Genome-wide association study of lung function phenotypes in a founder population

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Background: Lung function is a long-term predictor of mortality and morbidity.
Objective: We sought to identify single nucleotide polymorphisms (SNPs) associated with lung function.
Methods: We performed a genome-wide association study (GWAS) of FEV_1, forced vital capacity (FVC), and FEV_1/FVC in 1144 Hutterites aged 6 to 89 years, who are members of a founder population of European descent. We performed least absolute shrinkage and selection operation regression to select the minimum set of SNPs that best predict FEV_1/FVC in the Hutterites and used the GRAIL algorithm to mine the Gene Ontology database for evidence of functional connections between genes near the predictive SNPs.
Results: Our GWAS identified significant associations between FEV_1/FVC and SNPs at the *THSD4-UACA-TLE3* locus on chromosome 15q23 ($P = 5.7 \times 10^{-8}$ to 3.4×10^{-9}). Nine SNPs at or near 4 additional loci had $P < 10^{-5}$ with FEV_1/FVC. Only 2 SNPs were found with $P < 10^{-5}$ for FEV_1 or FVC. We found nominal levels of significance with SNPs at 9 of the 27 previously reported loci associated with lung function measures. Among a predictive set of 80 SNPs, 6 loci were identified that had a significant degree of functional connectivity (GRAIL $P < .05$), including 3 clusters of β-defensin genes, 2 chemokine genes (*CCL18* and *CXCL12*), and *TNFRSF13B*.
Conclusion: This study identifies genome-wide significant associations and replicates results of previous GWASs. Multimarker modeling implicated for the first time common variation in genes involved in antimicrobial immunity in airway mucosa that influences lung function. (J Allergy Clin Immunol 2014;133:248-55.)

Key words: *FEV_1/FVC, FEV_1, FVC, GWAS, LASSO regression, GRAIL*

Chronic lower respiratory diseases are the third leading cause of death in the United States, resulting in 137,082 deaths in 2009.[1] Lung function, as assessed by the spirometric measures of FEV_1, forced vital capacity (FVC) and the FEV_1-to-FVC ratio (FEV_1/FVC), is an objective indicator of general respiratory health, as well as an important long-term predictor of morbidity and mortality.[2-6] Family- and twin-based studies provide consistent evidence of genetic contributions to lung function, with estimates of heritability ranging as high as 85% for FEV_1, 91% for FVC, and 45% for FEV_1/FVC.[7-20]

Recently, genome-wide association studies (GWASs) have begun to shed light on the complex genetic architecture of lung function measures. Two large meta-analyses of lung function GWAS in subjects of European ancestry who participated in the SpiroMeta[21] or CHARGE[22] consortium reported 11 loci associated with FEV_1/FVC or FEV_1. A subsequent combined meta-analysis of 48,201 persons from both consortia reported 16 additional loci that influence lung function.[23] However, variants at these highly significant loci in the SpiroMeta-CHARGE meta-analysis explained only 3.2% of the variance for FEV_1/FVC and 1.5% of the variance for FEV_1.[23] Thus, similar to studies of other complex phenotypes, a significant proportion of the heritability remains unexplained by individual variants identified in GWASs.[24-26]

This "missing heritability" after GWAS has been attributed to numerous potential causes,[24-27] many or all of which likely contribute. In particular, the assumptions about the genetic model underlying complex phenotypes that are inherent in standard

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Abbreviations used
COPD: Chronic obstructive pulmonary disease
FVC: Forced vital capacity
GWAS: Genome-wide association study
LASSO: Least absolute shrinkage and selection operation
SNP: Single nucleotide polymorphism

GWAS approaches may not reflect the true genetic architecture for many phenotypes. GWASs typically assess the effect of each (common) single nucleotide polymorphism (SNP) individually with the use of stringent thresholds of significance. Although this strategy has been effective in minimizing false-positive associations and capturing the "low hanging fruit," the inability to identify genetic variation that accounts for significant proportions of human phenotypic variation suggests that alternative analytic strategies are required to differentiate the true from false-positive associations among the variants with more modest *P* values. For example, considering 294,831 SNPs simultaneously in a linear model, Yang et al[28] found that common SNPs accounted for as much as 45% of the phenotypic variance and 50% of the heritability of height in 3925 subjects compared with only 5% of the variance of height explained by approximately 50 SNPs that reached genome-wide thresholds of significance in earlier studies.[29-32]

Here, we conducted a GWAS of lung function phenotypes in members of a founder population, the Hutterites.[20,33,34] In addition to loci reported in previous GWASs, multimarker modeling identified a novel set of airway epithelial cell–derived host defense genes.

METHODS

The Hutterites

The Hutterites are a young founder population that originated in the South Tyrol in the 16th century and migrated from Europe to the United States in the 1870s.[35,36] Today, >40,000 Hutterites live on communal farms (called colonies) in the north central United States and western Canada. We have been conducting genetic studies of complex phenotypes in the Hutterites of South Dakota for >15 years.[20,34,37-40] Overall, their communal farming lifestyle minimizes environmental heterogeneity. In particular, smoking is prohibited and rare in this population, and air quality is excellent in rural South Dakota (see Table E1 in this article's Online Repository at www.jacionline.org), eliminating environmental exposures that have profound effects on lung function.

Subjects were recruited for this study if they were (1) at least 6 years of age, (2) at home on the days of our visit to their colony, and (3) able to perform spirometry. Participation rates within each colony are typically around 95%, thus minimizing ascertainment biases that could affect our results. The final sample included 1180 S-leut Hutterites who live on or were visiting 1 of 10 South Dakota colonies on the days of our visits; 187 persons (15.8%) were diagnosed with asthma, as previously defined.[39,40] These subjects are related to each other through multiple lines of descent in a 3673-person, 13-generation pedigree with 64 founders. Adult participants provided written informed consent for themselves and their children younger than 18 years; participants who were younger than 18 years provided written assent. These studies were approved by The University of Chicago Institutional Review Board.

Measures of lung function

Spirometry was performed in the Hutterites during 2 phases of field trips, the first in 1996-1997 and the second in 2006-2009, using identical protocols. Briefly, subjects underwent lung function tests with the use of spirometry in the sitting position while breathing through a mouthpiece and wearing a nose clip in accordance with the American Thoracic Society/European Respiratory Society recommendations.[41,42] The best FEV_1 and FVC were recorded. Of the 1180 persons, 335 were studied in phase 1 only, 524 in phase 2 only, and 321 in both phases. For the persons studied in both phases, we included measurements from the more recent time only and excluded 36 persons (24 used asthma rescue medications before spirometry, 4 had cystic fibrosis, and 8 had poor quality spirometry).

Genotyping and quality control

Hutterite persons were genotyped with the Affymetrix GeneChip 500k, Genome-Wide SNP 5.0, or Genome-Wide SNP 6.0 arrays (Affymetrix, Santa Clara, Calif). An overlapping set of 369,487 autosomal SNPs were present on the 500k, 5.0, and 6.0 arrays; 94,552 of those SNPs were not studied because they were monomorphic (n = 31,246) or had minor allele frequency of <5% (n = 63,306) in the Hutterites. Of the remaining 274,935 SNPs, 28,925 were excluded because they had call rates of <95% (n = 6,456), generated ≥5 Mendelian errors (n = 15,912), or deviated from Hardy-Weinberg expectations at $P < 10^{-3}$ (correcting for inbreeding and relatedness)[43] (n = 6,557), yielding a final set of 246,010 autosomal markers with a median intermarker spacing of 5.1 kb. The positions of SNPs shown in all figures and tables are based on NCBI release 36 (dbSNP build 129).

Heritability estimates and GWAS in the Hutterites

FEV_1, FVC, and FEV_1/FVC were transformed to normally distributed *z*-scores within each phase, and then adjusted for age, sex, age × sex, height, and inbreeding. The residuals of each trait from the 2 phases were then combined for further analyses. The distributions of these traits by age and sex and the correlations between them are shown in Fig E1 (in the Online Repository available at www.jacionline.org). The heritabilities of lung function measures were estimated with variance-component methods, as previously described.[44] Association testing was performed with a regression-based test for large, complex pedigrees.[37] Briefly, at each SNP, we used the general 2-allele model test of association in the entire pedigree, keeping all inbreeding loops intact; at each SNP we tested an additive model of association. SNP-specific *P* values were determined according to Gaussian theory. Genomic inflation was weak or absent (genomic inflation factor $\lambda = 1.10$ for FEV_1, 1.09 for FVC, and 1.00 for FEV_1/FVC). The GWAS *P* values for FEV_1 and FVC were adjusted by using their genomic control.[45] The Bonferroni-corrected genome-wide significance threshold was $P < 2.0 \times 10^{-7}$ (ie, 0.05/246,010). The proportion of the residual variance explained by each SNP or a group of SNPs was determined by comparing the residual sum of squares in the regression model with that obtained without a SNP (or a group of SNPs), as implemented in the general 2-allele model.

