Large-Scale Genome-Wide Association Studies and Meta-Analyses of Longitudinal Change in Adult Lung Function

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Accessibility
Large-Scale Genome-Wide Association Studies and Meta-Analyses of Longitudinal Change in Adult Lung Function

Wenbo Tang1, Matthew Kowgier2, Daan W. Loth3,4, Maria Soler Artigas5,6, Bonnie R. Joubert7, Emily Hodge8, Sina A. Gharib9, Albert V. Smith10,11, Ing Ruczinski12, Vilmundur Gudnason10,11, Rasika A. Mathias13, Tamara B. Harris14, Nadia N. Hansel13, Lenore J. Launer14, Kathleen C. Barnes13, Joyanna G. Hansen1, Eva Albrecht15, Melissa C. Aldrich16, Michael Allerhand17, R. Graham Barr18,19, Guy G. Brusselle20,21,22, David J. Couper, Ivan Curjuric23,24, Gail Davies17,25,26, Ian J. Deary17,26, Josép Dupuis27,28, Tove Fall29, Millennia Foy30, Nora Franceschini31, Wei Gao32, Sven Gläser32, Xiangjun Gu30, Dana B. Hancock7,33, Joachim Heinrich34, Albert Hofman3,35, Medea Imboden23,24, Erik Ingelsson29,36, Alan James37, Stefan Karrasch38,39,40, Beate Koch32, Stephen B. Kritchovsky41, Ashish Kumar23,24,36, Lies Lahousse3,20, Guo Li42, Lars Lind43, Cecilia Lindgren36,44, Yongmei Liu45, Kurt Lohman46, Thomas Lumley47, Wendy L. McArindle48, Bernd Meibohm49, Andrew P. Morris36, Alanna C. Morrison50,51, Bill Musk37, Kari E. North31, Lyle J. Palmer2,51,52, Nicole M. Probst-Hensch33,24, Bruce M. Psaty42,53,54,55, Fernando Rivadeneira35,56, Jerome I. Rotter57, Holger Schulz34, Lewis J. Smith38, Akshay Sood39, John M. Starr17,60, David P. Strachan61, Alexander Teumer52, André G. Uitterlinden35,56, Henry Völzke63, Arend Voorman64, Louise V. Wain5,6, Martin T. Wells65, Jemma B. Wilk38,66, O. Dale Williams67, Susan R. Heckbert42,53,54, Bruno H. Stricker3,4, Stephanie J. Lodon7, Myriam Fornage3,4, Martin D. Tobin5,6, George T. O’Connor28,68, Ian P. Hall9, Patricia A. Cassano1,69,†

