



## NIH PUBLIC ACCESS

## Author Manuscript

*Nat Genet.* Author manuscript; available in PMC 2015 January 01.

Published in final edited form as:

*Nat Genet.* 2014 July ; 46(7): 669–677. doi:10.1038/ng.3011.

## Genome-wide association analysis identifies six new loci associated with forced vital capacity

Correspondence regarding this manuscript should be directed to: Stephanie J. London, london2@niehs.nih.gov, Martin D. Tobin, mt47@le.ac.uk.

\*These authors contributed equally to this work

\*\*These authors jointly directed this work

**URLs**Korean HapMap, <http://www.khapmap.org/>; RegulomeDB <http://regulome.stanford.edu/>; Human protein atlas <http://www.proteinatlas.org/>; METAL <http://www.sph.umich.edu/csg/abecasis/metal/>; matSpD <http://gump.qimr.edu.au/general/daleN/matSpD/>; Gene Expression Omnibus <http://www.ncbi.nlm.nih.gov/geo/>.**Accession numbers**

NCBI Gene Expression Omnibus: GSE14334.

**Contributions****Project conception, design and management.** Stage 1 GWAs. Ages: G.E., M.G., V.G., T.B.H., L.J.L. ARIC: S.J.L., N.F., D.C., D.B.H., B.R.J., A.C.M., K.E.N. B58C T1DGC: D.P.S. B58C WTCCC: D.P.S. CARDIA: A.S., L.J.S. CHS: S.A.G., S.R.H., B.M.P. CROATIA-Korcula: O.P., A.F.W. CROATIA-Vis: I.R. ECRHS: D.J., E.O., I.P., M.W. EPIC: R.A.S., N.J.W., J.H.Z. FHS: J.B.W., G.T.O., J.D. FTC: J.K., K.H.P., T.R., A.V. Health ABC: P.A.C., T.B.H., S.B.K., Y.L. Health 2000: M.H., M.K. HCS: J.R.A., R.J.S. KORA S3: C.G., J.H. MESA-Lung: R.G.B. NFBC1966: M-R.J., A.P. NSPHS: U.G. ORCADES: H.C., J.F.W., S.H.W. RS I, II & III: A.H., B.H.S. G.G.B. SHIP: S.G., B.K., H.V. Twins UK-I: C.J.H., T.D.S. Stage 2 follow-up studies. BHS 1&2: A.L.J., A.B.M., J.B. CROATIA-Split: N.D.H., C.H. Generation Scotland: D.J.P., B.H. Smith. KORA F4: H.S. LBC1936: I.J.D., J.M.S. LifeLines: H.M.B., D.S.P., J.M.V., C. W. LLFS: A.N., B.T., M. Wojczynski, R.L.M. PIVUS: E.I., L.L., L.L., Twins UK-II&III: C.J.H., T.D.S. Multi-ethnic follow-up studies. CARE: R.G.B., K.M.B., D.J.L., R.K., L.J.S., J.B.W., N.H., M.F.P., K.M.B., S.R., E.G.B., G.T.O., L.R.L., W.B.W. KARE3 and Healthy Twin Study: J.S., W.K., Y.O. Gene expression analyses. Rt-PCR: K.R.B., G.G.B. eQTLs: J.B.J.M., A.G.U. Fetal lung expression: I.P.H., I.S., E.M.**Phenotype collection and data management.** Stage 1 GWAs. Ages: T.A. ARIC: D.C., N.F., A.C.M., K.E.N. B58C T1DGC: A.R.R., D.P.S. B58C WTCCC: A.R.R., D.P.S. CARDIA: L.J.S., O.D.W. CHS: S.A.G., S.R.H., B.M.P., T.L. CROATIA-Korcula: O.P., L.Z. CROATIA-Vis: S.C., I.K. ECRHS: D.L.J., E.O., I.P., M.W. EPIC: J.H.Z. FHS: J.B.W., G.T.O., J.D. FTC: J.K., K.H.P., T.R., A.V. Health ABC: P.A.C., W.T. Health 2000: M.H., M.K. HCS: J.R.A. KORA S3: J.H. MESA-Lung: R.G.B. NFBC1966: M-R.J., J.P., A.P. NSPHS: A.J. S.E. U.G. ORCADES: S.H.W., J.F.W. RS I, II & III: G.G.B., L. Lahousse, D.W.L., B.H.S. SHIP: S.G., B.K., H.V. Twins UK-I: P.G.H., A. Vi uela. Stage 2 follow-up studies. BHS 1&2: A.L.J., A.B.M., J.B. CROATIA-Split: C.H., T.Z. Generation Scotland: D.J.P., B.H. Smith. KORA F4: R.H., S.K., H.S. LBC1936: I.J.D., J.M.S. LifeLines: D.S.P., J.M.V. LLFS: A.N., B.T., R.M. PIVUS: E.I., L.L. Twins UK-II&III: P.G.H., A. Vi uela Multi-ethnic follow-up studies. CARE: R.G.B., T.P., K.M.B., D.J.L., R.K., L.J.S., J.B.W., N.H., M.F.P., K.M.B., S. Ripatti, E.G.B., G.T.O., L.R.L., W.B.W. KARE3 and Healthy Twin Study: J.S., W.K., Y.O. Gene expression analyses. Rt-PCR: K.R.B., G.G.B., F.M.V., P.S.H. eQTLs: J.B.J.M., M.J.P. Fetal lung expression: I.P.H., I. Sayers, E.M.**Genotyping** Stage 1 GWAs. B58C T1DGC: W.L.M. B58C WTCCC: W.L.M. CARDIA: M.F., X.G. CHS: J.I.R., B.M.P. CROATIA-Korcula: J.E.H. CROATIA-Vis: S.C. ECRHS: M.W. EPIC: J.H.Z. FTC: J.K. Health ABC: Y.L., K.L. Health 2000: S. Ripatti, I. Surakka. HCS: R.J.S. KORA S3: H.G. MESA-Lung: S.S.R. NFBC1966: M-R.J. NSPHS: A.J. S.E. U.G. Orcades: H.C., J.F.W. RS I, II & III: F.R., A.G.U. SHIP: S.G., B.K., A.T., H.V. Twins UK-I: C.J.H., T.D.S. Stage 2 follow-up studies. BHS 1&2: J. Hui, J.B. CROATIA-Split: C.H., P.N., T.Z. Generation Scotland: D.J.P., B.H. Smith, H.T. LBC1936: G.D. LifeLines: C.W. LLFS: A.N., B.T. PIVUS: E.I., A.P.M. Twins UK-II&III: C.J.H., T.D.S.**Data analysis.** Stage 1 GWAs. Ages: A.V.S. ARIC: N.F., D.B.H. B58C T1DGC: A.R.R., D.P.S. B58C WTCCC: A.R.R., D.P.S. CARDIA: X.G. CHS: S.A.G., G.L., S.R.H., T.L. CROATIA-Korcula: J.E.H. CROATIA-Vis: V.V. ECRHS: D.L.J., A.R. EPIC: J.H.Z. FHS: J.B.W., J.D., W.G. Health ABC: P.A.C., Y.L., K.L., W.T. Health 2000: M.K., S. Ripatti, I. Surakka. HCS: C.O., E.G.H. KORA S3: E.A. MESA-Lung: A.M., S.S.R. NFBC1966: A.C.A. NSPHS: S.E. ORCADES: P.K.J. RS I, II & III: L. Lahousse, D.W.L. SHIP: A.T. Twins UK-I: P.G.H. Stage 2 follow-up studies. KORA F4: C.F., R.H. LBC1936: L.M.L. LifeLines: K.J., H.M.B. LLFS: M. Wojczynski, B.T. PIVUS: T.F. Twins UK-II&III: P.G.H. Multi-ethnic follow-up studies. N.C.G. CARE: T.P., Q.D., L.A.L., X.Q.W. KARE3 and Healthy Twin Study: M.K.L. Gene expression analyses. Rt-PCR: K.R.B., F.M.V. eQTLs: M.J.P. Fetal lung expression: I.P.H., I. Sayers, E.M.**Analysis group:** CHARGE consortium: D.W.L., S.A.G., S.J.L., J.D., N.F., A.V.S. CARE: T.P., Q.D. SpiroMeta consortium: M.S.A., L.V.W., B.K., I.P.H., M.D.T.**Writing group:** CHARGE consortium: S.J.L., D.W.L., S.A.G., N.F., J.D., G.G.B., A.S. SpiroMeta consortium: I.P.H., M.S.A., M.D.T., L.V.W., C.H.**Conflict of interest**

JBW is employed by Pfizer, Inc. None of the other authors have declared a possible conflict of interest.

A full list of authors and affiliations appears at the end of the article.

## Abstract

Forced vital capacity (FVC), a spirometric measure of pulmonary function, reflects lung volume and is used to diagnose and monitor lung diseases. We performed genome-wide association study meta-analysis of FVC in 52,253 individuals from 26 studies and followed up the top associations in 32,917 additional individuals of European ancestry. We found six new regions associated at genome-wide significance ( $P < 5 \times 10^{-8}$ ) with FVC in or near *EFEMP1*, *BMP6*, *MIR-129-2/HSD17B12*, *PRDM11*, *WVX*, and *KCNJ2*. Two (*GSTCD* and *PTCH1*) loci previously associated with spirometric measures were related to FVC. Newly implicated regions were followed-up in samples of African American, Korean, Chinese, and Hispanic individuals. We detected transcripts for all six newly implicated genes in human lung tissue. The new loci may inform mechanisms involved in lung development and pathogenesis of restrictive lung disease.

## Introduction

Pulmonary function is a heritable trait that can be reliably measured by spirometry and reflects the physiological state of the lungs and airways<sup>1</sup>. Forced vital capacity (FVC), one of the most widely used pulmonary function measures, approximates vital capacity. In conjunction with forced expiratory volume in 1 second (FEV<sub>1</sub>), FVC is used to diagnose various respiratory diseases. A reduced ratio of FEV<sub>1</sub> to FVC (FEV<sub>1</sub>/FVC) indicates airflow obstruction when FEV<sub>1</sub> is reduced disproportionately relative to FVC. In contrast, a decreased FVC in the face of a normal to elevated FEV<sub>1</sub>/FVC suggests a restrictive ventilatory defect. In clinical practice, FVC is often used as a surrogate measure of disease progression in patients with established restrictive lung disorders, such as idiopathic pulmonary fibrosis<sup>2,3</sup>. Reduced FVC is a strong predictor of mortality in the general population, independently of FEV<sub>1</sub> and standard risk factors such as age and cigarette smoking<sup>4-8</sup>.