In silico replication

We investigated in the Hutterites the associations between lung function measures and SNPs at the 27 previously identified loci associated with lung function.[21-23] If the previously reported SNP was not genotyped in the Hutterites, a surrogate SNP with the strongest linkage equilibrium to the reported SNP was investigated.

Multimarker modeling

To select the minimum set of SNPs that best predict FEV_1/FVC in the Hutterites from among SNPs with $P < 10^{-3}$ in the GWAS, we performed least absolute shrinkage and selection operation (LASSO) regression,[46-48] as implemented in the R package glmnet.[49] These studies were conducted in 604 Hutterites without missing genotypes at all 312 SNPs with $P < 10^{-3}$ (87 SNPs had missing data in at least 1 person and were not included in the LASSO regression). Of the 540 subjects that had missing genotypes in these

SNPs and not included in the LASSO regression, 261 had no missing genotypes in the 80 SNPs selected by LASSO and were used in subsequent analyses. The minimum set of best-predicting SNPs was selected by running a 10-fold cross-validation procedure after choosing the glmnet parameter $\alpha = 1.0$. The cross-validation procedure selected a LASSO penalty parameter of $\lambda = 3.3 \times 10^{-3}$. *K*-fold cross-validation was used to minimize the effects of overfitting the model to our data by randomly dividing the full data set into *K*-subsamples where *K-1* subsamples are used to develop the model and the remaining subsample is used for testing the model. LASSO regression uses SNPs as predictors of the phenotype (FEV_1/FVC), while minimizing the number of SNPs in the model. Genotypes were coded as 0, 1, or 2 doses of the minor allele. After the 10-fold cross-validation procedure the LASSO regression selected 108 SNPs in the model. However, 28 of these SNPs had negligible effect sizes (absolute value of fixed effect size < .005) and were removed from the model, resulting in a final set of 80 SNPs.

Identifying related sets of genes

To identify related sets of genes and common pathways for genes near the SNPs that best predicted FEV_1/FVC, we used the GRAIL algorithm[50] to mine the Gene Ontology database. Briefly, GRAIL assesses the degree of relatedness among genes within regions that harbor predictive SNPs, selecting the most connected gene that corresponds to 1 or more SNPs as the likely implicated gene. GRAIL assigns a *P* value for each region that reflects the relatedness of the gene(s) in each region to all other regions, correcting for the number of genes in the region.

RESULTS

A total of 1144 Hutterites (613 females; 53.6%) aged 6 to 89 years (mean ± SD, 30.6 ± 18.4 years) with both genome-wide genotyping and spirometry phenotypes were included in the GWAS (Table I). These same data are shown for the nonasthmatic and asthmatic sample subsets in Table E2 (in the Online Repository available at www.jacionline.org).

Heritability of lung function in the Hutterites

The broad (H^2) and narrow (h^2) heritabilities of lung function measures in the Hutterites were $h^2 = H^2 = 40.2\%$ (SE 5.4%) for FEV_1, $h^2 = 17.8\%$ (SE 3.7%) and $H^2 = 70.4\%$ (SE 11.2%) for FVC, and $h^2 = 22.1\%$ (SE, 8.0%) and $H^2 = 91.5\%$ (SE, 12.9%) for FEV_1/FVC. These estimates indicate that 40.2%, 70.4%, and 91.5% of the phenotypic variances in FEV_1, FVC, and the FEV_1/FVC, respectively, are attributable to genetic variation in the Hutterites. The heritabilities of FVC and FEV_1/FVC included both additive and nonadditive (ie, dominance) genetic variance components, whereas the heritability of FEV_1 was attributed entirely to additive genetic variance.

GWAS of lung function traits

We identified genome-wide significant associations between FEV_1/FVC and 5 SNPs at the *THSD4-UACA-TLE3* locus on chromosome 15q23 (see Fig E2, *A*, in this article's Online Repository at www.jacionline.org), replicating results from previous GWASs.[21,23] Overall, there were 21 SNPs at this locus with $P < 10^{-5}$ (see Table E3 in this article's Online Repository at www.jacionline.org). The most significant SNP at this locus, rs12441227, explained 2.9% of the residual variance in FEV_1/FVC in the Hutterites. The evidence for association with SNPs at this locus remained when the persons with asthma were excluded (Fig E2, *D*), and when the sample was stratified by age (see Table E4 in this article's Online Repository at www.jacionline.org).

Nine additional SNPs at 4 loci had *P* values $< 10^{-5}$ with FEV_1/FVC, including SNPs downstream of the *C10orf11* gene on chromosome 10q22.3, which was associated with FEV_1 in a meta-analysis of lung function GWAS.[23] When a subanalysis was performed that excluded the Hutterites with asthma, the evidence for association at this locus increased to genome-wide levels of significance (Table E4 and Fig E2, *F*). The evidence for associations with SNPs at 3 of these loci with *P* values $< 10^{-5}$, *CCL23-CCL18* on chromosome 17q12 (Fig E2, *B* and *E*), *PITPNC1* locus on chromosome 17q24.2, and *CHAF1B* on chromosome 21q22.13, remained in subanalyses that excluded persons with asthma. The evidence for association at all locus with *P* values $< 10^{-5}$ remained in subset analyses stratified by age (Table E4). Only 2 SNPs had *P* values $< 10^{-5}$ in the GWAS for the other 2 phenotypes: 1 SNP 7 k downstream of the *IL37* gene on chromosome 2q13 was associated with FEV_1 and 1 SNP in an intron of *ASXL3* on chromosome 18q12.1 was associated with FVC.

The Manhattan and Q–Q plots of *P* values for the GWAS of the 3 phenotypes are shown in Fig 1; results for all SNPs with $P < 10^{-5}$ are shown in Table E3. The GWAS *P* values in the Hutterites for the 27 loci associated with lung function in previous meta-analyses[21-23] are shown in Table E5 (in the Online Repository available at www.jacionline.org). Overall, we found nominal evidence ($P < .05$) of association with at least 1 of the 3 phenotypes for 15 SNPs at 9 of the 27 previously reported loci.

Multimarker modeling

We assumed that there were additional true associations among the GWAS SNPs that did not reach genome-wide levels of significance because their effects are too small to detect in single SNP analyses, especially in a sample size of approximately 1000 subjects. Therefore, to assess a multimarker model of risk that included all SNPs with $P < 10^{-3}$, we performed LASSO regression to identify minimum sets of SNPs that provided the smallest mean square error of FEV_1/FVC in the Hutterites. A set of 80 SNPs yielded the best predictive value and were used for further study (see Table E6 in this article's Online Repository at www.jacionline.org).

First, we assessed the phenotypic effects of these 80 SNPs by binning persons by the total number of alleles associated with reduced FEV_1/FVC that they carried (total possible = 160) and calculated the mean ± SE residual FEV_1/FVC for Hutterites in each bin. The mean residual FEV_1/FVC decreased with increasing number of low FEV_1/FVC alleles, consistent with an additive genetic architecture (Fig 2).

Next, we used the GRAIL algorithm[50] to mine the Gene Ontology database for evidence of functional connections between genes near the 80 predictive SNPs. We identified a subset of 6 SNPs with significantly related genes (GRAIL $P < .05$), including 3 clusters of β-defensin genes, 2 chemokine genes (*CCL18* and *CXCL12*), and *TNFRSF13B* (Table II and Fig 3). Notably, the associated GWAS SNPs at 2 replicated loci, *THSD4-UACA-TLE3* and *C10orf11*, were not functionally connected to any other genes defined by the 80 SNPs. However, a SNP at the *CCL23-CCL18* locus, the second most significant locus in the Hutterite GWAS (see Fig E2, *B*, in this article's Online Repository at www.jacionline.org), was significantly

TABLE I. Characteristics of the Hutterite sample

	Males		Females	
	6-17 y	>17 y	6-17 y	>17 y
Sample size	180	351	195	418
No. with asthma (%)	25 (13.9)	48 (13.6)	38 (19.5)	51 (12.2)
No. with atopy (%)	76 (42.2)	191 (54.4)	93 (47.7)	194 (46.4)
Mean age ± SD (y)	11.3 ± 3.2	40.1 ± 15.3	12.2 ± 2.9	39.6 ± 15.7
Mean FEV_1 ± SD (L)	2.60 ± 1.05	3.90 ± 0.77	2.56 ± 0.78	2.94 ± 0.59
Mean FVC ± SD (L)	3.04 ± 1.29	4.91 ± 0.91	2.86 ± 0.90	3.61 ± 0.70
Mean FEV_1/FVC ± SD (%)	86.8 ± 7.5	79.6 ± 7.8	90.0 ± 6.5	81.5 ± 7.1

Asthma and atopy are defined as described in Ober et al.[40]