1 Division of Nutritional Sciences, Cornell University, Ithaca, New York, United States of America, 2 Ontario Institute for Cancer Research and Biostatistics Division, Dalla Lana School of Public Health, University of Toronto, Toronto, Ontario, Canada, 3 Department of Epidemiology, Erasmus Medical Center, Rotterdam, the Netherlands, 4 Netherlands Healthcare Inspectorate, The Hague, the Netherlands, 5 University of Leicester, Genetic Epidemiology Group, Department of Health Sciences, Leicester, United Kingdom, 6 National Institute for Health Research (NIHR) Leicester Respiratory Biomedical Research Unit, Glenfield Hospital, Leicester, United Kingdom, 7 Epidemiology Branch, National Institute of Environmental Health Sciences, National Institutes of Health, U.S. Department of Health and Human Services, Research Triangle Park, North Carolina, United States of America, 8 Division of Respiratory Medicine, University of Nottingham, Nottingham, United Kingdom, 9 Computational Medicine Core, Center for Lung Biology, Division of Pulmonary & Critical Care Medicine, Department of Medicine, University of Washington, Seattle, Washington, United States of America, 10 Icelandic Heart Association, Kopavogur, Iceland, 11 University of Iceland, Reykjavik, Iceland, 12 Department of Biostatistics, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland, United States of America, 13 Department of Medicine, School of Medicine, Johns Hopkins University, Baltimore, Maryland, United States of America, 14 Laboratory of Lung Biology, National Institutes on Aging, National Institutes of Health, Bethesda, Maryland, United States of America, 15 Institute of Genetic Epidemiology, Helmholtz Zentrum München - German Research Center for Environmental Health, Neuherberg, Germany, 16 Department of Thoracic Surgery and Division of Epidemiology, Vanderbilt University Medical Center, Nashville, Tennessee, United States of America, 17 Centre for Cognitive Ageing and Cognitive Epidemiology, University of Edinburgh, Edinburgh, United Kingdom, 18 Division of General Medicine, Pulmonary, Allergy and Critical Care, Department of Medicine, College of Physicians and Surgeons, Columbia University, New York, New York, United States of America, 19 Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, New York, United States of America, 20 Department of Respiratory Medicine, Ghent University Hospital, Ghent, Belgium, 21 Department of Respiratory Medicine, Erasmus Medical Center, Rotterdam, the Netherlands, 22 Department of Biostatistics, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States of America, 23 Swiss Tropical and Public Health Institute, Basel, Switzerland, 24 University of Basel, Basel, Switzerland, 25 Medical Genetics Section, University of Edinburgh Molecular Medicine Centre and MRC Institute of Genetics and Molecular Medicine, Western General Hospital, Edinburgh, United Kingdom, 26 Department of Psychology, University of Edinburgh, Edinburgh, United Kingdom, 27 Biostatistics Department, Boston University School of Public Health, Boston, Massachusetts, United States of America, 28 The National Heart, Lung, and Blood Institute’s Framingham Heart Study, Framingham, Massachusetts, United States of America, 29 Department of Medical Sciences, Molecular Epidemiology and Science for Life Laboratory, Uppsala University, Uppsala, Sweden, 30 Institute of Molecular Medicine, University of Texas Health Science Center at Houston, Houston, Texas, United States of America, 31 Gillings School of Global Public Health, Department of Epidemiology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States of America, 32 Department of Internal Medicine B; Pneumology, Cardiology, Intensive Care Medicine; Field of Research: Pneumology and Pneumological Epidemiology, University Medicine Greifswald, Greifswald, Germany, 33 Behavioral Health Epidemiology Program, Research Triangle Institute, Research Triangle Park, North Carolina, United States of America, 34 Institute of Epidemiology I, Helmholtz Zentrum München - German Research Center for Environmental Health, Neuherberg, Germany, 35 Institute for Epidemiology I, Helmholtz Zentrum München - German Research Center for Environmental Health, Neuherberg, Germany, 36 Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK and Department of Biostatistics, University of Liverpool, Liverpool, United Kingdom, 37 School of Medicine and Pharmacology, University of Western Australia, Perth, Western Australia, 38 Institute and Outpatient Clinic for Occupational, Social and Environmental Medicine, Ludwig-Maximilians-Universität, Munich, Germany, 39 Institute of General Practice, University Hospital Klinikum rechts der Isar, Technische Universität München, Munich, Germany, 40 Institute of Epidemiology I, Helmholtz Zentrum München - German Research Center for Environmental Health, Neuherberg, Germany, 41 Sticht Center on Aging, Wake Forest School of Medicine, Winston-Salem, North Carolina, United States of America, 42 Cardiovascular Health Research Unit, University of Washington, Seattle, Washington, United States of America, 43 Department of Medical Sciences, Uppsala University, Uppsala, Sweden, 44 Broad Institute of MIT and Harvard, Cambridge, Massachusetts, United States of America, 45 Department of Epidemiology and Prevention, Division of Public Health Sciences, Wake Forest School of Medicine, Winston-Salem, North Carolina, United States of America, 46 Department of Biostatistical Sciences, Division of Public Health Sciences, Wake Forest School of Medicine, Winston-Salem, North Carolina, United States of America, 47 Department of Statistics, University of Auckland, Auckland, New Zealand, 48 School of Social and Community Medicine, University of Bristol, Bristol, United Kingdom, 49 College of Pharmacy, University of Tennessee Health Science Center, Memphis, Tennessee, United States of America, 50 Human Genetics Center, School of Public Health, University of Texas Health Science Center, Houston, Texas, United States of America, 51 Department of Psychology, University of Edinburgh, Edinburgh, United Kingdom, 52 Department of Biostatistics, University of Liverpool, Liverpool, United Kingdom, 53 Department of Epidemiology and Health Services Research, University of Oxford, Oxford, UK, 54 Department of Biostatistics, University of Liverpool, Liverpool, United Kingdom, 55 Department of Health Sciences, University of Liverpool, Liverpool, United Kingdom, 56 Department of Biostatistics, University of Liverpool, Liverpool, United Kingdom.
Abstract

**Background:** Genome-wide association studies (GWAS) have identified numerous loci influencing cross-sectional lung function, but less is known about genes influencing longitudinal change in lung function.

**Methods:** We performed GWAS of the rate of change in forced expiratory volume in the first second (FEV1) in 14 longitudinal, population-based cohort studies comprising 27,249 adults of European ancestry using linear mixed effects model and combined cohort-specific results using fixed effect meta-analysis to identify novel genetic loci associated with longitudinal change in lung function. Gene expression analyses were subsequently performed for identified genetic loci. As a secondary aim, we estimated the mean rate of decline in FEV1 by smoking pattern, irrespective of genotypes, across these 14 studies using meta-analysis.