Pulmonary function measures show familial aggregation, with evidence for genetic effects in twin and family studies<sup>9,10</sup>. We previously reported associations between FEV<sub>1</sub> or FEV<sub>1</sub>/FVC and at least 27 genetic loci using large-scale meta-analyses of genome-wide association studies (GWAS)<sup>11-14</sup>. To date, the genetic determinants of FVC have not been studied using GWAS methods. We conducted a comprehensive GWAS meta-analysis across two large consortia of European ancestry—the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) and SpiroMeta—to identify common genetic variants associated with cross-sectional measures of FVC in 52,253 subjects of European descent. In each of six new loci, we confirmed expression levels of genes nearest the novel variants in lung tissue and performed expression quantitative trait locus (eQTL)-analyses in 762 whole blood samples. We evaluated the new loci in 6,070 African Americans in the National Heart, Lung and Blood Institute (NHLBI)-sponsored Candidate gene Association Resource (CARE) Project, in a Chinese subset (n=563) of the Multi-Ethnic Study of Atherosclerosis (MESA), in a Hispanic subset (n = 849) of MESA, and in Koreans (n = 8,074) from two cohort studies; Healthy Twin<sup>15-17</sup> and Korea Association Resource 3 (KARE3)<sup>18</sup>.

## Results

The study consisted of two stages. Stage 1 was a meta-analysis of study-specific genome-wide analyses of FVC conducted in 26 studies with a total of 52,253 individuals of European ancestry. The study characteristics are shown in Supplementary Table 1. Individual cohorts performed a GWAS analysis using linear regression models with FVC (in milliliters) as the outcome, stratified by never/ever smoking. Adjustment factors included age, age<sup>2</sup>, sex, height, height<sup>2</sup> and weight. If applicable, cohorts adjusted for center, cohort or principal components to adjust for population stratification. Stage 1 results are in Supplementary Data Set 1. In stage 2, we followed-up SNPs showing association with FVC ( $P < 5 \times 10^{-7}$ ) and meta-analyzed beta estimates and standard errors across stages 1 and 2 (Figure 1). Stage 2 encompassed 32,917 subjects of European ancestry from 9 independent cohorts. The study characteristics are shown in Supplementary Table 2. The follow-up studies used the same models. Effect estimates for each study were corrected using genomic control<sup>19</sup> separately within smoking strata. Study-specific lambda estimates are shown in Supplementary Table 1. The test statistic inflation ( $\lambda_{GC}$ ) before applying genomic control at the meta-analysis level was 1.12 (Supplementary Figure 1). The test statistic inflation standardized for a sample size of 1,000 individuals was 1.002.

Regions around SNPs reaching genome-wide significance after the meta-analysis of the two stages were followed up in diverse ancestry samples of African Americans, Koreans, Chinese and Hispanics. The characteristics for these multi-ethnic follow-up studies are shown in Supplementary Table 2. Following the meta-analysis, we investigated mRNA expression of the nearest gene for each of the new SNPs in human lung tissue, human airway smooth muscle cells (HASM), human bronchial epithelial cells (HEBC) and peripheral mononuclear blood cells (PMBC) (Supplementary Note). We assessed whether these SNPs were associated with gene expression in whole blood cells (Supplementary Note, Supplementary Table 3) and queried databases to assess whether these SNPs are located within known or predicted regulatory regions.

### Stage 1 and 2 results

There were 9 regions containing at least one SNP associated with FVC at  $P < 5 \times 10^{-7}$  in stage 1. Of these, two (*GSTCD* and *PTCHI*) had previously been reported in GWAS<sup>12,13</sup> of spirometric traits (FEV<sub>1</sub> and FEV<sub>1</sub>/FVC), thus were not evaluated further, leaving 7 SNPs in 7 loci for follow-up in stage 2.

Six loci reached genome-wide significance ( $P < 5 \times 10^{-8}$ ) for FVC in the meta-analysis of stages 1 and 2 (Table 1, Figure 2). The loci were in or near the following genes: *BMP6* (rs6923462, 6p24, intronic), *EFEMP1* (rs1430193, 2p16.1, intronic), *MIR-129-2/HSD17B12* (rs4237643, 11p11.2, 54 kb upstream), *PRDM11* (rs2863171, 11p11.2, 3 kb downstream), *WWOX* (rs1079572, 16q23.1, intronic) and *KCNJ2* (rs6501431, 17q24.3, 800 kb downstream) (Supplementary Figures 2a–g). Effect sizes were generally consistent across studies (Supplementary Figures 3 a–g and 4 a–g). Three of these regions (*BMP6*, *EFEMP1* and *PRDM11*) also showed independent replication in stage 2 European ancestry samples, with  $P$  values below a Bonferroni corrected threshold for 7 tests ( $P < 7.14 \times 10^{-3}$ ) (Table 1).

The lowest  $P$  value ( $5.89 \times 10^{-13}$ ) for the meta-analyzed effect estimate across stage 1 and 2 was found for SNP rs6923462 (intronic SNP in *BMP6*).

We evaluated the effect of the six new loci separately in ever-smokers and in never-smokers, and the effect sizes were consistent across smoking strata for all the variants (Supplementary Table 4).

### Multi-ethnic follow-up

To examine the portability of the identified loci to other ethnic groups, we evaluated the regions of our newly identified SNPs in African Americans, Hispanics, Chinese, and Koreans. For three of these samples (African Americans, Hispanics, and Chinese), we looked up the SNPs with minor allele frequency (MAF)  $\geq 0.05$  within 200 kb in either direction from the sentinel SNP in Europeans in the 1000 Genomes (1000G) Project all ancestries imputation panel<sup>20</sup>. For Koreans, we looked up the sentinel SNP  $\pm 200$  kb for SNPs with MAF  $\geq 0.05$  according to imputation to HapMap and the Korean panel. To determine the appropriate Bonferroni-corrected  $P$  value threshold for declaring statistical significance in each ethnic group, we used the Nyholt method to calculate the effective number of independent variants, based on pairwise linkage disequilibrium among the follow-up SNPs<sup>21,22</sup>.

African Americans were participants of the Candidate gene Association Resource consortium (CARE)<sup>23</sup>. Baseline characteristics of the 6,070 African Americans in CARE are shown by cohort in Supplementary Table 2b. We performed regional meta-analyses of the 7,470 SNPs (MAF  $\geq 0.05$ ) within  $\pm 200$  kb of the sentinel SNPs in Europeans using 1000G imputed data. Using the  $P$  value threshold of  $4.42 \times 10^{-5}$  (based on 1,132 independent tests), 78 SNPs in the region of *EFEMP1* were significantly associated with FVC (lowest  $P = 1.63 \times 10^{-7}$ ) in African Americans. Our top hit at the *EFEMP1* locus in European samples (SNP rs1430193, T allele frequency 0.37, Table 1) had a very different allele frequency in our replication African American samples (T allele frequency 0.71) and was not statistically significant ( $P = 0.13$ ). As has been recently noted regarding extension of GWAS SNPs discovered in Europeans to African-American populations<sup>24</sup>, the effect size was in the same direction but attenuated in our African-Americans ( $\beta = -15.79$  ml versus  $-23.75$  ml). The top hit in African Americans for the *EFEMP1* region (rs62164511,  $P = 1.63 \times 10^{-7}$ ) showed a decrease in FVC of 84.7 ml per each copy of the A-allele (A-allele frequency: 0.9) (Supplementary Table 5a). The  $r^2$  between the most significant SNP (rs62164511) in African Americans and the most significant SNP (rs1430193) in Europeans was low (0.16), further supporting evidence for allelic heterogeneity at this locus (Supplementary Figures 5a–b).

Baseline characteristics of the 563 Chinese subjects from MESA are shown in Supplementary Table 2c. We performed regional analyses ( $\pm 200$  kb, SNPs with MAF  $\geq 0.05$ ) around the sentinel SNPs in Europeans using 1000 Genomes imputation. The  $P$  value threshold was determined at  $4.41 \times 10^{-5}$  (based on 1,133 independent tests). None of the 7,436 investigated SNPs reached a statistical significance level below the predefined threshold.

Baseline characteristics of the 849 Hispanics from MESA are shown in Supplementary Table 2d. We performed regional analyses of SNPs (MAF  $\geq 0.05$ ) within  $\pm 200$  kb of the sentinel SNPs in Europeans using 1000 Genomes imputation. None of the 7,473 investigated SNPs reached a statistical significance level below the predefined threshold ( $4.41 \times 10^{-5}$ , based on 1,133 independent tests).

Baseline characteristics of the 8,074 Koreans from the Healthy Twin<sup>15–17</sup> and KARE3<sup>18</sup> studies are shown in Supplementary Table 2e. In this sample, only HapMap imputed (HapMap3 Phase 2 and Korean HapMap) data were available. There were 72 SNPs (MAF  $\geq 0.05$ ) within  $\pm 200$  Kb of the sentinel SNPs in Europeans. Using the threshold  $P$  value of  $1.52 \times 10^{-3}$  (based on 26 independent tests), two SNPs (rs12449659 and rs4793331, both located approximately 700kb upstream of *KCNJ2*) were associated with FVC in Koreans with a decrease of 32.5 ml (rs12449659, per T allele,  $P = 7.92 \times 10^{-4}$ ) and 22.5 ml (rs4793331, per A allele,  $P = 1.22 \times 10^{-3}$ ). These SNPs did not show a significant association with FVC in Europeans ( $P = 0.17$  and  $P = 0.34$ , Supplementary Table 5b).

### Gene set enrichment analysis

To identify plausible pathways associated with FVC, we broadened our focus beyond genome-wide significant variants by performing gene set enrichment analysis<sup>25</sup> on the entire meta-analyzed GWAS. We queried approximately 2,000 gene sets including canonical pathways and Gene Ontology functional categories. Using a false discovery rate (FDR)  $< 0.01$ , we identified 65 enriched pathways (Supplementary Table 6). While these over-represented gene sets encompassed diverse functions, many involved processes critical to organ development and tissue remodeling including epithelial morphogenesis, cell proliferation, extracellular matrix, Notch signaling, and cell adhesion. Other prominent pathways included acetylcholine binding and channel activity, smooth muscle contraction, glutamate receptor activity, immunity, and transcriptional/DNA repair processes.

### Gene expression

Expression profiles of genes from the six loci that were significant in the meta-analysis of stages 1 and 2 (*EFEMP1*, *BMP6*, *WVWX*, *KCNJ2*, *PRDM11* and *HSD17B12*), and the housekeeping gene *GAPDH*, were measured in human lung tissue and primary cell samples using RT-PCR. We detected transcripts for all six newly implicated genes in lung tissue, human bronchial epithelial cells (HBEC) and human airway smooth muscle (HASM). Transcripts for five of the 6 genes (excluding *EFEMP1*) were present in peripheral blood mononuclear cells (PBMCs) (Table 2 and Supplementary Figures 6 and 7).