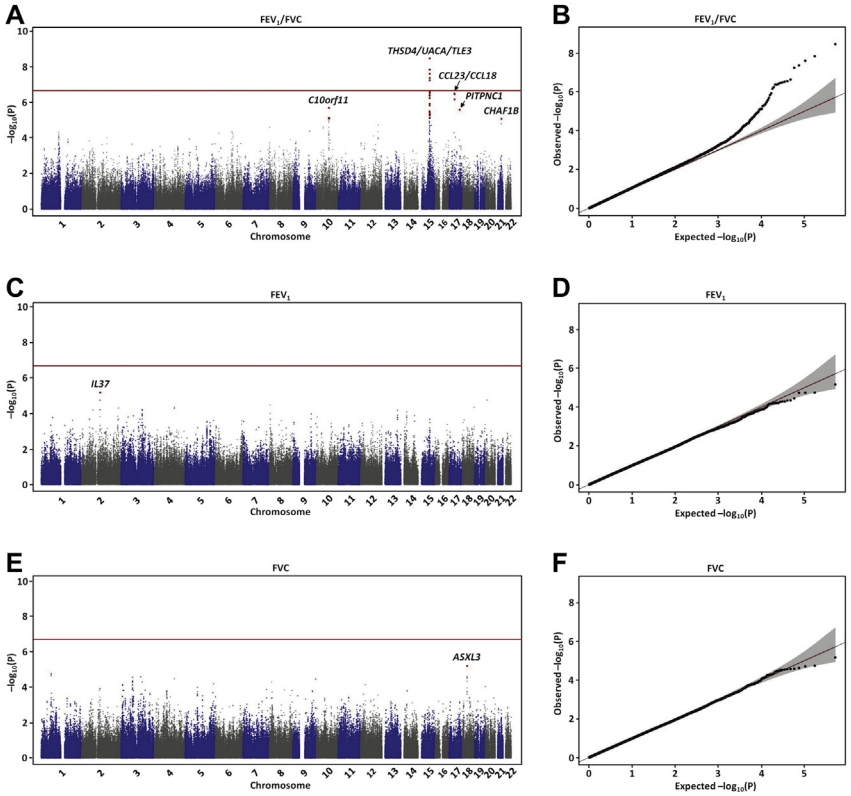

FIG 1. Manhattan and Q–Q plots of *P* values from the GWAS of FEV_1/FVC **(A** and **B)**, FEV_1 **(C** and **D)**, and FVC **(E** and **F)**. SNPs with $P < 10^{-5}$ are shown in *red*. The *horizontal red line* shows the genome-wide significance threshold ($P < 2.0 \times 10^{-7}$).

connected to the β-defensin genes, as well as to *CXCL12* and *TNFRSF13B* in the GRAIL analysis. These 6 SNPs by themselves explained 5.8% of the residual variance in FEV_1/FVC in the Hutterites.

DISCUSSION

The success of GWAS for unraveling the genetic architecture of complex phenotypes has been widely debated.[24-27,51-53] Although many robust associations have been discovered for a wide spectrum of diseases and phenotypes,[54] the associated variants typically explain relatively little of the phenotypic variation. Several recent studies have highlighted the importance of approaches that consider multiple variants simultaneously,[28,48,55-58] a more suitable approach if the genetic architecture of common phenotypes is polygenic with many contributing loci with small effects. However, the best way to identify multiple contributing loci is at present unclear.

The GWAS of the FEV_1/FVC in the Hutterites revealed 2 previously reported associations with measures of lung function. Associations with multiple SNPs at the highly replicated locus on 15q23[21,23] reached genome-wide significance in the combined sample, and SNPs at the *C10orf11* on chromosome 10q22.3[23] reached genome-wide significance in the nonasthmatic subset of the Hutterite sample. These results were robust to age, with evidence for association present in both the child and adult subsets of the population. Moreover, we detected nominal levels of significance with SNPs at 9 previously reported loci associated with lung function measures. Together, these results indicate that genes influencing lung function in Europeans and European

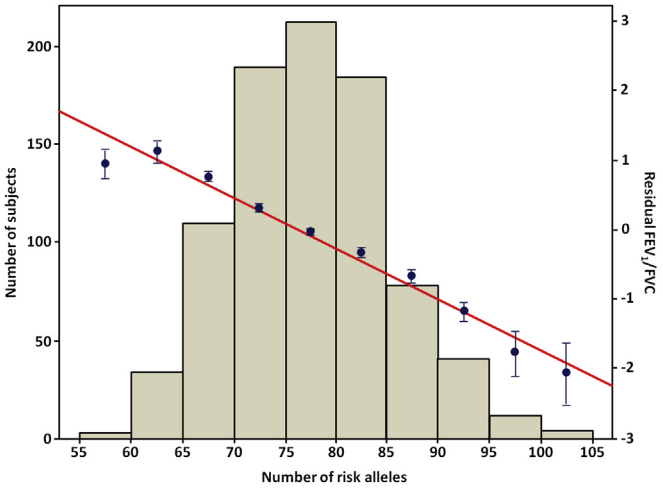

FIG 2. The combined effects of genotypes for 80 SNPs on the residual FEV_1/FVC in the Hutterites. Hutterites were binned by their total number of alleles associated with reduced FEV_1/FVC (x-axis); the mean ± SE residual FEV_1/FVC for each bin is plotted on the right y-axis (*blue dots* and *bars*), and the number of subjects in each bin is on the left y-axis. The linear regression line through these points is shown in *red*.

Americans from the general population also contribute to lung function phenotypes in the Hutterites.

To assess the combined effects of these and other SNPs with less significant evidence of association, we used LASSO regression to select the minimum set of SNPs from among the 312 with $P < 10^{-3}$. The LASSO regression selected 80 independent SNPs as the best predictor of the FEV_1/FVC. Consistent with an additive genetic model, the mean phenotypic value decreases with increasing number of "risk" alleles (Fig 2). Moreover, this approach led to the discovery of additional genes, including 3 independent clusters of β-defensin genes, 2 chemokine genes, and a TNF family receptor, suggesting an important link between host defense mechanisms and lung function. Defensins are antimicrobial peptides that recruit inflammatory cells and modulate innate and adaptive immune responses, participating in both the promotion and resolution of inflammatory responses.[59] There are 3 classes of defensins, but only the β-defensins are specifically expressed in epithelial cells, including those lining the respiratory tract. Genetic studies have implicated the β-defensin genes on chromosome 8p23 in lung function in patients with asthma,[60] chronic obstructive pulmonary disease (COPD)[61] and with cystic fibrosis.[62] In particular, *DEFB1* mRNA in bronchial epithelial cell biopsies was significantly elevated in patients with COPD compared with controls and significantly associated with both reduced FEV_1 and FEV_1/FVC in patients with COPD and in controls.[61] The results of our studies would further suggest that all 3 clusters of β-defensin genes on chromosomes 8p23, 20p13, and 20p11 contribute to lung function in healthy, unselected subjects. Chemokines are small proteins that bind to G-protein–coupled receptors and orchestrate the migration of circulating leukocytes to sites of inflammation. CCL18 (also named pulmonary and activated-regulated cytokine) is constitutively and highly expressed in the human lung[63] and can generate regulatory T cells from $CD4^+CD25^-$ T cells in healthy persons via direct induction of TGF-β1.[64] Functional polymorphisms in the promoter of the *TGFB1* gene have been associated with airway responsiveness and asthma exacerbations, and haplotypes that comprise polymorphisms and specific coding variants in this gene have been associated with lung function in patients with cystic fibrosis,[65,66] although the exact variants and direction of effect are inconsistent across studies. Moreover, both β-defensin-2 and CCL18 were significantly elevated in peripheral blood from patients with COPD compared to in smoking and nonsmoking controls.[67] CXCL12 (also name stromal derived growth factor 1) is critical to bone marrow–derived stem cell production and shows increased expression in bronchial alveolar lavage fluid after bleomycin-induced lung fibrosis in a murine model and in airway tissues in patients with idiopathic pulmonary fibrosis compared with controls.[68] The *TNFRSF13B* gene encodes the transmembrane activator and calcium modulator and cyclophilin ligand interactor, which binds 2 ligands, B-cell activating factor and a proliferating-inducing ligand. It is thought that the transmembrane activator and calcium modulator and cyclophilin ligand interactor plays a key role in B-cell activation and differentiation into plasma cells. In a recent study, rare mutations in *TNFRSF13B* were associated with asthma symptoms in Swedish children.[69] Moreover, expression of B-cell activating factor in alveolar macrophages was inversely correlated with lung function in patients with COPD.[70] Our study extends the roles of these 2 chemokines and TNF-family receptor to interindividual variability in normal lung function.