**Results:** The overall meta-analysis produced suggestive evidence for association at the novel IL16/STARD5/TMC3 locus on chromosome 15 ($P = 5.71 \times 10^{-7}$). In addition, meta-analysis using the five cohorts with ≥3 FEV1 measurements per participant identified the novel ME3 locus on chromosome 11 ($P = 2.18 \times 10^{-8}$) at genome-wide significance. Neither locus was associated with FEV1 decline in two additional cohort studies. We confirmed gene expression of IL16, STARD5, and ME3 in multiple lung tissues. Publicly available microarray data confirmed differential expression of all three genes in lung samples from COPD patients compared with controls. Irrespective of genotypes, the combined estimate for FEV1 decline was 26.9, 29.2 and 35.7 mL/year in never, former, and persistent smokers, respectively.

**Conclusions:** In this large-scale GWAS, we identified two novel genetic loci in association with the rate of change in FEV1 that harbor candidate genes with biologically plausible functional links to lung function.

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GWAS of Longitudinal Change in Adult Lung Function

Introduction
Forced expiratory volume in the first second (FEV₁) is a reliable spirometric parameter that reflects the physiological state of the lungs and airways. Reduced FEV₁ is relative to forced vital capacity (FVC), indicating a diagnosis of chronic obstructive pulmonary disease (COPD), a leading cause of death globally.[1] FEV₁ is also used as a measure of morbidity and mortality in the general population.[2,3] Lung function reaches its peak in early adulthood, followed by a plateau, and then subsequently declines. As first reported by Fletcher and Peto,[4] decline in lung function is accelerated in smokers, leading to increased risks of COPD and premature death. While cigarette smoking is a key risk factor for accelerated loss of lung function, genetic variation is hypothesized to also play an important role.[5,6] Family and twin studies of the longitudinal change in lung function report heritability estimates between 10 and 39%.[7,8]

Recent large-scale genome-wide association studies (GWAS) identified 26 novel loci for cross-sectional lung function,[9-11] demonstrating the power of GWAS with large sample size to identify common genetic variants with modest effect sizes. However, cross-sectional measurements in adults reflect the combination of maximal attained lung growth and subsequent decline. GWAS that specifically study the longitudinal change in lung function are needed to distinguish the genetic contributions to age-related decline. To date, only one population-based GWAS meta-analysis of longitudinal change in lung function has been reported.[12] Separate analyses were conducted in 1,441 asthmatic and 2,667 non-asthmatic participants; association was found at one novel locus in each analysis, though only the locus in non-asthmatic participants; association was found.[12] FEV₁ measurements meeting acceptability criteria were included in the current study.

Study populations
All 14 cohort studies are members of the CHARge or SpiroMeta Consortium (Table 1). The respective local Institutional Review Boards approved all study protocols, and written informed consent for genetic studies was obtained from all participants. Spirometry tests were performed at baseline and at least one follow-up time point by trained technicians and in accordance with the American Thoracic Society or European Respiratory Society recommendations (Methods S1 in File S1 for further details).[13] FEV₁ measurements meeting acceptability criteria were included in the current study.

Statistical analysis
For the analysis of repeated measurement data such as longitudinal change in lung function, mixed effects models offer more flexibility and statistical power than alternative approaches; the model allows for the use of unbalanced data and does not exclude individuals with incomplete records. Each cohort study performed the GWAS using a linear mixed effects model. The model included a random intercept and a random slope, and fixed effects for time (a continuous variable quantifying the time distance between each FEV₁ measurement and baseline), SNP and its interaction with time (SNP-by-time), baseline age, gender, standing height, smoking pattern during follow-up and its interaction with time (smoking-by-time), baseline smoking pack-years, study site, and principal components for genetic ancestry (as needed). Cohort-specific results for the SNP-by-time interaction term, which estimates the effect of genotype on the rate of change in FEV₁, were shared, and two meta-analyses, one using all 14 studies and the other using the five studies with ≥3 FEV₁ expression studies, and expression quantitative trait loci (eQTL) look-up.