### Expression quantitative trait locus analysis in peripheral blood cells

We investigated whether the top SNPs or their proxies ( $r^2 \geq 0.7$ ) in the six newly implicated FVC loci were associated with gene expression using expression Quantitative Trait Loci (eQTL) data as described in the methods. Multiple SNPs in or near *HSD17B12* showed significant cis-eQTL associations ( $P < 10^{-4}$ ) in peripheral blood, with the strongest association represented by rs11037676 (a proxy of rs4237643,  $r^2 = 0.7$ ) at a  $P$  value of  $8.42 \times 10^{-81}$  (Supplementary Table 3). The sentinel SNP associated with FVC in this region (rs4237643) also exhibited a strong cis-effect on *HSD17B12* ( $P = 1.82 \times 10^{-35}$ )



(Supplementary Figure 8). Furthermore, this SNP showed a significant cis-eQTL association in lymphoblastoid cell lines ( $P = 6.7 \times 10^{-11}$ )<sup>26</sup> and brain tissue ( $P = 1.2 \times 10^{-8}$ )<sup>27</sup>. Another FVC-associated SNP located in the intronic region of *EFEMP1* (rs1430189) demonstrated local effects on this gene's expression based on eQTL data from human fibroblasts ( $P = 4.8 \times 10^{-6}$ )<sup>28</sup>. We did not find statistically significant cis-eQTLs for the other FVC-associated variants and loci.

### eQTL analysis in lung tissue

To better assess the relevance of cis-eQTLs to lung biology, we queried a publically available database that included lung tissue (The Genotype-Tissue Expression project, GTEx)<sup>29</sup> to further investigate the top SNPs and their proxies. Multiple SNPs mapped to *HSD17B12*, including rs11037676 and the sentinel SNP, rs4237643, demonstrated highly significant cis-eQTLs in human lung samples ( $P = 2.8 \times 10^{-26}$  and  $P = 7.2 \times 10^{-14}$ , respectively).

### Fetal lung mRNA expression for genes associated with FVC

Studies were performed to investigate whether or not the genes we identified were differentially expressed during normal human fetal lung development (Supplementary Table 7). There was strong evidence ( $P$  controlling for false discovery rate =  $6.7 \times 10^{-6}$ ) for differential expression of *PRDM11*, suggesting this gene may play an important role *in utero* in lung development. One probe for *WWOX* also showed correlation between lung expression and fetal age although this was not seen with other probes for the gene.

### Putative regulatory variants

We queried the RegulomeDB<sup>30</sup> database to assess whether any of the newly identified FVC-associated SNPs ( $P < 10^{-7}$ ,  $n = 150$  SNPs) were located within known or predicted regulatory elements, including regions of DNAase hypersensitivity, binding sites of transcription factors, and promoter regions that have been biochemically characterized to regulate transcription. Five SNPs received high likelihood scores (based on the amount of supporting data) for mapping to regulatory regions and affecting gene expression; these were 4 variants upstream of *HSD17B12* (rs9783304, rs2862996, rs10768966, and rs6485443) and one variant downstream of *EFEMP1* (rs1430189). These variants showed evidence for eQTL, transcription factor binding and/or DNase peak.

### Discussion

In a two-stage meta-analysis across 35 cohorts encompassing 85,170 individuals of European ancestry, we found 6 new loci associated with FVC that had not been identified in previous GWAS of spirometric measures of airflow obstruction ( $FEV_1$  or  $FEV_1/FVC$ ). The six new loci showed consistent associations across the European ancestry studies in discovery and replication stages. The meta-analysis effect estimates range from 13 to 31 ml per allele, which is similar to the annual rate of decline of FVC ranging from 12 to 47 ml in the general population<sup>31</sup>. Expression analyses showed that all the top candidate genes at these loci were expressed in lung tissue and primary lung cells (HBEC and HASM). Two additional loci associated with FVC at genome-wide significance in the stage 1 analysis

were previously associated with FEV<sub>1</sub>/FVC (*PTCHI*) or FEV<sub>1</sub> (*GSTCD*)<sup>12–14</sup> at genome-wide levels of significance in GWAS.

The six new associations found in this analysis explain only a modest proportion of the additive polygenic variance of FVC (0.74%). Stage 2 effect size estimates were used to calculate the proportion of the variance explained by the six new loci, to avoid the effect of winner's curse bias. When we take the other known loci for pulmonary function into account, the proportion of the additive polygenic variance explained is 1.78%, a finding that is comparable to many other complex traits<sup>32</sup>. Unexplained heritability has become a well-known phenomenon in genetic epidemiology<sup>33</sup> and possible explanations include multiple effects of common variants, rare variants, gene-by-environment interactions, gene-gene interactions and epigenetic regulation - mechanisms that are not captured by existing GWAS platforms.

We, and others, previously identified 27 regions associated at genome wide significance with FEV<sub>1</sub>, FEV<sub>1</sub>/FVC or both<sup>11–14</sup>. Although FEV<sub>1</sub> and FVC are statistically correlated ( $r = 0.83$  in the Rotterdam Study, adjusted for age, sex, height and height<sup>2</sup>), these are clinically different entities. FVC is used for the evaluation of restrictive ventilatory defects and is a predictor of mortality independent of FEV<sub>1</sub>, standard risk factors, and even prior cardiovascular disease<sup>5,6</sup>. In contrast, FVC and FEV<sub>1</sub>/FVC have a very low correlation ( $r = -0.08$  in the Rotterdam Study, adjusted for age, sex, height and height<sup>2</sup>). In this analysis, we were able to identify 6 new loci that are associated with FVC at genome-wide significance. Only two of the loci that were previously associated with FEV<sub>1</sub> or FEV<sub>1</sub>/FVC showed genome-wide significant association with FVC in our study (*GSTCD* and *PTCHI*)<sup>12,13</sup>. The sentinel SNPs at each of the six novel loci showed consistent directions of effect on both FEV<sub>1</sub> and FVC (Supplementary Table 8). Among our FVC-associated SNPs, the smallest *P* value for FEV<sub>1</sub> ( $9.43 \times 10^{-7}$ ) was observed for rs1079572 (*WVOX*). An intragenic SNP (rs11654749) in the region between *KCNJ2* and *SOX9* was associated with FEV<sub>1</sub> at genome-wide significance in our previous meta-analysis of SNP and SNP-by-smoking effects<sup>34</sup>. To assess whether the variant (rs11654749) identified in that analysis of FEV<sub>1</sub> and the sentinel SNP (rs6501431) from our current FVC analysis are pointing to the same signal, we fitted both variants together in the model using the software GCTA<sup>35</sup>. Their effects sizes increased slightly when fitted together as expected given that the marginal correlation ( $r=0.03$ ) of their alleles was positive and the effects of these alleles on lung function were in opposite directions. Thus, these SNPs appear to be independent signals.

Two of the top loci for FVC (*BMP6* and *EFEMP1*) have been associated with height in a previous GWAS<sup>36</sup>. The FVC-associated SNPs in or near these two genes show a modest to weak correlation with the top SNPs from the height GWAS<sup>36</sup>. For *EFEMP1*, the  $r^2$  between rs1430193 (for FVC) and rs3791675 (for height) was 0.45. For *BMP6*, the correlation between rs6923462 (for FVC) and two SNPs associated with height (rs3812163 and rs1219896) was low. For rs6923462 and rs3812163, the  $r^2$  was 0.01 and for rs6923462 and rs1219896 the  $r^2$  was 0.02. Since we adjusted for height in our analysis, our findings are likely to be independent of height, but may reflect genetic effects on body or organ size.

To assess whether our identified loci are associated with FVC across populations of different ancestries, we assessed the associations of our main findings in African Americans, Koreans, Hispanics, and Chinese subjects. Despite the limited size of these samples, 78 SNPs in the region of *EFEMP1* reached the significance threshold of  $P < 4.42 \times 10^{-5}$  in African Americans. These results support the involvement of this locus in lung function in individuals of both European and African descent, although there was evidence for allelic heterogeneity. In the Korean dataset, we found two SNPs in the region of our locus near *KCNJ2* (*LOC101928165*) to be significantly associated with FVC. In the smaller samples of Chinese and Hispanic participants, none of the investigated SNPs were significantly associated with FVC which may not be surprising given the greatly reduced power. In summary, despite the smaller sample size, we were able to show significant evidence of association with FVC for the *EFEMP1* locus in African Americans and for the locus downstream of *KCNJ2* in Koreans.

A literature review identified candidate genes within the newly identified associated loci plausibly involved in lung growth and pathogenesis. For example, *BMP6* is a member of the bone morphogenetic proteins (BMPs) that represent a key canonical signaling pathway in the regulation of lung development, repair and response to injury<sup>37</sup>. *BMP6* expression in bronchial epithelial cells has been reported to increase during experimental models of allergic airway inflammation<sup>38</sup>. *EFEMP1* is part of the fibulin family of extracellular matrix glycoproteins and encodes fibulin-3. Targeted disruption of a member of this -family, fibulin-4, has been shown to cause reduced elasticity and emphysematous morphology in the lungs of mice<sup>39</sup>. Expression of other members of the fibulin family (fibulin-1 and fibulin-5) seems to be influenced by transforming growth factor beta 1 (TGF- $\beta$ 1), a gene previously linked to inflammation in COPD patients<sup>40,41</sup>. *WWOX* may influence protein-induced apoptosis and behaves as a tumor suppressing gene in various types of neoplasms, including small cell lung cancer<sup>42</sup>. Our analysis in fetal lung data showed strong evidence for differential expression of *PRDM11* suggesting a role in *in utero* development. In addition, nuclear expression of *PRDM11* has been shown in respiratory epithelial cells of the human bronchus in 3 subjects Human Protein Atlas<sup>43</sup>. Interestingly, our eQTL-analysis revealed highly significant cis-effects of FVC-associated variants on *HSD17B12* expression in multiple tissues including lung. Furthermore, several of these SNPs were located within regulatory sites upstream of *HSD17B12*, suggesting that these variants may play a regulatory role in gene function. Exploratory pathway analysis using the all SNPs in the meta-analysis of FVC implicated multiple processes, including several involved in tissue development and remodeling. These findings suggest that distinct and identifiable biological pathways underlie the genetic basis of lung's vital capacity in the general population.

There are some limitations of our analysis. With cross-sectional measures of FVC, we cannot determine whether the identified signals are due to influence on lung growth or age-related lung function decline<sup>44</sup>. The primary analyses were not adjusted for pack-years to avoid attrition in sample size, but within the CHARGE cohorts, estimates from meta-analysis with and without adjustment for pack years were very similar (results not shown).

An important strength of our study is its considerable sample size of European ancestry individuals. Our application of genomic control at the three stages is likely to be overly



conservative because it has recently been shown that in large meta-analyses, test statistics are expected to be elevated under polygenic inheritance even when there is no population structure. Genomic inflation estimates increase with sample size, as has been shown for other traits<sup>14,36,45,46</sup>. Following the two staged meta-analysis, we were able to test the SNPs and their regions for tissue specific expression.