Despite conducting this study in a relatively small sample (~1000 Hutterites) and the absence of a major locus that influenced variation in lung function compared with other traits (eg, see Ober et al[39] and Ober et al[71]), we were successful in identifying both genome-wide significant associations with replicated loci on chromosome 15 in the combined sample and on chromosome 10 in the nonasthmatic subset, in addition to a set of novel variants that are highly predictive for lung function in the Hutterites. The power of our study was likely enhanced by the homogeneity of the Hutterite population compared with the larger population samples that have been included in previous studies of lung function.[21-23] The advantages of this population for genetic studies of complex phenotypes are primarily 2-fold. On the one hand, it is possible that there are fewer lung function-associated alleles segregating in the Hutterites because of the population bottleneck that occurred before their emigration to the United States.[35,36] This would result in a simpler genetic architecture due to both overall reduced genetic variation and increased frequencies of some variants with potentially larger phenotypic effects that are rare in other European populations. On the other hand, their communal lifestyle and shared environmental exposures,[33] which include the absence of exposure to cigarette smoke and air pollution, may have enhanced the effects of genetic variation in general, and on specific pathways in particular, on lung development and subsequent lung function. In this population, exposures are remarkably similar during critical periods of lung development both *in utero* and in early life. Hutterite women and young children are not directly involved in farming activities, and their homes are generally distant from the agricultural fields and animal barns. Meals are prepared in a communal kitchen, using traditional recipes that are shared among the colonies. There are no pets, televisions, radios, or computers in the homes, and, as a result, Hutterite children spend significant proportions of each day playing outside. Thus, the absence of important environmental exposures that affect lung development and lung function, combined with a shared environment throughout life, not only reduces nongenetic heterogeneity but also allows for the detection

TABLE II. High-scoring regions from the GRAIL analysis, sorted by the GRAIL *P* value

SNP	Chromosome	NCBI36 position	P_{GWAS}	Beta (SE)	P_{GRAIL}	Implicated gene
rs365548	20	185,618	$5.61E^{-04}$	0.003 (0.046)	$6.4E^{-04}$	*DEFB129, C20orf96*
rs2921026	8	8,384,658	$6.14E^{-04}$	−0.133 (0.046)	$6.6E^{-04}$	*DEFB107A*
rs4815436	20	25,521,423	$9.45E^{-04}$	0.181 (0.069)	$9.7E^{-04}$	*DEFB115, DEFB116, DEFB123, DEFB124*
rs854679	17	31,382,952	$3.40E^{-07}$	−0.049 (0.059)	.020	*CCL18*
rs1570846	10	43,776,486	$7.06E^{-04}$	−0.139 (0.055)	.037	*CXCL12*
rs7216399	17	16,797,303	$5.96E^{-04}$	−0.065 (0.054)	.043	*TNFRSF13B*

P_{GWAS} is the *P* value from the FEV_1/FVC GWAS in the Hutterites; Beta (SE) is that of the predictive SNP in the regression model for the 865 Hutterites; and P_{GRAIL} is the region's *P* value given by GRAIL. The last column shows the candidate gene identified by GRAIL.

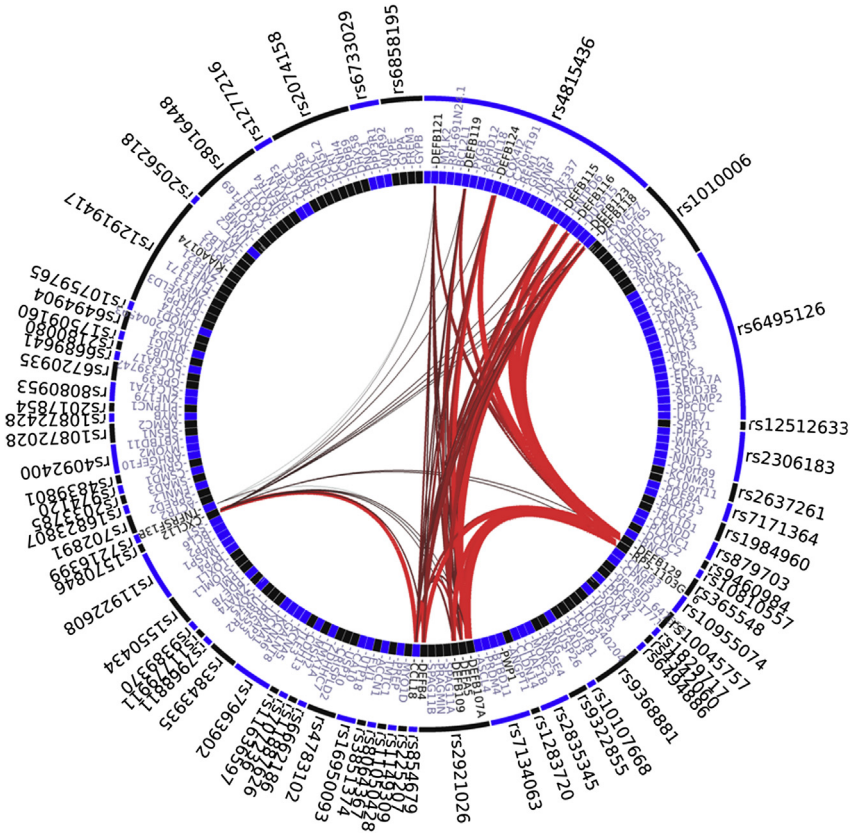

FIG 3. GRAIL functional connections between the 80 predictive SNPs. Six SNPs with no nearby genes defined by GRAIL are not shown. GRAIL identified 6 pairs of SNPs that implicated the same genes; only 1 SNP from each these 6 pairs is shown in the figure. The regions (SNPs; *outer ring*) and genes *(inner ring)* are optimally ordered to display connections with a minimal number of intersections. Only the genes with $P_{GRAIL} < .05$ have connections displayed. The *thickness* and *redness* of the connectors reflects the significance of the connections. Three clusters of β-defensin genes are the most connected sets.

of lung function alleles that are not confounded with those related to socioeconomic factors or behavior, such as cigarette smoking, or to ecogenetic pathways that are important in metabolizing inhaled particles. These population characteristics possibly enabled the novel finding in this study of an enrichment of genes involved in antimicrobial immunity in the airways among those associated with lung function.

In summary, this study identifies genome-wide significant associations between lung function and SNPs at the *THSD4-UACA-TLE3* locus on chromosome 15q23 and the *C10orf11* on chromosome 10q22.3, and replicates many other previous GWAS results. Moreover, with the use of LASSO regression, we identified 80 independent SNPs as the best predictor of FEV_1/FVC, with the mean phenotypic value decreasing with increasing number of risk alleles, consistent with an additive genetic architecture. Of note is that multimarker modeling implicated for the first time common variation in 3 independent clusters of β-defensin genes, 2 chemokine genes, and a TNF family receptor that involved in antimicrobial immunity in airway mucosa and influences lung function.

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Clinical implications: Three independent clusters of β-defensin genes, 2 chemokine genes (*CCL18* and *CXCL12*), and *TNFRSF13B* that are involved in antimicrobial immunity in airway mucosa contribute to lung function phenotypes in healthy, unselected subjects.

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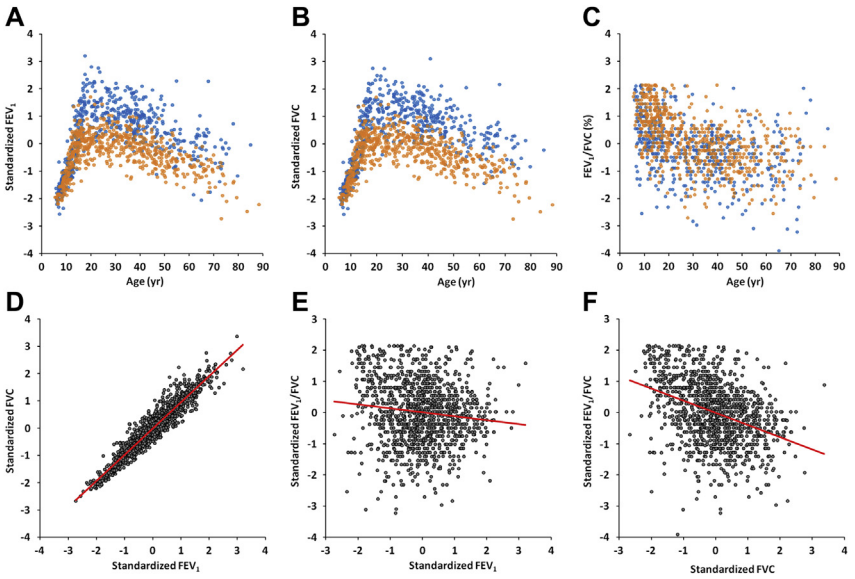

FIG E1. Measures of lung function in the Hutterites. Distributions of standardized values of FEV_1 **(A)**, FVC **(B)**, and FEV_1/FVC **(C)** by age and sex (*blue*, male; *orange*, female). Correlations between measures of lung function: FEV_1 and FVC **(D)**, FEV_1 and FEV_1/FVC **(E)**, and FVC and FEV_1/FVC **(F)**. The linear regression line is shown in *red*.

