presented as median (interquartile range) or mean (SD) unless otherwise indicated.

Results

Fourteen patients who received a double-lung transplantation between 2011 and 2012 were included in the study (Table 1). Eleven patients received NAVA for a median of 9 days (interquartile range, 3–30 d) after bilateral lung transplantation because of primary graft dysfunction and/or pneumonia. Three other patients were ventilated 4 years (interquartile range, 4–5 years) after transplantation ("late NAVA") because of pneumonia and/or chronic rejection. Mean $F_{I_{O_2}}$ and positive end-expiratory pressure were 0.4 (SD, 0.1) and 4.1 (2.5) cm H_2O, respectively. No patients developed respiratory distress under NAVA, and they were all ventilated using the initial settings.

Average V_T was 5.3 (1.3) ml · kg^{-1} predicted body weight (ranging from 2.9 to 7.5 ml · kg^{-1}), with a coefficient of variation of 0.25 (0.14). Seven of the 14 transplanted patients were hypercapnic: V_T was 4.8 (1.2) and 5.9 (1.2) ml · kg^{-1} predicted body weight for hypercapnic and nonhypercapnic patients ($P = 0.10$), respectively. V_T was not correlated to patients' height ($P = 0.48$; Figure 1A).

Comparison with controls. Surgical patients who failed the SBT and were ventilated with NAVA were used as controls (n = 22; *see* Table E2 in the online supplement). Nontransplanted patients were older, with lower $PaCO_2$. Mean V_T in the control group was significantly higher than in transplanted patients, at 6.5 (1.5) versus 5.3 (1.3) ml · kg^{-1} predicted body weight ($P = 0.02$), and their V_T correlated with height ($r = 0.46$; $P = 0.03$; *see* Table E2).

Correlation with lung volume. There was a significant correlation between V_T under NAVA for transplanted patients and their most recently measured TLC (Pearson coefficient of correlation $r = 0.66$ [95% confidence interval, 0.20–0.88]; $P = 0.009$; Figure 1). TLC and height were not correlated ($P = 0.17$; Figure 1B), and V_T was not correlated to any other variable presented in Table 1.

Discussion

We demonstrate that the V_T freely adopted by patients after bipulmonary transplantation under NAVA was not elevated, was lower than in a control group of surgical patients at a similar stage of weaning, and correlated to their most recently measured TLC. This is the first description of a correlation between V_T and TLC under a proportional ventilator mode.

One of the theoretical advantages expected with the clinical use of NAVA is to allow patients to control their breathing pattern (7, 8). Increasing NAVA levels reduces the diaphragmatic electrical