In conclusion, using a large-scale staged meta-analysis, we report six new loci associated with FVC and show that all are expressed in lung tissue and primary lung cells. Our findings point to previously unexplored pathways and mechanisms underlying lung function. Improvement of the understanding of the role that these genes play in normal lung development and disease pathogenesis could lead to novel therapeutic targets for lung diseases.

## Online Methods

### Study design

The study consisted of two stages. Stage 1 was a meta-analysis of study-specific genome-wide analyses of FVC conducted in 26 studies with a total of 52,253 individuals of European ancestry. The study characteristics are shown in Supplementary table 1. In stage 2, we followed-up SNPs showing association with FVC ( $P < 5 \times 10^{-7}$ ) and meta-analyzed betas and standard errors across stages 1 and 2. Stage 2 encompassed 32,917 subjects of European ancestry from 9 independent cohorts.

### Cohorts included in stage 1

Stage 1 included a total of 26 studies, 15 from the SpiroMeta consortium and 11 from the CHARGE consortium: AGES, ARIC, B58C T1DGC, B58C WTCCC, CARDIA, CHS, ECRHS, EPIC (obese cases and population-based studies), the EUROSPAN studies (CROATIA-Korcula, ORCADES, CROATIA-Vis and NSPHS), FHS, FTC (incorporating the FinnTwin16 and Finnish Twin Study on Aging), Health 2000, Health ABC, HCS, KORA S3, MESA, NFBC 1966, RS-I, RS-II, RS-III, SHIP and Twins UK-I. The genotyping platforms and quality-control criteria implemented by each study are described in Supplementary Table 9.

### Cohorts included in stage 2

A total of 9 studies were included in our stage 2 follow-up: BHS 1 & 2, CROATIA-Split, Generation Scotland, KORA F4, LBC1936, LifeLines, LLFS, Pivus, Twins UK-II & III. Study descriptions can be found in the Supplementary Note.

### Imputation

Imputation to the HapMap CEU panel was conducted using either MACH<sup>47</sup>, IMPUTE<sup>48</sup>, Beagle<sup>49,50</sup> or BIMBAM<sup>51</sup> with filters and quality control parameters as shown in Supplementary Table 9. SNPs were excluded on a cohort basis if the imputation score, assessed using r2.hat (MACH), .info (IMPUTE) or OEvar (BIMBAM), was  $< 0.3$ . In total, 2,762,059 SNPs were analyzed.

## Statistical analysis

Individual studies performed a GWAS analysis using linear regression models with FVC (in milliliters) as the outcome, stratified by never/ever smoking. Adjustment factors were age, age<sup>2</sup>, sex and height (plus height<sup>2</sup> and weight for CHARGE and replication cohorts). If applicable, cohorts adjusted for center, cohort or principal components to adjust for population stratification. The follow-up studies used the same models. Effect estimates for each study were corrected using genomic control<sup>19</sup> separately within smoking strata. Study-specific lambda estimates are shown in Supplementary Table 1.

## Meta-analysis of stage 1 data

Variants with imputation quality below 0.3 or minor allele frequency below 0.03 were excluded from each dataset before the meta-analysis. Study-specific effect estimates and standard errors for ever-smokers and never-smokers were combined using METAL<sup>52</sup> with fixed-effects inverse variance weighted meta-analysis, which takes directionality into account by aligning study results according to the same effect allele. Genomic control was applied to the resulting combined (ever-smokers and never-smokers) effect estimates for each study. These combined effect estimates for each study were then combined across studies, again using fixed-effect inverse variance weighted meta-analysis with METAL, and genomic control was applied again to the final meta-analysis estimates. Manhattan plots, quantile-quantile-plots, forest-plots, gene annotation, and additional statistics were produced using R version 2.9.2<sup>53</sup>. Stage 1 results are in Supplementary Data Set 1.

## Selection of SNPs for stage 2 and stage 2 meta-analysis

For every region containing at least one SNP showing evidence for association with FVC ( $P < 5 \times 10^{-7}$ ), the SNP with the smallest  $P$  value that also had a  $N_{\text{effective}} \geq 80\%$  (the  $N_{\text{effective}}$  is the product of sample size and the imputation quality summed across studies) of the total stage 1 sample size was followed up in a second stage using *in silico* data from 9 cohorts (Figure 1, Supplementary Table 2a). In total, 7 SNPs were followed up. Results for variants with imputation quality (Supplementary Table 10) below 0.3 in a given study were excluded from the meta-analysis. Results across stage 2 studies were meta-analyzed using fixed-effects inverse variance weighted meta-analysis with METAL<sup>52</sup>.

Regions were defined as independent if the leading SNP from one region was  $> 500$  kilobases (kb) from the leading SNP of any other region. We excluded two regions (*GSTCD* and *PTCHI*) from follow-up, that were previously associated with FEV<sub>1</sub> or FEV<sub>1</sub>/FVC<sup>12–14</sup>.

## Combined analysis of stage 1 and stage 2

We performed an inverse variance weighted fixed effects meta-analysis across stages 1 and 2 using METAL<sup>52</sup> and obtained two-sided  $P$  values for the resulting effect estimates.

## Follow-up in other ethnicities

To evaluate these loci across ethnicities, we studied association with FVC in four samples of non-European ancestry. We used data from the National Heart, Lung, and Blood Institute

(NHLBI)-sponsored Candidate gene Association Resource (CARE) Project<sup>23,54</sup>, which genotyped African Americans in ARIC, MESA, CHS, CARDIA, the Jackson Heart Study and the Cleveland Family Studies. The analyses in CARE were carried out per cohort and meta-analyzed using METAL<sup>52</sup>. Furthermore, we assessed the SNPs in Hispanic and Chinese participants from the MESA study. Lastly, we investigated the loci in Korean participants from the Healthy Twin<sup>15–17</sup> and KARE3<sup>18</sup> studies. For the Korean studies, the analyses were carried out per cohort and meta-analyzed. Individual studies performed a GWAS analysis using linear regression models with FVC (in milliliters) as the outcome. Adjustment factors were age, age<sup>2</sup>, sex, height, height<sup>2</sup>, ever/never smoking and weight. We assessed the sentinel SNPs from the HapMap CEU reference panel that were available and SNPs from the regions of the identified loci based on location of the sentinel SNPs  $\pm$  200 kb. Only SNPs with a MAF  $\geq$  0.05 were included. The estimated number of independent tests per population sample and corresponding Bonferroni *P* values are shown in Supplementary Table 11. The effective number of independent variants being tested in each replication population was estimated based on linkage disequilibrium between SNPs using the technique of Li and Ji<sup>22</sup>, which is a modification of the technique originally proposed by Cheverud<sup>55</sup> and implemented by Nyholt<sup>21</sup>. This calculation was performed using “matSpD”<sup>21</sup> based on the linkage disequilibrium structures of the 1,000 Genomes “all ancestries” sample.

### Gene set enrichment analysis

We applied an algorithm known as improved gene set enrichment analysis for GWAS (*i*-GSEA4GWAS) to place variants associated with FVC within curated pathways and functional categories<sup>25</sup>. SNPs from the stage 1 and 2 meta-analyzed GWAS were mapped to genes if within a 100 kb distance (upstream or downstream). For a given SNP, if multiple genes were located within this range, the closest gene was selected and assigned the association *P* value. Since multiple SNPs can map to the same gene, a SNP label permutation was used to reduce biases caused by larger loci having disproportionately higher number of SNPs. Log-transformed association *P* values were used to rank order the resulting gene list (18,454 genes) and calculate gene set enrichment scores. Approximately 2,000 gene sets were used. These were limited to curated pathways derived from multiple resources such as KEGG, BioCarta, REACTOME, and functional annotations extracted from the Gene Ontology database. A modified version of GSEA procedure was performed and adjusted for multiple testing using false discovery rate (FDR). Significant enrichment of gene sets was set at FDR < 0.01.

### Gene expression analysis

Following the meta-analysis, we investigated mRNA expression of the nearest gene for each of the new SNPs in: human lung tissue, human airway smooth muscle cells (HASM), human bronchial epithelial cells (HEBC) and peripheral mononuclear blood cells (PMBC). Lung resection specimens were obtained from patients diagnosed with solitary pulmonary tumors at Ghent University Hospital (Ghent, Belgium). Primary human bronchial epithelial cells (HBEC) and human airway smooth muscle cells (HASM) were prepared from lung resection specimens obtained from anonymous donors during surgery for lung cancer at the Leiden University Medical Center (LUMC, Leiden, The Netherlands). All assays were done at the

Ghent University Hospital. Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood using Ficoll gradients. Written informed consent was obtained from all subjects according to protocols approved by the local ethics committees. Total RNA was extracted from samples using the miRNeasy Mini kit (Qiagen) and cDNA was prepared from 1 µg RNA template using the Transcriptor Universal cDNA Master kit (Roche) following manufacturer's instructions. Expression of the candidate genes and the housekeeping gene *GADPH* was analyzed using TaqMan Gene Expression Assays (Applied Biosystems, Forster City, CA, USA; Assay ID numbers are given in Supplementary Table 12 in the Supplementary Note).

### Expression Quantitative Trait Loci

We assessed whether top SNPs or their proxies, based on an  $r^2 > 0.7$ , in the six new regions were associated with gene expression in whole blood cells in a sample of 762 individuals from the Rotterdam Study III (RS-III)<sup>56</sup>. Expression was assessed using Illumina Whole-Genome Expression Beadchips (HumanHT-12 v4). For eQTL-analysis, we used the eQTL mapping pipeline called MegaQTL<sup>57</sup>. eQTLs were deemed cis when the distance between the SNP chromosomal position and the probe midpoint was less than 250 kb. eQTLs were mapped using Spearman's rank correlation, using the imputation dosage values as genotypes. Resultant correlations were then converted to *P* values and their respective *z*-scores weighted with the square root of the sample size. The model was adjusted for the first 40 eigenvectors of the principal component analysis. We corrected for multiple testing by using Bonferroni correction: associations with  $P < 10^{-4}$  were considered statistically significant.

Finally, we also queried publicly available eQTL databases derived from multiple cell and tissue types (lymphoblastoid cell lines, brain tissue and human fibroblasts)<sup>26–29</sup>. We corrected for multiple testing by using Bonferroni correction: associations with  $P < 10^{-4}$  were considered statistically significant.

### Expression in fetal lung

We used methods previously described<sup>58</sup> to mine publicly available data<sup>59,60</sup> to identify whether differential expression of relevant genes occurs during normal human lung development. Previously, human fetal lung tissues were obtained from National Institute of Child Health and Human Development tissue databases and microarray profiles used to investigate the expression spanning different gestational ages. RNA samples from 38 subjects (estimated gestational age 7–22 weeks or 53–154 days post conception) representing the pseudoglandular (gestational age, 7–16 weeks) and canalicular (17–26 weeks) stages of lung development were included within the dataset. These data are available at NCBI Gene Expression Omnibus.

### Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Authors

Daan W. Loth<sup>1,2,\*</sup>, María Soler Artigas<sup>3,4,\*</sup>, Sina A. Gharib<sup>5,6,\*</sup>, Louise V. Wain<sup>3,4,\*</sup>, Nora Franceschini<sup>7,8,\*</sup>, Beate Koch<sup>9,\*</sup>, Tess Pottinger<sup>10,\*</sup>, Albert Vernon Smith<sup>11,12,\*</sup>, Qing Duan<sup>13,\*</sup>, Chris Oldmeadow<sup>14,15</sup>, Mi Kyeong Lee<sup>16</sup>, David P. Strachan<sup>17</sup>, Alan L. James<sup>18,19,20</sup>, Jennifer E. Huffman<sup>21</sup>, Veronique Vitart<sup>21</sup>, Adaikalavan Ramasamy<sup>22,23</sup>, Nicholas J. Wareham<sup>24</sup>, Jaakko Kaprio<sup>25,26,27</sup>, Xin-Qun Wang<sup>28</sup>, Holly Trochet<sup>21</sup>, Mika Kähönen<sup>29</sup>, Claudia Flexeder<sup>30</sup>, Eva Albrecht<sup>31</sup>, Lorna M. Lopez<sup>32,33</sup>, Kim de Jong<sup>34,35</sup>, Bharat Thyagarajan<sup>36</sup>, Alexsander Couto Alves<sup>23</sup>, Stefan Enroth<sup>37,38</sup>, Ernst Omenaas<sup>39,40</sup>, Peter K. Joshi<sup>41</sup>, Tove Fall<sup>38,42</sup>, Ana Vi uela<sup>43</sup>, Lenore J. Launer<sup>44</sup>, Laura R. Loehr<sup>7,8</sup>, Myriam Fornage<sup>45,46</sup>, Guo Li<sup>47</sup>, Jemma B. Wilk<sup>48</sup>, Wenbo Tang<sup>49</sup>, Ani Manichaikul<sup>50,51</sup>, Lies Lahousse<sup>1,52</sup>, Tamara B. Harris<sup>44</sup>, Kari E. North<sup>7</sup>, Alicja R. Rudnicka<sup>17</sup>, Jennie Hui<sup>53</sup>, Xiangjun Gu<sup>45,46</sup>, Thomas Lumley<sup>54</sup>, Alan F. Wright<sup>21</sup>, Nicholas D. Hastie<sup>21</sup>, Susan Campbell<sup>21</sup>, Rajesh Kumar<sup>55</sup>, Isabelle Pin<sup>56,57,58</sup>, Robert A. Scott<sup>24</sup>, Kirsi H. Pietiläinen<sup>27,59,60</sup>, Ida Surakka<sup>27,61</sup>, Yongmei Liu<sup>62</sup>, Elizabeth G. Holliday<sup>14,15</sup>, Holger Schulz<sup>30</sup>, Joachim Heinrich<sup>30</sup>, Gail Davies<sup>32,33,63,64</sup>, Judith M. Vonk<sup>34,35</sup>, Mary Wojczynski<sup>65</sup>, Anneli Pouta<sup>66,67</sup>, Åsa Johansson<sup>37,38,68</sup>, Sarah H. Wild<sup>41</sup>, Erik Ingelsson<sup>38,42,69,70</sup>, Fernando Rivadeneira<sup>71,72</sup>, Henry Völzke<sup>73</sup>, Pirro G. Hysi<sup>43</sup>, Gudny Eiriksdottir<sup>11</sup>, Alanna C. Morrison<sup>74</sup>, Jerome I. Rotter<sup>75,76</sup>, Wei Gao<sup>77</sup>, Dirkje S. Postma<sup>35,78</sup>, Wendy B. White<sup>79</sup>, Stephen S. Rich<sup>50,51</sup>, Albert Hofman<sup>1,72</sup>, Thor Aspelund<sup>11,12</sup>, David Couper<sup>80</sup>, Lewis J. Smith<sup>55</sup>, Bruce M. Psaty<sup>6,47,81,82</sup>, Kurt Lohman<sup>83</sup>, Esteban G. Burchard<sup>84,85</sup>, André G. Uitterlinden<sup>71,72</sup>, Melissa Garcia<sup>44</sup>, Bonnie R. Joubert<sup>86</sup>, Wendy L. McArdle<sup>87</sup>, A. Bill Musk<sup>88</sup>, Nadia Hansel<sup>89</sup>, Susan R. Heckbert<sup>47,81,82</sup>, Lina Zgaga<sup>90,91</sup>, Joyce B.J. van Meurs<sup>71,72</sup>, Pau Navarro<sup>21</sup>, Igor Rudan<sup>92</sup>, Yeon-Mok Oh<sup>93,94</sup>, Susan Redline<sup>95</sup>, Deborah Jarvis<sup>22,96</sup>, Jing Hua Zhao<sup>24</sup>, Taina Rantanen<sup>97</sup>, George T. O'Connor<sup>98,99</sup>, Samuli Ripatti<sup>27,61,100</sup>, Rodney J. Scott<sup>14,15</sup>, Stefan Karrasch<sup>30,101,102</sup>, Harald Grallert<sup>103</sup>, Nathan C. Gaddis<sup>104</sup>, John M. Starr<sup>32,105</sup>, Cisca Wijmenga<sup>106</sup>, Ryan L. Minster<sup>107</sup>, David J. Lederer<sup>10,108</sup>, Juha Pekkanen<sup>109,110</sup>, Ulf Gyllensten<sup>37,38</sup>, Harry Campbell<sup>41</sup>, Andrew P. Morris<sup>70</sup>, Sven Gläser<sup>9</sup>, Christopher J. Hammond<sup>43</sup>, Kristin M. Burkart<sup>10</sup>, John Beilby<sup>53</sup>, Stephen B. Kritchevsky<sup>111</sup>, Vilmundur Gudnason<sup>11,12</sup>, Dana B. Hancock<sup>112</sup>, O. Dale Williams<sup>113</sup>, Ozren Polasek<sup>114</sup>, Tatijana Zemunik<sup>115</sup>, Ivana Kolcic<sup>114</sup>, Marcy F. Petrini<sup>116</sup>, Matthias Wjst<sup>117</sup>, Woo Jin Kim<sup>118,119</sup>, David J. Porteous<sup>63</sup>, Generation Scotland<sup>76</sup>, Blair H. Smith<sup>75</sup>, Anne Viljanen<sup>97</sup>, Markku Heliövaara<sup>26</sup>, John R. Attia<sup>14,15</sup>, Ian Sayers<sup>120</sup>, Regina Hampel<sup>121</sup>, Christian Gieger<sup>31</sup>, Ian J. Deary<sup>32,33</sup>, H. Marika Boezen<sup>34,35</sup>, Anne Newman<sup>122</sup>, Marjo-Riitta Jarvelin<sup>23,123,124,125,126</sup>, James F. Wilson<sup>41</sup>, Lars Lind<sup>69</sup>, Bruno H. Stricker<sup>1,2,71,72</sup>, Alexander Teumer<sup>127</sup>, Timothy D. Spector<sup>43</sup>, Erik Melén<sup>128</sup>, Marjolein J. Peters<sup>71,72</sup>, Leslie A. Lange<sup>13</sup>, R. Graham Barr<sup>108</sup>, Ken R. Bracke<sup>52</sup>, Fien M. Verhamme<sup>52</sup>, Joonhoon Sung<sup>16,129</sup>, Pieter S. Hiemstra<sup>130</sup>, Patricia A. Cassano<sup>49,131</sup>, Akshay Sood<sup>132,\*\*</sup>, Caroline Hayward<sup>21,\*\*</sup>, Josée Dupuis<sup>77,99,\*\*</sup>, Ian P. Hall<sup>120,\*\*</sup>, Guy G. Brusselle<sup>1,52,133,\*\*</sup>, Martin D. Tobin<sup>3,4,\*\*</sup>, and Stephanie J. London<sup>86,\*\*</sup>



## Affiliations

<sup>1</sup>Department of Epidemiology, Erasmus MC, Rotterdam, The Netherlands. <sup>2</sup>Netherlands Health Care Inspectorate, The Hague, The Netherlands. <sup>3</sup>Genetic Epidemiology Group, Department of Health Sciences, University of Leicester, Leicester, UK. <sup>4</sup>National Institute for Health Research (NIHR) Leicester Respiratory Biomedical Research Unit, Glenfield Hospital, Leicester, UK. <sup>5</sup>Computational Medicine Core, Center for Lung Biology, University of Washington, Seattle, WA, USA. <sup>6</sup>Department of Medicine, University of Washington, Seattle, WA, USA. <sup>7</sup>Department of Epidemiology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA. <sup>8</sup>Carolina Center for Genome Sciences University of North Carolina at Chapel Hill, Chapel Hill, NC, USA. <sup>9</sup>Department of Internal Medicine B - Pneumology, Cardiology, Intensive Care and Infectious Diseases, University Hospital Greifswald, Greifswald, Germany. <sup>10</sup>Department of Medicine, College of Physicians and Surgeons, Columbia University, New York, NY, USA. <sup>11</sup>Iceland Heart Association, Kopavogur, Iceland. <sup>12</sup>University of Iceland, Reykjavik, Iceland. <sup>13</sup>Department of Genetics, University of North Carolina, Chapel Hill, NC, USA. <sup>14</sup>Hunter Medical Research Institute, University of Newcastle, Newcastle, NSW, Australia. <sup>15</sup>Faculty of Health, University of Newcastle, Newcastle, NSW, Australia. <sup>16</sup>Institute of Health and Environment, Seoul National University, Seoul, South Korea. <sup>17</sup>Division of Population Health Sciences and Education, St George's, University of London, London, UK. <sup>18</sup>Department of Pulmonary Physiology and Sleep Medicine/West Australian Sleep Disorders Research Institute, Nedlands, Australia. <sup>19</sup>School of Medicine and Pharmacology, The University of Western Australia, Perth, Australia. <sup>20</sup>Busselton Population Medical Research Institute, Busselton, Australia. <sup>21</sup>MRC Human Genetics, MRC IGMM, University of Edinburgh, Edinburgh, Scotland, UK. <sup>22</sup>Respiratory Epidemiology and Public Health Group, National Heart and Lung Institute, Imperial College London, London, UK. <sup>23</sup>Department of Epidemiology and Biostatistics, MRC Health Protection Agency (HPA) Centre for Environment and Health, School of Public Health, Imperial College London, London, UK. <sup>24</sup>MRC Epidemiology Unit, Institute of Metabolic Science, Addenbrooke's Hospital, Cambridge, United Kingdom. <sup>25</sup>Hjelt Institute, Department of Public Health, University of Helsinki, Helsinki, Finland. <sup>26</sup>The National Institute for Health and Welfare (THL), Helsinki, Finland. <sup>27</sup>Institute for Molecular Medicine Finland (FIMM), University of Helsinki, Helsinki, Finland. <sup>28</sup>Department of Public Health Sciences, University of Virginia, Charlottesville, VA, USA. <sup>29</sup>Department of Clinical Physiology, University of Tampere and Tampere University Hospital, Tampere, Finland. <sup>30</sup>Institute of Epidemiology I, Helmholtz Zentrum München - German Research Center for Environmental Health, Neuherberg, Germany. <sup>31</sup>Institute of Genetic Epidemiology, Helmholtz Zentrum München - German Research Center for Environmental Health, Neuherberg, Germany. <sup>32</sup>Centre for Cognitive Ageing and Cognitive Epidemiology, University of Edinburgh, Edinburgh, UK. <sup>33</sup>Department of Psychology, The University of Edinburgh, Edinburgh, UK. <sup>34</sup>University of Groningen, University Medical Center Groningen, Department of Epidemiology, Groningen, The Netherlands. <sup>35</sup>GRIAC research institute, University