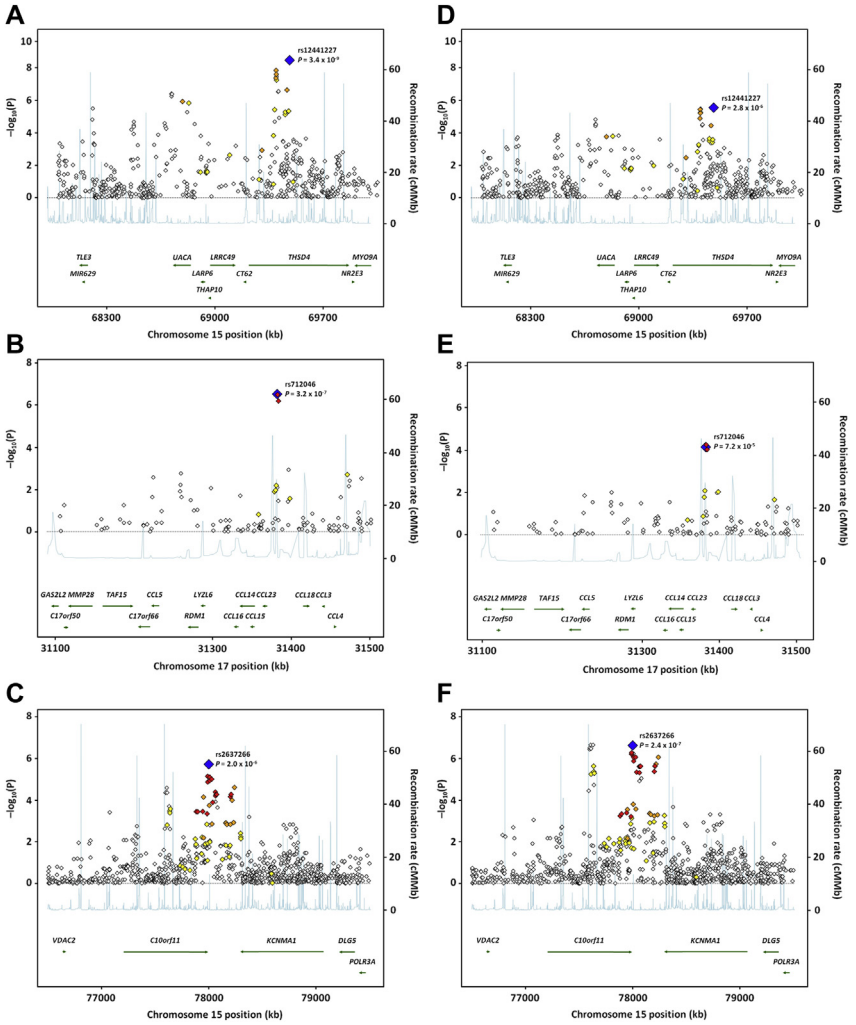

FIG E2. Regional association plots for the 3 most significant associations in the GWAS of FEV_1/FVC in the Hutterites: *THSD4-UACA-TLE3* locus on chromosome 15q23, *CCL23-CCL18* locus on chromosome 17q12, and *C10orf11* locus on chromosome 10q22.3 in the full sample (**A-C**, respectively) and in subanalyses that excluded persons with asthma (**D-F**, respectively). In each plot the most significantly associated SNP is shown as a *large blue diamond*. The colors of the other SNPs reflect the linkage disequilibrium with that SNP based on r^2 values in the Hutterites (*red*, $r^2 \geq 0.8$; *orange*, $0.5 \leq r^2 < 0.8$; *yellow*, $0.2 \leq r^2 < 0.5$; *white*, $r^2 < 0.2$).

TABLE E1. Air quality data for the 10 Hutterite colonies in South Dakota participating in these studies

ZIP code	No. of colonies at this ZIP code	Range of air quality values	Overall air quality	Overall rating
57042	1	5.0-9.9	9.2	Outstanding
57076	1	5.7-9.9	9.2	Outstanding
57301	2	6.8-9.9	9.6	Outstanding
57311	2	6.8-9.9	9.6	Outstanding
57314	1	5.0-9.9	9.2	Outstanding
57334	1	6.8-9.9	9.2	Outstanding
57334	1	6.8-9.9	9.6	Outstanding
57366	1	6.8-9.9	9.6	Outstanding

These data are gathered from measuring stations across the country. A higher number (on a scale of 1-10) reflects fewer amounts of pollutants (ie, a 9.0 means that 90% of the stations around the country are measuring higher amounts than the local station). The 6 air pollutants reported are ozone, carbon monoxide, nitrogen dioxide, sulfur dioxide, particulate matter (PM) 10, and PM 2.5. The range of values for the 6 pollutants and overall ratings are shown. Values for the individual pollutants can be found at http://www.homefacts.com/airquality/South-Dakota.html.

TABLE E2. Characteristics of the nonasthmatic and asthmatic sample subsets

	Males		Females	
	6-17 y	**>17 y**	**6-17 y**	**>17 y**
Persons with asthma				
Sample size	25	48	38	51
No. with atopy (%)	13 (52.0)	34 (70.8)	20 (52.6)	24 (47.1)
Mean age ± SD (y)	10.8 ± 3.2	39.4 ± 14.6	12.0 ± 2.9	38.2 ± 15.6
Mean FEV_1 ± SD (L)	2.28 ± 0.92	3.76 ± 0.78	2.56 ± 0.81	2.80 ± 0.53
Mean FVC ± SD (L)	2.75 ± 1.13	5.03 ± 0.90	2.93 ± 0.95	3.64 ± 0.70
Mean FEV_1/FVC ± SD (%)	83.5 ± 7.1	74.9 ± 7.5	87.8 ± 6.7	77.2 ± 8.0
Persons without asthma				
Sample size	155	303	157	367
No. with atopy (%)	63 (40.6)	157 (51.8)	73 (46.5)	170 (46.3)
Mean age ± SD (y)	11.5 ± 3.2	40.2 ± 15.4	12.2 ± 2.9	39.8 ± 15.6
Mean FEV_1 ± SD (L)	2.65 ± 1.06	3.92 ± 0.77	2.56 ± 0.77	2.96 ± 0.60
Mean FVC ± SD (L)	3.08 ± 1.31	4.89 ± 0.91	2.85 ± 0.90	3.61 ± 0.70
Mean FEV_1/FVC ± SD (%)	87.3 ± 7.5	80.4 ± 7.6	90.0 ± 6.3	82.1 ± 6.8

TABLE E3. SNPs associated with lung function at $P < 10^{-5}$ in the Hutterites

SNP	Chr	NCBI36 position	Gene	SNP-gene relationship	Major/minor allele	MAF	FEV_1/FVC *P* value	FEV_1 *P* value	FVC *P* value
rs10864907	2	113,400,346	*IL37*	7.4 k downstream	G/A	0.44	1.5E-01	6.7E-06	3.9E-04
rs2637260	10	77,990,352	*C10orf11*	3.2 k downstream	T/C	0.44	7.5E-06	1.4E-01	3.9E-01
rs2637261	10	77,990,599	*C10orf11*	3.5 k downstream	G/A	0.44	7.5E-06	1.4E-01	3.9E-01
rs2637266	10	78,001,324	*C10orf11*	14.2 k downstream	T/C	0.44	2.0E-06	1.1E-01	4.2E-01
rs10824425	10	78,010,316	*C10orf11*	23.2 k downstream	C/G	0.44	8.2E-06	1.1E-01	4.7E-01
rs11856830	15	68,211,555	*TLE3*	34.2 k upstream	A/G	0.40	3.2E-06	1.6E-01	8.7E-01
rs2114719	15	68,720,392	*UACA*	13.6 k downstream	G/A	0.12	4.2E-07	6.9E-04	2.5E-01
rs2162555	15	68,720,626	*UACA*	13.3 k downstream	G/A	0.12	5.7E-07	2.2E-04	1.6E-01
rs6494886	15	68,720,785	*UACA*	13.2 k downstream	C/A	0.12	3.9E-07	1.2E-03	2.5E-01
rs2162556	15	68,723,492	*UACA*	10.5 k downstream	C/T	0.12	4.2E-07	6.9E-04	2.5E-01
rs1991088	15	68,791,504	*UACA*	Intron	C/T	0.18	1.2E-06	3.8E-02	8.6E-01
rs1477439	15	68,821,634	*UACA*	Intron	G/C	0.28	5.3E-06	1.3E-01	5.4E-01
rs4777305	15	68,832,522	*UACA*	Intron	T/C	0.18	1.5E-06	2.5E-02	8.1E-01
rs11633212	15	69,387,305	*THSD4*	Intron	A/G	0.33	3.8E-06	1.6E-01	6.4E-01
rs17786786	15	69,395,673	*THSD4*	Intron	A/C	0.27	4.2E-08*	1.6E-01	4.2E-01
rs6494904	15	69,396,576	*THSD4*	Intron	T/C	0.25	1.4E-08*	5.8E-02	5.9E-01
rs11855326	15	69,397,889	*THSD4*	Intron	G/A	0.25	2.5E-08*	5.4E-02	6.6E-01
rs1837762	15	69,399,357	*THSD4*	Intron	C/T	0.11	5.7E-08*	1.7E-03	4.5E-01
rs11858540	15	69,409,840	*THSD4*	Intron	T/G	0.12	3.0E-07	1.5E-02	7.4E-01
rs1441361	15	69,412,176	*THSD4*	Intron	A/G	0.12	2.8E-07	1.5E-02	7.3E-01
rs1568010	15	69,455,566	*THSD4*	Intron	T/G	0.44	7.6E-06	3.8E-01	5.8E-02
rs11858454	15	69,456,169	*THSD4*	Intron	C/T	0.44	5.3E-06	3.8E-01	6.1E-02
rs8033889	15	69,467,134	*THSD4*	Intron	G/T	0.25	2.3E-07	4.4E-03	3.3E-01
rs4531689	15	69,476,033	*THSD4*	Intron	C/T	0.46	4.8E-06	5.0E-01	5.4E-02
rs4288952	15	69,477,937	*THSD4*	Intron	G/A	0.45	4.6E-06	4.6E-01	4.8E-02
rs12441227	15	69,483,940	*THSD4*	Intron	T/C	0.22	3.4E-09*	2.2E-03	3.7E-01
rs712046	17	31,382,410	*CCL23*	13.3 k upstream	G/A	0.32	3.2E-07	8.2E-02	5.0E-01
rs854679	17	31,382,952	*CCL23*	13.8 k upstream	G/T	0.32	3.4E-07	8.9E-02	5.2E-01
rs854674	17	31,384,085	*CCL23*	15.0 k upstream	T/C	0.32	6.8E-07	1.3E-01	4.6E-01
rs2017854	17	62,918,483	*PITPNC1*	Intron	G/C	0.41	2.6E-06	5.2E-01	8.5E-04
rs4799710	18	29,520,739	*ASXL3*	Intron	A/G	0.32	2.8E-01	6.4E-04	6.7E-06
rs2835345	21	36,723,304	*CHAF1B*	12.3 k downstream	G/T	0.25	8.3E-06	1.1E-01	4.9E-01

Chr, Chromosome; *MAF*, minor allele frequency.

*P values exceeded the threshold for Bonferroni-corrected genome-wide significance ($P < 2.0 \times 10^{-7}$).

TABLE E4. Results of analyses of FEV_1/FVC in combined sample, in sample excluding asthmatics (nonasthmatics), and in analyses stratified by age

			Combined sample		Nonasthmatics		Asthmatics		Adults (>17 y)		Children (≤17 y)	
SNP	**Chr**	**NCBI36 position**	**No.**	***P* value**	**No.**	***P* value**	**No.**	***P* value**	**No.**	***P* value**	**No.**	***P* value**
rs2637260	10	77,990,352	1106	7.50E-06	949	6.34E-07	157	9.82E-01	760	2.70E-04	346	2.69E-02
rs2637261	10	77,990,599	1106	7.50E-06	949	6.34E-07	157	9.82E-01	760	2.70E-04	346	2.69E-02
rs2637266	10	78,001,324	1065	2.00E-06	916	2.43E-07	149	8.31E-01	722	4.22E-05	343	3.38E-02
rs10824425	10	78,010,316	1099	8.23E-06	943	8.98E-07	156	9.99E-01	753	3.34E-04	346	2.58E-02
rs11856830	15	68,211,555	1055	3.17E-06	905	2.41E-04	150	1.42E-01	713	1.87E-03	342	1.40E-03
rs2114719	15	68,720,392	1106	4.15E-07	949	2.97E-05	157	1.93E-02	760	5.56E-05	346	1.98E-02
rs2162555	15	68,720,626	1095	5.68E-07	940	3.74E-05	155	2.38E-02	750	4.62E-05	345	2.38E-02
rs6494886	15	68,720,785	1092	3.87E-07	938	1.50E-05	154	4.28E-02	750	6.07E-05	342	2.58E-02
rs2162556	15	68,723,492	1106	4.15E-07	949	2.97E-05	157	1.93E-02	760	5.56E-05	346	1.98E-02
rs1991088	15	68,791,504	1097	1.18E-06	941	1.72E-04	156	1.64E-03	753	3.87E-04	344	5.35E-03
rs1477439	15	68,821,634	1101	5.34E-06	945	1.18E-03	156	9.70E-03	757	4.63E-03	344	5.08E-04
rs4777305	15	68,832,522	1100	1.47E-06	944	1.60E-04	156	3.22E-03	754	6.59E-04	346	4.03E-03
rs11633212	15	69,387,305	1103	3.80E-06	946	5.33E-04	157	7.29E-05	757	1.22E-04	346	2.31E-02
rs17786786	15	69,395,673	1100	4.23E-08	944	1.28E-05	156	7.28E-05	757	1.08E-05	343	1.09E-02
rs6494904	15	69,396,576	1105	1.43E-08	948	3.64E-06	157	1.57E-04	759	3.23E-06	346	9.91E-03
rs11855326	15	69,397,889	1104	2.47E-08	947	6.12E-06	157	1.57E-04	758	6.08E-06	346	9.91E-03
rs1837762	15	69,399,357	1104	5.65E-08	947	5.42E-06	157	2.36E-02	758	4.17E-05	346	1.59E-02
rs11858540	15	69,409,840	1106	2.98E-07	949	3.44E-05	157	1.26E-02	760	2.05E-04	346	2.12E-02
rs1441361	15	69,412,176	1105	2.76E-07	948	3.06E-05	157	1.26E-02	759	1.95E-04	346	2.12E-02
rs1568010	15	69,455,566	1102	7.56E-06	946	3.10E-04	156	1.45E-01	757	3.08E-05	345	1.15E-01
rs11858454	15	69,456,169	1106	5.26E-06	949	2.26E-04	157	1.38E-01	760	2.87E-05	346	1.04E-01
rs8033889	15	69,467,134	1103	2.31E-07	946	3.56E-05	157	1.08E-03	757	1.89E-05	346	2.07E-03
rs4531689	15	69,476,033	1104	4.77E-06	947	3.87E-04	157	1.05E-01	759	5.60E-05	345	4.50E-02
rs4288952	15	69,477,937	1099	4.58E-06	942	2.60E-04	157	1.03E-01	755	3.51E-05	344	8.37E-02
rs12441227	15	69,483,940	1091	3.38E-09	36	2.79E-06	155	2.05E-03	746	4.70E-06	345	5.86E-04
rs712046	17	31,382,410	1104	3.20E-07	947	7.23E-05	157	4.37E-02	759	6.73E-05	345	6.27E-03
rs854679	17	31,382,952	1097	3.40E-07	941	5.66E-05	156	6.64E-02	751	6.21E-05	346	7.38E-03
rs854674	17	31,384,085	1098	6.77E-07	941	9.55E-05	157	5.44E-02	752	1.39E-04	346	6.92E-03
rs2017854	17	62,918,483	1105	2.58E-06	948	3.87E-05	157	1.48E-03	759	1.63E-05	346	1.19E-02
rs2835345	21	36,723,304	1103	8.29E-06	947	1.92E-04	156	3.39E-03	758	2.45E-03	345	5.70E-03

All SNPs with $P < 10^{-5}$ in the combined sample are shown.
Chr, Chromosome.

TABLE E5. Evidence for associations with lung function in the Hutterites for the 27 previously reported lung function associated loci