of Groningen, University Medical Center Groningen, Groningen, The Netherlands. <sup>36</sup>Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN, USA. <sup>37</sup>Department of Immunology, Genetics, and Pathology, Rudbeck Laboratory, Uppsala University, Uppsala, Sweden. <sup>38</sup>Science for Life Laboratory, Uppsala University, Uppsala, Sweden. <sup>39</sup>Haukeland University Hospital, Bergen, Norway. <sup>40</sup>Department Clinical Sciences, University of Bergen, Bergen, Norway. <sup>41</sup>Centre for Population Health Sciences, University of Edinburgh, Teviot Place, Edinburgh, Scotland, UK. <sup>42</sup>Molecular Epidemiology, Department of Medical Sciences, Uppsala University, Uppsala, Sweden. <sup>43</sup>Department of Twins Research and Genetic Epidemiology, King's College London, London, UK. <sup>44</sup>Laboratory of Epidemiology, Demography, and Biometry, National Institute on Aging, National Institutes of Health, Bethesda, MD, USA. <sup>45</sup>Institute of Molecular Medicine, University of Texas Health Science Center at Houston, Houston, TX, USA. <sup>46</sup>Human Genetics Center, University of Texas Health Science Center at Houston, Houston, TX, USA. <sup>47</sup>Cardiovascular Health Research Unit, University of Washington, Seattle, WA, USA. <sup>48</sup>Precision Medicine, Pfizer Global Research and Development, Cambridge, MA, USA. <sup>49</sup>Division of Nutritional Sciences, Cornell University, Ithaca, NY, USA. <sup>50</sup>Center for Public Health Genomics, University of Virginia, Charlottesville, Virginia, USA. <sup>51</sup>Division of Biostatistics and Epidemiology, Department of Public Health Sciences, University of Virginia, Charlottesville, Virginia, USA. <sup>52</sup>Department of Respiratory Medicine, Ghent University Hospital, Ghent, Belgium. <sup>53</sup>PathWest Laboratory Medicine WA, Nedlands, Australia. <sup>54</sup>Department of Statistics, University of Auckland, Auckland, New Zealand. <sup>55</sup>Northwestern University Feinberg School of Medicine, Chicago, IL, USA. <sup>56</sup>Centre Hospitalier Universitaire de Grenoble, Grenoble, France. <sup>57</sup>INSERM U823, Institut Albert Bonniot, Grenoble, France. <sup>58</sup>Université Joseph Fourier, Grenoble, France. <sup>59</sup>Obesity Research Unit, Research Programs Unit, Diabetes and Obesity, University of Helsinki, Helsinki, Finland. <sup>60</sup>Department of Medicine, Division of Internal Medicine, Helsinki University Central Hospital, Helsinki, Finland. <sup>61</sup>Public Health Genomics Unit, Department of Chronic Disease Prevention, The National Institute for Health and Welfare (THL), Helsinki, Finland. <sup>62</sup>Department of Epidemiology and Prevention, Division of Public Health Sciences, Wake Forest School of Medicine, Winston-Salem, NC, USA. <sup>63</sup>Medical Genetics Section, University of Edinburgh Molecular Medicine Centre, Edinburgh, UK. <sup>64</sup>MRC Institute of Genetics and Molecular Medicine, Edinburgh, UK. <sup>65</sup>Department of Statistical Genomics, Washington University, St. Louis, MO, USA. <sup>66</sup>National Institute for Health and Welfare, Oulu, Finland. <sup>67</sup>Department of Clinical Sciences/Obstetrics and Gynecology, University of Oulu, Oulu, Finland. <sup>68</sup>Uppsala Clinical Research Center, Uppsala University, Uppsala, Sweden. <sup>69</sup>Department of Medical Sciences, Uppsala University, Uppsala, Sweden. <sup>70</sup>Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom. <sup>71</sup>Department of Internal Medicine, Erasmus MC, Rotterdam, The Netherlands. <sup>72</sup>Netherlands Genomics Initiative (NGI)-sponsored Netherlands Consortium for Healthy Aging (NCHA), Rotterdam, The Netherlands. <sup>73</sup>Institute for Community Medicine, University

Medicine Greifswald, Greifswald, Germany <sup>74</sup>School of Public Health, University of Texas Health Science Center at Houston, Houston, TX, USA. <sup>75</sup>Biomedical Research Institute, Harbor-UCLA Medical Center, Torrance, CA, USA. <sup>76</sup>Department of Pediatrics, Harbor-UCLA Medical Center, Torrance, CA, USA. <sup>77</sup>Department of Biostatistics, Boston University School of Public Health, Boston, MA, USA. <sup>78</sup>University of Groningen, University Medical Center Groningen, Department of Pulmonology, Groningen, The Netherlands. <sup>79</sup>Tougaloo College, Jackson, MS, USA. <sup>80</sup>Department of Biostatistics, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA. <sup>81</sup>Department of Epidemiology, University of Washington, Seattle, WA, USA. <sup>82</sup>Group Health Research Institute, Group Health Cooperative, Seattle, WA, USA. <sup>83</sup>Department of Biostatistical Sciences, Division of Public Health Sciences, Wake Forest School of Medicine, Winston-Salem, NC, USA. <sup>84</sup>Department of Bioengineering & Therapeutic Sciences, University of California, San Francisco, CA, USA. <sup>85</sup>Department of Medicine, University of California, San Francisco, CA, USA. <sup>86</sup>Epidemiology Branch, National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, Research Triangle Park, NC, USA. <sup>87</sup>School of Social and Community Medicine, University of Bristol, Bristol, United Kingdom. <sup>88</sup>Department of Respiratory Medicine, Sir Charles Gairdner Hospital, Nedlands, Australia. <sup>89</sup>Department of Medicine, Johns Hopkins University, Baltimore, MD, USA. <sup>90</sup>Department of Public Health and Primary Care, Trinity College Dublin, Dublin, Ireland. <sup>91</sup>Adrija Stampar School of Public Health, Medical School, University of Zagreb, Zagreb, Croatia. <sup>92</sup>Centre for Population Health Sciences, Medical School, University of Edinburgh, Edinburgh, Scotland, UK. <sup>93</sup>Department of Pulmonary and Critical Care Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul, South Korea. <sup>94</sup>Clinical Research Center for Chronic Obstructive Airway Diseases, Asan Medical Center, University of Ulsan College of Medicine, Seoul, South Korea. <sup>95</sup>Department of Medicine, Brigham Women's Hospital, Boston, MA, USA. <sup>96</sup>MRC-PHE Centre for Environment & Health, Imperial College London, London, UK. <sup>97</sup>Gerontology Research Centre, Department of Health Sciences, University of Jyväskylä, Jyväskylä, Finland. <sup>98</sup>Pulmonary Center, Boston University School of Medicine, Boston, MA, USA. <sup>99</sup>The NHLBI's Framingham Heart Study, Framingham, MA, USA. <sup>100</sup>Genetic Epidemiology Group, Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK. <sup>101</sup>Institute for Occupational, Social and Environmental Medicine, Ludwig-Maximilians-Universität, Munich, Germany. <sup>102</sup>Institute of General Practice, University Hospital Klinikum rechts der Isar, Technische Universität München, Munich, Germany. <sup>103</sup>Research Unit of Molecular Epidemiology, Helmholtz Zentrum München – German Research Center for Environmental Health, Neuherberg, Germany. <sup>104</sup>Research Computing Division, Research Triangle Institute International, Research Triangle Park, NC, USA. <sup>105</sup>Alzheimer Scotland Dementia Research Centre, University of Edinburgh, Edinburgh, UK. <sup>106</sup>Department of Genetics, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands. <sup>107</sup>Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA, USA. <sup>108</sup>Department of

Epidemiology, Mailman School of Public Health, Columbia University, New York, NY, USA. <sup>109</sup>Department of Environmental Health, National Institute for Health and Welfare (THL), Kuopio, Finland. <sup>110</sup>Public Health and Clinical Nutrition, University of Eastern Finland, Kuopio, Finland. <sup>111</sup>Sticht Center on Aging, Wake Forest School of Medicine, Winston-Salem, NC, USA. <sup>112</sup>Behavioral Health Epidemiology Program, Research Triangle Institute International, Research Triangle Park, NC, USA. <sup>113</sup>Florida International University, Miami, FL, USA. <sup>114</sup>Department of Public Health, Medical School, University of Split, Split, Croatia. <sup>115</sup>Department of Medical Biology, Medical School, University of Split, Split, Croatia. <sup>116</sup>Division of Pulmonary, Critical Care and Sleep Medicine, Department of Medicine, University of Mississippi Medical Center, Jackson, MS, USA. <sup>117</sup>Comprehensive Pneumology Center (CPC), Helmholtz Zentrum München (HMGU), München, Germany. <sup>118</sup>Department of Internal Medicine, Kangwon National University Hospital, School of Medicine, Kangwon National University, Chuncheon, South Korea. <sup>119</sup>Environmental Health Center, Kangwon National University Hospital, School of Medicine, Kangwon National University, Chuncheon, South Korea. <sup>120</sup>Division of Therapeutics and Molecular Medicine, University of Nottingham, Nottingham, UK. <sup>121</sup>Institute of Epidemiology II, Helmholtz Zentrum München - German Research Center for Environmental Health, Neuherberg, Germany. <sup>122</sup>Department of Epidemiology, Center for Aging and Population Health, University of Pittsburgh, Pittsburgh, PA, USA. <sup>123</sup>Institute of Health Sciences, University of Oulu, Oulu, Finland. <sup>124</sup>Biocenter Oulu, University of Oulu, Oulu, Finland. <sup>125</sup>Unit of Primary Care, Oulu University Hospital, Oulu, Finland. <sup>126</sup>Department of Children and Young People and Families, National Institute for Health and Welfare, Oulu, Finland. <sup>127</sup>Department for Genetics and Functional Genomics, Interfaculty Institute for Genetics and Functional Genomics, University Medicine Greifswald, Greifswald, Germany. <sup>128</sup>Institute of Environmental Medicine, Karolinska Institutet and Sachs' Children's Hospital, Stockholm, Sweden. <sup>129</sup>Complex Disease & Genetic Epidemiology Branch, Department of Epidemiology, Seoul National University School of Public Health, Seoul, South Korea. <sup>130</sup>Department of Pulmonology, Leiden University Medical Center, Leiden, The Netherlands. <sup>131</sup>Department of Public Health, Division of Biostatistics and Epidemiology, Weill Cornell Medical College, New York, NY, USA. <sup>132</sup>University of New Mexico Health Sciences Center School of Medicine, Albuquerque, NM, USA. <sup>133</sup>Department of Respiratory Medicine, Erasmus MC, Rotterdam, The Netherlands.