Chr	NCBI36 position	Reported SNP	Reported locus	Reported phenotype	Tested SNP	NCBI36 position	HapMap r^2	FEV_1/FVC *P* value	FEV_1 *P* value	FVC *P* value	Other SNPs at locus with *P* < .01
1	17,051,981	rs2284746	*MFAP2*	FEV_1/FVC	rs7545518	17,374,742	0.574	8.25E-01	9.74E-01	6.60E-01	
1	215,248,463	rs993925	*TGFB2*	FEV_1/FVC	rs642836	219,023,654	0.203	5.54E-01	4.65E-01	1.78E-01	rs1018040 (9.29E-03, FVC)
2	218,508,660	rs2571445	*TNS1*	FEV_1	rs3796028	218,695,102	0.530	7.22E-01	1.13E-01	9.09E-02	
2	229,336,434	rs1435867	*PID1*	FEV_1/FVC	rs3732192	229,592,304	1	5.18E-02	1.33E-01	5.11E-01	
	229,328,008	rs10498230	*PID1*	FEV_1/FVC	rs10498230	229,328,008	1	5.51E-01	5.01E-01	6.96E-01	
2	239,613,402	rs12477314*	*HDAC4*	FEV_1/FVC	rs12712295	239,914,718	0.281	3.04E-02	4.59E-01	7.20E-01	rs10186131 (5.27E-03, FEV_1/FVC)
3	25,495,586	rs1529672*	*RARB*	FEV_1/FVC	rs1153582	25,543,275	0.928	1.37E-02	7.66E-01	3.31E-01	rs2116703 (2.23E-03, FEV_1)
3	170,782,921	rs1344555*	*MECOM*	FEV_1	rs10513678	169,312,833	0.504	5.77E-03	5.57E-01	3.76E-02	rs6444855 (3.68E-04, FVC)
4	90,226,510	rs2869967	*FAM13A*	FEV_1/FVC	rs6849143	89,928,489	0.743	4.91E-01	6.75E-01	7.72E-01	
	90,134,259	rs6830970	*FAM13A*	FEV_1/FVC	rs6852928	89,926,193	0.468	5.87E-01	8.17E-01	6.36E-01	
4	107,046,508	rs10516526*	*FLJ20184-INTS12-GSTCD-NPNT*	FEV_1	rs11726124	106,766,496	1	6.52E-01	1.23E-02	7.62E-02	
	107,165,711	rs17331332*	*FLJ20184-INTS12-GSTCD-NPNT*	FEV_1	rs7693333	107,047,594	0.004	7.30E-01	3.85E-02	2.85E-01	
	107,154,433	rs17036341	*FLJ20184-INTS12-GSTCD-NPNT*	FEV_1	rs10021819	106,895,614	0.118	1.05E-01	9.86E-02	5.15E-01	
	106,976,744	rs11727189*	*FLJ20184-INTS12-GSTCD-NPNT*	FEV_1	rs11097903	106,866,077	0.004	5.79E-01	1.56E-01	1.53E-02	
	106,951,178	rs17036090	*FLJ20184-INTS12-GSTCD-NPNT*	FEV_1	rs10470990	106,821,578	0.159	7.16E-01	3.81E-01	7.08E-02	
	106,920,983	rs17036052	*FLJ20184-INTS12-GSTCD-NPNT*	FEV_1	rs17036076	106,575,269	0.702	2.43E-01	1.67E-02	1.96E-01	
	106,889,450	rs17035960*	*FLJ20184-INTS12-GSTCD-NPNT*	FEV_1	rs17036090	106,593,574	1	2.43E-01	1.60E-02	1.89E-01	
	107,087,537	rs11097901	*FLJ20184-INTS12-GSTCD-NPNT*	FEV_1	rs11728716	106,755,996	1	6.12E-01	6.00E-02	2.49E-01	
	107,113,600	rs11728716*	*FLJ20184-INTS12-GSTCD-NPNT*	FEV_1	rs11726124	106,766,496	1	6.52E-01	1.23E-02	7.62E-02	
4	145,793,929	rs12504628*	*HHIP*	FEV_1/FVC, FEV_1	rs13147758	145,460,230	0.965	2.46E-02	7.28E-01	5.27E-01	
	145,843,343	rs1980057*	*HHIP*	FEV_1/FVC	rs1980057	145,843,343	1	2.46E-02	7.28E-01	5.27E-01	
	145,792,189	rs1032295*	*HHIP*	FEV_1/FVC	rs13147758	145,460,230	0.743	2.46E-02	7.28E-01	5.27E-01	
5	95,062,456	rs153916	*SPATA9*	FEV_1/FVC	rs153916	95,062,456	1	2.50E-01	6.47E-01	3.19E-01	
5	147,822,546	rs11168048	*HTR4*	FEV_1/FVC	rs7735184	147,822,546	0.930	5.20E-01	2.55E-01	3.36E-01	rs6861078 (2.94E-03, FEV_1)
	147,824,585	rs7735184	*HTR4*	FEV_1/FVC	rs7735184	147,824,585	1	5.20E-01	2.55E-01	3.36E-01	
	147,826,008	rs3995090	*HTR4*	FEV_1	rs3995090	147,826,008	1	9.3E-01	1.38E-01	2.50E-01	
	147,826,900	rs6889822	*HTR4*	FEV_1	rs6889822	147,826,900	1	4.36E-01	3.3E-01	4.33E-01	
5	156,864,954	rs2277027	*ADAM19*	FEV_1/FVC	rs1422795	156,936,364	1	7.83E-01	4.06E-01	2.02E-01	rs9313633 (3.07E-03, FVC)
	156,868,942	rs1422795	*ADAM19*	FEV_1/FVC	rs1422795	156,868,942	1	7.83E-01	4.06E-01	2.02E-01	

(Continued)

TABLE E5. *(Continued)*

Chr	NCBI36 position	Reported SNP	Reported locus	Reported phenotype	Tested SNP	NCBI36 position	HapMap r^2	FEV_1/FVC *P* value	FEV_1 *P* value	FVC *P* value	Other SNPs at locus with *P* < .01
6	28,430,275	rs6903823	*ZKSCAN3/ZNF323*	FEV_1	rs6922111	28,325,308	0.945	7.46E-01	4.97E-01	2.98E-01	
6	31,676,448	rs2857595	*NCR3*	FEV_1/FVC	rs2857595	31,676,448	1	6.17E-01	9.31E-01	7.84E-01	
6	32,259,421	rs2070600	*PPT2-AGER-NOTCH4*	FEV_1/FVC	rs206015	32,182,759	0.649	8.48E-01	2.31E-01	1.87E-01	
	32,232,402	rs10947233*	*PPT2-AGER-NOTCH4*	FEV_1/FVC	rs10947233	32,232,402	1	2.42E-01	1.45E-01	8.64E-03	
6	109,374,743	rs2798641*	*ARMC2*	FEV_1/FVC	rs2798641	109,374,743		2.22E-03	8.37E-01	2.15E-01	rs6904998 (3.25E-03, FVC)
6	142,792,209	rs3817928	*GPR126*	FEV_1/FVC	rs6906468	142,769,386	1	1.59E-01	6.80E-01	8.07E-01	
	142,818,757	rs7776375	*GPR126*	FEV_1/FVC	rs595184	143,012,314	0.147	4.47E-01	7.77E-01	8.55E-01	
	142,748,826	rs6937121	*GPR126*	FEV_1/FVC	rs6937121	142,748,826	1	1.60E-01	6.47E-01	2.88E-01	
	142,733,242	rs11155242	*GPR126*	FEV_1/FVC	rs6906468	142,769,386	1	1.59E-01	6.80E-01	8.07E-01	
9	95,310,563	rs16909898	*PTCH1*	FEV_1/FVC	rs10512249	98,256,309	1	3.00E-01	4.54E-01	3.29E-01	
	95,335,864	rs10512249	*PTCH1*	FEV_1/FVC	rs10512249	95,335,864	1	3.00E-01	4.54E-01	3.29E-01	
10	12,317,998	rs7068966	*CDC123*	FEV_1/FVC, FEV_1	rs7068966	12,317,998	1	5.98E-01	8.57E-01	7.36E-01	
10	77,985,230	rs11001819*	*C10orf11*	FEV_1	rs2256413	78,315,334	0.755	4.67E-04	3.62E-01	5.40E-01	rs2637266 (2.00E-06, FEV_1/FVC)
12	55,813,550	rs11172113	*LRP1*	FEV_1/FVC	rs1466535	57,534,470	0.721	5.31E-01	5.70E-02	5.26E-02	
12	94,773,896	rs1036429	*CCDC38*	FEV_1/FVC	rs4762637	96,282,655	0.691	4.79E-01	9.81E-01	3.41E-01	
15	69,432,174	rs12899618*	*THSD4*	FEV_1/FVC	rs12102112	71,655,735	1	3.54E-04	8.39E-01	1.37E-01	rs12441227 (3.38E-09, FEV_1/FVC; 2.99E-04, FEV_1)
16	56,632,783	rs12447804†	*MMP15*	FEV_1/FVC	-†			-†	-†	-†	
16	73,947,817	rs2865531	*CFDP1*	FEV_1/FVC	rs12444589	75,454,404	1	1.96E-01	7.80E-02	3.13E-01	rs10871308 (5.07E-03, FEV_1)
21	34,574,109	rs9978142	*KCNE2*	FEV_1/FVC	rs10470171	35,652,644	0.857	5.26E-01	8.64E-01	9.41E-01	rs2834455 (8.53E-03, FEV_1/FVC)

If the reported SNP was not genotyped in the Hutterites, a surrogate SNP with the strongest LD to the reported SNP and the amount of LD in HapMap (r^2) are shown. One SNP, rs12447804, did not have any surrogate SNPs in the Hutterites. Other SNPs at the same locus with $P < .01$ in the Hutterites are shown in the last column.

Chr, Chromosome.

*SNPs replicated at $P < .05$.

†HapMap linkage disequilibrium data are not available for rs12447804; therefore, no surrogate marker has been selected for rs12447804.

TABLE E6. Eighty SNPs that best predicted FEV_1/FVC in the Hutterites, sorted by the chromosome location

SNP	Chr	NCBI36 position	P_{GWAS}	Beta (SE)	P_{GRAIL}	Implicated gene
rs6694986	1p13.3	107,631,201	4.0E-04	0.090 (0.049)	0.340	*NTNG1*
rs17509160	1p13.3	107,656,536	6.7E-05	0.074 (0.077)	0.340	*NTNG1*
rs1277216	1p13.3	109,160,754	4.7E-04	0.105 (0.048)	0.961	*STXBP3*
rs6689641	1p13.3	110,521,9233	7.1E-04	0.090 (0.051)	0.756	*SLC6A17*
rs6662186	1q23.3	163,531,357	1.2E-04	0.092 (0.077)	0.250	*LMX1A*
rs11887626	2p16.3	47,709,832	1.4E-04	0.137 (0.062)	0.737	*MSH2*
rs702891	2p14	65,611,954	4.6E-04	-0.015 (0.049)	0.242	*SPRED2*
rs6733029	2p14	68,287,661	3.7E-04	0.057 (0.046)	0.516	*PNO1*
rs2707549	2q14.3	124,120,027	2.3E-04	0.040 (0.047)	NA	
rs6720935	2q21.2	132,882,030	6.9E-04	0.067 (0.050)	0.758	*LOC339742*
rs16823807	2q23.3	153,146,906	9.2E-05	0.044 (0.088)	0.489	*FMNL2*
rs4342060	3q22.3	138,698,510	7.6E-04	0.050 (0.059)	0.363	*SOX14*
rs3851374	3q26.2	170,184,342	7.0E-04	−0.102 (0.048)	0.089	*EVI1*
rs11922608	3q27.3	188,719,180	5.4E-04	0.090 (0.069)	0.482	*SST*
rs10517456	4p14	37,631,127	9.0E-04	0.046 (0.051)	0.971	*PTTG2*
rs1984960	4p14	37,644,419	3.0E-04	0.175 (0.080)	0.971	*PTTG2*
rs12512633	4q28.1	124,506,365	5.2E-04	−0.115 (0.052)	0.149	*SPRY1*
rs6858195	4q31.21	144,909,047	3.1E-04	0.076 (0.046)	0.978	*GYPE*
rs10045757	5p14.2	23,306,226	1.0E-03	0.094 (0.059)	0.423	*LOC391771*
rs12659895	5p14.1	27,801,997	6.4E-04	−0.022 (0.057)	NA	
rs245610	5q34	162,032,345	8.9E-04	−0.134 (0.083)	NA	
rs9460984	6p22.2	24,355,227	5.6E-05	0.144 (0.059)	0.412	*DCDC2*
rs9368881	6p21.31	35,742,266	3.5E-04	−0.061 (0.047)	0.641	*C6orf81*
rs4839801	6q16.3	102,353,321	9.6E-04	0.004 (0.052)	0.777	*GRIK2*
rs1149309	6q21	105,856,616	4.9E-05	0.031 (0.067)	0.574	*PREP*
rs10872028	6q21	109,420,421	2.7E-04	−0.055 (0.060)	0.256	*ARMC2*
rs10872428	6q23.3	135,533,565	4.4E-04	0.207 (0.057)	0.324	*MYB*
rs9389370	6q23.3	136,472,958	5.4E-04	−0.072 (0.044)	0.620	*PDE7B*
rs4092400	8p23.2	2,371,110	9.9E-04	0.065 (0.048)	0.616	*MYOM2*
rs974120	8p23.2	2,634,025	3.2E-04	0.038 (0.079)	0.937	*CSMD1*
rs2921026	8p23.1	8,384,658	6.1E-04	−0.133 (0.046)	0.001	*DEFB107A*
rs10107668	8p12	33,643,721	8.0E-04	0.038 (0.048)	0.208	*DUSP26*
rs11779911	8p11.21	40,301,135	7.0E-04	−0.173 (0.049)	0.905	*ZMAT4*
rs7001967	8q21.13	83,905,913	8.1E-04	0.189 (0.079)	NA	
rs10955074	8q21.3	87,925,578	9.6E-04	−0.045 (0.045)	0.680	*CNGB3*
rs1283720	8q23.1	108,541,504	5.2E-04	−0.099 (0.048)	0.392	*ANGPT1*
rs9886419	8q24.21	129,353,533	7.1E-04	−0.119 (0.050)	NA	
rs10810557	9p22.3	16,326,250	3.4E-04	0.077 (0.044)	0.648	*BNC2*
rs3843935	9p13.3	33,777,871	9.0E-04	0.029 (0.048)	0.573	*PRSS3*
rs2306183	9q22.31	95,092,000	9.7E-04	−0.118 (0.045)	0.816	*PHF2*
rs10759765	9q22.33	99,347,314	4.2E-05	−0.065 (0.048)	0.261	*TMOD1*
rs1570846	10q11.21	43,776,486	7.1E-04	−0.139 (0.055)	0.037	*CXCL12*
rs2637261	10q22.3	77,990,599	7.5E-06	0.044 (0.044)	0.766	*KCNMA1*
rs1010006	10q24.2	99,562,286	4.5E-04	0.051 (0.046)	0.662	*ANKRD2*
rs7963902	12p13.32	4,988,938	1.9E-04	0.090 (0.043)	0.932	*KCNA6*
rs3925064	12p12.1	23,985,031	4.2E-04	−0.099 (0.048)	0.271	*SOX5*
rs7968811	12p12.1	24,428,104	2.8E-04	−0.021 (0.045)	0.271	*SOX5*
rs11050428	12p12.1	29,823,051	2.2E-04	−0.112 (0.065)	0.818	*TMTC1*
rs1829717	12q21.31	80,433,224	7.8E-05	0.009 (0.048)	0.899	*PPFIA2*
rs2056218	12q21.31	82,056,671	4.5E-04	0.147 (0.048)	0.903	*TMTC2*
rs879703	12q22	92,946,123	2.4E-04	0.049 (0.047)	0.467	*CRADD*
rs7134063	12q23.3	106,723,461	4.4E-05	0.083 (0.047)	0.167	*PWP1*
rs2329247	13q31.1	82,160,171	8.0E-04	0.055 (0.077)	NA	
rs9322855	14q11.2	20,223,139	7.6E-04	−0.048 (0.046)	0.171	*RNASE4*
rs8016448	14q24.3	72,961,640	5.9E-04	−0.071 (0.055)	0.316	*C14orf169*
rs2180080	14q32.13	93,560,454	7.2E-04	−0.132 (0.067)	0.213	*OTUB2*
rs2033785	15q22.33	65,228,920	2.9E-04	0.044 (0.057)	0.262	*SMAD3*
rs11636597	15q23	68,208,095	1.5E-05	0.117 (0.059)	0.642	*TLE3*
rs12907875	15q23	68,217,654	4.6E-04	−0.024 (0.054)	0.642	*TLE3*
rs6494886	15q23	68,720,785	3.9E-07	−0.018 (0.098)	0.557	*UACA*
rs1477439	15q23	68,821,634	5.3E-06	0.110 (0.071)	0.557	*UACA*
rs6494904	15q23	69,396,576	1.4E-08	−0.016 (0.069)	0.739	*THSD4*

(Continued)

TABLE E6. *(Continued)*

SNP	Chr	NCBI36 position	P_{GWAS}	Beta (SE)	P_{GRAIL}	Implicated gene
rs12592370	15q24.1	72,098,742	4.5E-04	−0.027 (0.065)	0.738	*PML*
rs1550434	15q24.1	72,118,264	2.0E-05	−0.067 (0.057)	0.738	*PML*
rs6495126	15q24.1	72,962,079	8.0E-04	−0.060 (0.056)	0.976	*RPP25*
rs7171364	15q25.3	83,803,031	3.4E-04	0.180 (0.064)	0.367	*PDE8A*
rs12919417	16q22.3	70,137,212	3.2E-04	0.043 (0.068)	0.345	*KIAA0174*
rs707236	16q23.3	82,360,661	6.3E-04	0.143 (0.045)	0.910	*CDH13*
rs4783102	16q24.1	83,535,549	1.4E-04	−0.106 (0.054)	0.687	*USP10*
rs7216399	17p11.2	16,797,303	6.0E-04	−0.065 (0.054)	0.043	*TNFRSF13B*
rs8080953	17p11.2	19,381,812	1.0E-03	−0.233 (0.100)	0.748	*ZNF179*
rs225207	17q11.2	27,918,837	4.9E-05	−0.096 (0.065)	0.980	*MYO1D*
rs8064367	17q12	29,496,973	9.1E-04	−0.018 (0.062)	0.521	*ACCN1*
rs854679	17q12	31,382,952	3.4E-07	−0.049 (0.059)	0.020	*CCL18*
rs2074158	17q21.2	37,510,689	8.8E-05	−0.132 (0.083)	0.486	*HSPB9*
rs16950093	17q21.33	46,995,183	5.6E-04	−0.085 (0.054)	0.205	*UTP18*
rs2017854	17q24.2	62,918,483	2.6E-06	−0.097 (0.048)	0.828	*PITPNC1*
rs365548	20p13	185,618	5.6E-04	0.003 (0.046)	0.001	*DEFB129, C20orf96*
rs4815436	20p11.21	25,521,423	9.4E-04	0.181 (0.069)	0.001	*DEFB115, DEFB116, DEFB123, DEFB124*
rs2835345	21q22.13	36,723,304	8.3E-06	−0.108 (0.055)	0.900	*CHAF1B*

P_{GWAS} is the *P* value from the FEV_1/FVC GWAS in the Hutterites; Beta (SE) is that of the predictive SNP in the regression model for the 865 Hutterites; and P_{GRAIL} is the region's *P* value given by GRAIL.
Chr, Chromosome; *NA*, not available.