## Acknowledgments

### *Funding and Acknowledgments*

For all studies, information on funding and acknowledgments can be found in the Supplementary Note.

## References

1. Wilk JB, et al. Evidence for major genes influencing pulmonary function in the NHLBI family heart study. *Genet Epidemiol.* 2000; 19:81–94. [PubMed: 10861898]

2. Zappala CJ, et al. Marginal decline in forced vital capacity is associated with a poor outcome in idiopathic pulmonary fibrosis. *Eur Respir J*. 2010; 35:830–836. [PubMed: 19840957]
3. Raghu G, et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. *Am J Respir Crit Care Med*. 2011; 183:788–824. [PubMed: 21471066]
4. Ashley F, Kannel WB, Sorlie PD, Masson R. Pulmonary function: relation to aging, cigarette habit, and mortality. *Ann Intern Med*. 1975; 82:739–745. [PubMed: 1094879]
5. Burney PG, Hooper R. Forced vital capacity, airway obstruction and survival in a general population sample from the USA. *Thorax*. 2011; 66:49–54. [PubMed: 20980245]
6. Lee HM, Le H, Lee BT, Lopez VA, Wong ND. Forced vital capacity paired with Framingham Risk Score for prediction of all-cause mortality. *Eur Respir J*. 2010; 36:1002–1006. [PubMed: 20562119]
7. Lange P, Nyboe J, Appleyard M, Jensen G, Schnohr P. Spirometric findings and mortality in never-smokers. *J Clin Epidemiol*. 1990; 43:867–873. [PubMed: 2213076]
8. Kannel WB, Hubert H, Lew EA. Vital capacity as a predictor of cardiovascular disease: the Framingham study. *Am Heart J*. 1983; 105:311–315. [PubMed: 6823813]
9. Palmer LJ, et al. Familial aggregation and heritability of adult lung function: results from the Busselton Health Study. *Eur Respir J*. 2001; 17:696–702. [PubMed: 11401066]
10. van Putte-Katier N, et al. Relationship between parental lung function and their children's lung function early in life. *Eur Respir J*. 2011; 38:664–671. [PubMed: 21233268]
11. Wilk JB, et al. A genome-wide association study of pulmonary function measures in the Framingham Heart Study. *PLoS Genet*. 2009; 5:e1000429. [PubMed: 19300500]
12. Repapi E, et al. Genome-wide association study identifies five loci associated with lung function. *Nat Genet*. 2010; 42:36–44. [PubMed: 20010834]
13. Hancock DB, et al. Meta-analyses of genome-wide association studies identify multiple loci associated with pulmonary function. *Nat Genet*. 2010; 42:45–52. [PubMed: 20010835]
14. Soler Artigas M, et al. Genome-wide association and large-scale follow up identifies 16 new loci influencing lung function. *Nat Genet*. 2011; 43:1082–1090. [PubMed: 21946350]
15. Sung J, et al. The Korean Twin Registry--methods, current stage, and interim results. *Twin Res*. 2002; 5:394–400. [PubMed: 12537866]
16. Sung J, et al. Healthy Twin: a twin-family study of Korea--protocols and current status. *Twin Res Hum Genet*. 2006; 9:844–848. [PubMed: 17254419]
17. Gombojav B, et al. The Healthy Twin Study, Korea updates: resources for omics and genome epidemiology studies. *Twin Res Hum Genet*. 2013; 16:241–245. [PubMed: 23218411]
18. Cho YS, et al. A large-scale genome-wide association study of Asian populations uncovers genetic factors influencing eight quantitative traits. *Nat Genet*. 2009; 41:527–534. [PubMed: 19396169]
19. Devlin B, Roeder K. Genomic control for association studies. *Biometrics*. 1999; 55:997–1004. [PubMed: 11315092]
20. The 1000 Genomes Project Consortium et al. A map of human genome variation from population-scale sequencing. *Nature*. 2010; 467:1061–1073. [PubMed: 20981092]
21. Nyholt DR. A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. *Am J Hum Genet*. 2004; 74:765–769. [PubMed: 14997420]
22. Li J, Ji L. Adjusting multiple testing in multilocus analyses using the eigenvalues of a correlation matrix. *Heredity (Edinb)*. 2005; 95:221–227. [PubMed: 16077740]
23. Musunuru K, et al. Candidate gene association resource (CARE): design, methods, and proof of concept. *Circ Cardiovasc Genet*. 2010; 3:267–275. [PubMed: 20400780]
24. Carlson CS, et al. Generalization and dilution of association results from European GWAS in populations of non-European ancestry: the PAGE study. *PLoS Biol*. 2013; 11:e1001661. [PubMed: 24068893]
25. Zhang K, Cui S, Chang S, Zhang L, Wang J. i-GSEA4GWAS: a web server for identification of pathways/gene sets associated with traits by applying an improved gene set enrichment analysis to genome-wide association study. *Nucleic Acids Res*. 2010; 38:W90–W95. [PubMed: 20435672]

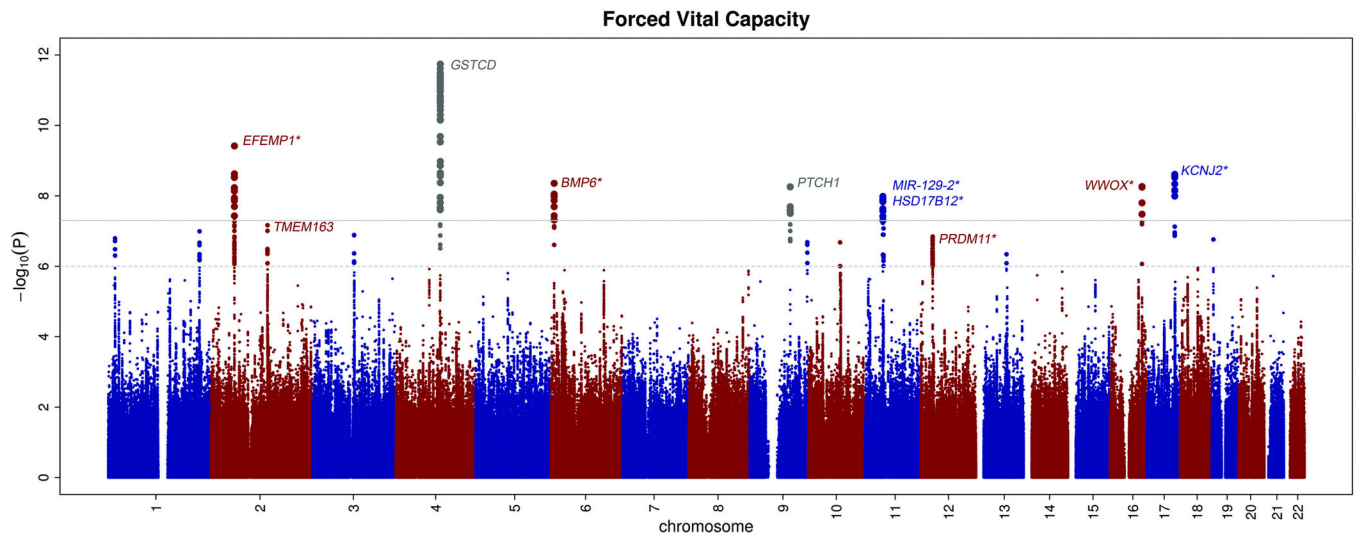


26. Dixon AL, et al. A genome-wide association study of global gene expression. *Nat Genet.* 2007; 39:1202–1207. [PubMed: 17873877]
27. Gibbs JR, et al. Abundant quantitative trait loci exist for DNA methylation and gene expression in human brain. *PLoS Genet.* 2010; 6:e1000952. [PubMed: 20485568]
28. Dimas AS, et al. Common regulatory variation impacts gene expression in a cell type-dependent manner. *Science.* 2009; 325:1246–1250. [PubMed: 19644074]
29. The Genotype-Tissue Expression Consortium. The Genotype-Tissue Expression (GTEx) project. *Nat Genet.* 2013; 45:580–585. [PubMed: 23715323]
30. Boyle AP, et al. Annotation of functional variation in personal genomes using RegulomeDB. *Genome Res.* 2012; 22:1790–1797. [PubMed: 22955989]
31. Gottlieb DJ, et al. Heritability of longitudinal change in lung function. The Framingham study. *Am J Respir Crit Care Med.* 2001; 164:1655–1659. [PubMed: 11719305]
32. Park JH, et al. Estimation of effect size distribution from genome-wide association studies and implications for future discoveries. *Nat Genet.* 2010; 42:570–575. [PubMed: 20562874]
33. Eichler EE, et al. Missing heritability and strategies for finding the underlying causes of complex disease. *Nat Rev Genet.* 2010; 11:446–450. [PubMed: 20479774]
34. Hancock DB, et al. Genome-wide joint meta-analysis of SNP and SNP-by-smoking interaction identifies novel loci for pulmonary function. *PLoS Genet.* 2012; 8:e1003098. [PubMed: 23284291]
35. Yang J, et al. Conditional and joint multiple-SNP analysis of GWAS summary statistics identifies additional variants influencing complex traits. *Nat Genet.* 2012; 44:369–375. S1–S3. [PubMed: 22426310]
36. Lango Allen H, et al. Hundreds of variants clustered in genomic loci and biological pathways affect human height. *Nature.* 2010; 467:832–838. [PubMed: 20881960]
37. Sountoulidis A, et al. Activation of the canonical Bone Morphogenetic Protein (BMP) pathway during lung morphogenesis and adult lung tissue repair. *PLoS One.* 2012; 7:e41460. [PubMed: 22916109]
38. Rosendahl A, et al. Activation of bone morphogenetic protein/Smad signaling in bronchial epithelial cells during airway inflammation. *Am J Respir Cell Mol Biol.* 2002; 27:160–169. [PubMed: 12151307]
39. McLaughlin PJ, et al. Targeted disruption of fibulin-4 abolishes elastogenesis and causes perinatal lethality in mice. *Mol Cell Biol.* 2006; 26:1700–1709. [PubMed: 16478991]
40. de Boer WI, et al. Transforming growth factor beta1 and recruitment of macrophages and mast cells in airways in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med.* 1998; 158:1951–1957. [PubMed: 9847291]
41. Takizawa H, et al. Increased expression of transforming growth factor-beta1 in small airway epithelium from tobacco smokers and patients with chronic obstructive pulmonary disease (COPD). *Am J Respir Crit Care Med.* 2001; 163:1476–1483. [PubMed: 11371421]
42. Kimura M, et al. Bmi1 regulates cell fate via tumor suppressor WWOX repression in small-cell lung cancer cells. *Cancer Sci.* 2011; 102:983–990. [PubMed: 21276135]
43. Uhlen M, et al. Towards a knowledge-based Human Protein Atlas. *Nat Biotechnol.* 2010; 28:1248–1250. [PubMed: 21139605]
44. Weiss ST. Lung function and airway diseases. *Nat Genet.* 2010; 42:14–16. [PubMed: 20037613]
45. Elks CE, et al. Thirty new loci for age at menarche identified by a meta-analysis of genome-wide association studies. *Nat Genet.* 2010; 42:1077–1085. [PubMed: 21102462]
46. Lindgren CM, et al. Genome-wide association scan meta-analysis identifies three Loci influencing adiposity and fat distribution. *PLoS Genet.* 2009; 5:e1000508. [PubMed: 19557161]
47. Li Y, Willer CJ, Ding J, Scheet P, Abecasis GR. MaCH: using sequence and genotype data to estimate haplotypes and unobserved genotypes. *Genet Epidemiol.* 2010; 34:816–834. [PubMed: 21058334]
48. Marchini J, Howie B, Myers S, McVean G, Donnelly P. A new multipoint method for genome-wide association studies by imputation of genotypes. *Nat Genet.* 2007; 39:906–913. [PubMed: 17572673]

49. Browning BL, Browning SR. A unified approach to genotype imputation and haplotype-phase inference for large data sets of trios and unrelated individuals. *Am J Hum Genet.* 2009; 84:210–223. [PubMed: 19200528]
50. Browning SR, Browning BL. High-resolution detection of identity by descent in unrelated individuals. *Am J Hum Genet.* 2010; 86:526–539. [PubMed: 20303063]
51. Servin B, Stephens M. Imputation-based analysis of association studies: candidate regions and quantitative traits. *PLoS Genet.* 2007; 3:e114. [PubMed: 17676998]
52. Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics.* 2010; 26:2190–2191. [PubMed: 20616382]
53. R Development Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria: (R foundation for Statistical Computing; 2009.
54. Lettre G, et al. Genome-wide association study of coronary heart disease and its risk factors in 8,090 African Americans: the NHLBI CARE Project. *PLoS Genet.* 2011; 7:e1001300. [PubMed: 21347282]
55. Cheverud JM. A simple correction for multiple comparisons in interval mapping genome scans. *Heredity (Edinb).* 2001; 87:52–58. [PubMed: 11678987]
56. Hofman A, et al. The Rotterdam Study: 2012 objectives and design update. *Eur J Epidemiol.* 2011; 26:657–686. [PubMed: 21877163]
57. Westra HJ, et al. Systematic identification of trans eQTLs as putative drivers of known disease associations. *Nat Genet.* 2013; 45:1238–1243. [PubMed: 24013639]
58. Hodge E, et al. HTR4 gene structure and altered expression in the developing lung. *Respir Res.* 2013; 14:77. [PubMed: 23890215]
59. Melen E, et al. Expression analysis of asthma candidate genes during human and murine lung development. *Respir Res.* 2011; 12:86. [PubMed: 21699702]
60. Kho AT, et al. Transcriptomic analysis of human lung development. *Am J Respir Crit Care Med.* 2010; 181:54–63. [PubMed: 19815808]

**Figure 1. Study design**

Overview of our staged analysis to identify new variants influencing FVC. After a large-scale meta-analyzed GWAS of stage 1 cohorts (n = 52,253), we followed-up a total of 7 SNPs showing evidence of association with FVC ( $P < 5 \times 10^{-7}$ ) in stage 2. The studies included in stage 2, encompassing a total sample size of n = 32,917, undertook *in silico* testing of the 7 loci, which were not previously associated to any pulmonary phenotype. See Supplementary Tables 1 and 2 for definitions of all study abbreviations



**Figure 2. Manhattan plot**

Manhattan plot for the association results for FVC. The plot shows all the loci analyzed in stage 1, where the two loci previously associated with either FEV<sub>1</sub> or FEV<sub>1</sub>/FVC are indicated in grey. The previously unassociated loci were in or near the presented adjacent gene. The loci reaching genome-wide significance, after the combined analysis of stage 1 and stage 2, are marked with an asterisk.

Table 1

Main results from stage 1, stage 2 and the meta-analysis of stage 1 and 2 for loci associated with forced vital capacity

SNP ID	Chr.	NCBI 36 Position	Nearest gene	Coded Allele	Analysis Stage	$\beta$ (ml)	S.E. <sub>gc</sub>	P value	Coded Allele freq.	N effective
rs1430193	2	55974357	<i>EFEMP1</i> (intronic)	T	Stage 1	-23.75	4.022	$3.52 \times 10^{-9}$	0.370	45,852
					Stage 2	-17.839	4.500	<b><math>7.36 \times 10^{-5}</math></b>	0.361	28,103
					Joint meta-analysis	-21.125	2.999	<b><math>1.86 \times 10^{-12}</math></b>		
rs1942055	2	135215394	<i>TMEM163</i> (50 kb upstream)	G	Stage 1	-19.943	3.919	$3.60 \times 10^{-7}$	0.458	46,365
					Stage 2	-3.13	4.447	0.482	0.470	24,984
					Joint meta-analysis	-12.594	2.940	$1.84 \times 10^{-5}$		
rs6923462	6	7746111	<i>BMP6</i> (intronic)	T	Stage 1	28.828	5.208	$3.11 \times 10^{-8}$	0.843	48,680
					Stage 2	35.204	7.552	<b><math>3.14 \times 10^{-6}</math></b>	0.846	17,271
					Joint meta-analysis	30.883	4.288	<b><math>5.89 \times 10^{-13}</math></b>		
rs4237643	11	43604944	<i>HSD17B12</i> (54 kb upstream)	T	Stage 1	-21.366	3.957	$6.66 \times 10^{-8}$	0.311	51,977
					Stage 2	-10.073	4.686	0.032	0.305	30,119
					Joint meta-analysis	-16.666	3.023	<b><math>3.53 \times 10^{-8}</math></b>		
rs2863171	11	45207308	<i>PRDM11</i> (3 kb downstream)	C	Stage 1	25.343	5.015	$4.33 \times 10^{-7}$	0.158	51,758
					Stage 2	21.755	6.23	<b><math>4.79 \times 10^{-3}</math></b>	0.160	25,121
					Joint meta-analysis	23.924	3.906	<b><math>8.97 \times 10^{-10}</math></b>		
rs1079572	16	76744639	<i>WWOX</i> (intronic)	G	Stage 1	20.539	3.733	$3.76 \times 10^{-8}$	0.417	51,049
					Stage 2	10.41	4.364	0.017	0.419	28,103
					Joint meta-analysis	16.258	2.837	<b><math>9.95 \times 10^{-9}</math></b>		
rs6501431	17	66488010	<i>KCNJ2</i> (800 kb downstream)	T	Stage 1	26.729	4.751	$1.84 \times 10^{-8}$	0.798	47,576
					Stage 2	15.641	6.746	0.020	0.789	17,694
					Joint meta-analysis	23.053	3.884	<b><math>2.94 \times 10^{-9}</math></b>		

Shown are FVC results for the leading SNPs, ordered by chromosome and position for each independent locus associated ( $P < 5 \times 10^{-8}$ ) with FVC in a joint analysis of up to 85,170 individuals of European ancestry from the CHARGE-SpiroMeta GWAS (stage 1) and follow-up (stage 2). Two-sided P values are given for stage 1, stage 2 and the joint meta-analysis of all stages. P values reaching genome-wide significance ( $P < 5 \times 10^{-8}$ ) in the joint meta-analysis of all stages are indicated in bold. SNPs reaching independent replication in stage 2 ( $P = 0.05/7 = 7.14 \times 10^{-3}$ ) are indicated with their stage 2 P value in bold. The sample sizes (N) shown are the effective sample sizes. The effective sample size (N effective) is the product of sample size and the imputation quality summed across studies. The joint meta-analysis includes data from stage 1 and stage 2.  $\beta$  values reflect effect-size estimates in milliliters (ml).



Table 2

Expression profiling of candidate genes in the lung and periphery

Sentinel SNP (relationship to gene)	Chr.	Gene	Putative function of encoded protein	Tissue					
				Lung	HASM	HBEC	PBMC		
rs1430193 (intron)	2	<i>EFEMP1</i>	Binds EGFR, the EGF receptor, inducing EGFR autophosphorylation and the activation of downstream signaling pathways. May play a role in cell adhesion and migration. May function as a negative regulator of chondrocyte differentiation.	+	+	+	-		
rs6923462 (intron)	6	<i>BMP6</i>	The bone morphogenetic proteins (BMPs) are a family of secreted signaling molecules that can induce ectopic bone growth. Many BMPs are part of the transforming growth factor-beta (TGF $\beta$ ) superfamily.	+	+	+	+		
rs4237643 (intergenic)	11	<i>MIR129-2</i> ( <i>downstream</i> )/ <i>HSD17B12</i>	This gene encodes a very important 17beta-hydroxysteroid dehydrogenase (17 $\beta$ -HSD)	+	+	+	+		
rs2863171 (downstream)	11	<i>PRDM11</i>	PR domain-containing protein 11	+	+	+	+		
rs1079572 (intron)	16	<i>WWOX</i>	WW domain-containing proteins are found in all eukaryotes and play an important role in the regulation of a wide variety of cellular functions such as protein degradation, transcription, and RNA splicing. This gene encodes a protein which contains 2 WW domains and a short-chain dehydrogenase/reductase domain (SRD).	+	+	+	+		

Sentinel SNP (relationship to gene)	Chr.	Gene	Putative function of encoded protein	Tissue				
				Lung	HASM	HBEC	PBMC	
rs6501431 (downstream)	17	<i>KCNJ2</i>	The protein encoded by this gene is an integral membrane protein and inward-rectifier type potassium channel. The encoded protein, which has a greater tendency to allow potassium to flow into a cell rather than out of a cell, probably participates in establishing action potential waveform and excitability of neuronal and muscle tissues.	+	+	+	+	

+ indicates that the gene is expressed in the cell type used, and – indicates that we did not detect gene expression at the mRNA level following 70 cycles of PCR. Amplification was followed in real-time by gene specific TaqMan probes and final PCR products were visualized by gel electrophoresis. We used *GAPDH* (encoding glyceraldehyde-3-phosphate dehydrogenase) as a positive control for the complementary DNA, and this gene was expressed in all tissues. Chr., chromosome; HASM, human airway smooth muscle; HBEC, human bronchial epithelial cells; PBMC, peripheral blood mononuclear cells.