Methods

Competing Interests: All authors have read the journal’s policy, and 57 of the 81 authors have declared that no competing interests exist. The following 24 authors have possible conflicts, as follows: Dr. Aldrich reports grants from NIH, during the conduct of the study; Dr. Barnes reports grants from NIH, during the conduct of the study; Dr. Barr reports grants from NIH and US—EPA, during the conduct of the study; Dr. Couper reports grants from NIH, during the conduct of the study; Dr. Deary reports grants from Age UK, grants from BBSRC, during the conduct of the study; Dr. Dupuis reports grants from Boston University, during the conduct of the study; Dr. Fall reports personal fees from MSD (Merck), outside the submitted work; Dr. Gudnason reports other from NIH, during the conduct of the study; Dr. Glaser reports grants from BMBF (German Ministry for Research and Education), during the conduct of the study; personal fees from Actelion Pharma, personal fees from Novartis Pharma, personal fees from GSK, personal fees from Pfizer, personal fees from Boehringer Ingelheim, personal fees from Bayer Pharma, all those apply outside of the submitted work; Dr. Hall reports grants received from MRC and Pfizer, and Vertex sponsored lecture at ERS, outside the submitted work; Dr. Hodge reports grants from The Medical Research Council, UK, during the conduct of the study; Dr. Koch reports grants from BMBF, during the conduct of the study, travel fees from Actelion Pharma, Pfizer, Bayer Pharma, the German Academic Exchange Service, and the Research Network for Community Medicine of the University of Greifswald, one research prize of the Society of Internal Medicine Mecklenburg-Vorpommern, Germany, outside the submitted work; Dr. Lahousse reports grant from Belgian Society of Pneumology, during the conduct of the study; Dr. London is funded in full by the Division of Intramural Research, NIEHS, NIH, DHHG; Dr. Lumley reports grants from NIH, during the conduct of the study; Dr. Mathias reports grants from NIH, during the conduct of the study; Dr. Melbohm reports grants from NIH, during the conduct of the study; Dr. O’Connor reports personal fees from Sunovion, Inc., outside the submitted work; Dr. Psaty reports grants from NIH, during the conduct of the study, and Dr. Psaty serves on the DSMB for a clinical trial of a device, which is funded by the manufacturer (Zoll LifeCor), and he is on the Steering Committee of the Yale Open Access Project funded by Johnson & Johnson; Dr. L Smith reports personal fees as member of Merck Data Safety and Monitoring Board, outside the submitted work; Dr. Tobin reports grants from Medical Research Council grant G09023313, grants from National Institute for Health Research (NIHR), Leicester Respiratory Biomedical Research Unit, during the conduct of the study (the views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health), grants from Pfizer for collaborative research project (on rare sequence variants and the smoking resistant lung, Nov. 2010 to Nov 2012), outside the submitted work; Dr. Wain reports grants from Pfizer collaborative research project (onrare sequence variants and the smoking resistant lung, Nov. 2010 to Nov 2012), outside the submitted work; Dr. Wilk reports grants from FAMRI, grants from NIH, during the conduct of the study; personal fees from Pfizer, outside the submitted work. This does not alter the authors’ adherence to all PLOS ONE policies on sharing data and materials.

* Email: pac6@cornell.edu
† These authors are joint first authors on this work.
‡ These authors are joint last authors on this work.
### Table 1. Baseline characteristics of cohort studies included in the meta-analysis.

| Cohort:       | ARIC     | BS8C     | BHS      | CARDIA   | CHS      | FHS      | Health ABC |
|---------------|----------|----------|----------|----------|----------|----------|------------|
| No. of participants | 8,242    | 827      | 1,009    | 1,492    | 3,159    | 3,230    | 1,586      |
| No. of FEV₁ measurements | 15,582   | 1,653    | 3,073    | 6,140    | 1,174    | 11,275   | 4,426      |
| No. of FEV₁ per person | 227.5     | 35.9     | 43.5     | 21.3     | 39.2     | 246.2    | 6.4        |
| Follow-up duration, yr | 5.6       | 10       | 29       | 7.6      | 3.3      | 5        | 4          |
| Males, %     | 46.5     | 48.6     | 41.6     | 46.9     | 46.9     | 47       | 52.7       |
| Baseline age, yr | 54.6 (5.7)| 35.0 (0.2)| 37.5 (12.8)| 27.5 (2.3)| 72.5 (5.4)| 50.9 (10.3)| 73.8 (2.8) |
| Baseline height, cm | 168.7 (9.4)| 170.1 (9.5)| 168.1 (8.9)| 171.2 (9.3)| 164.6 (9.4)| 168.4 (9.3)| 166.8 (9.3) |
| Current smokers, % | 20.2      | 27.1     | 20.9     | 24.8     | 10.8     | 246.2    | 6.4        |
| Former smokers, % | 32.6      | 41.5     | 16.5     | 35.7     | 39.8     | 49.9     | 49.9       |
| Baseline FEV₁, mL | 2972 (758) | 3631 (744) | 3230 (927)| 3818 (781)| 2123 (652)| 2989 (806)| 2308 (649) |
| Baseline FEV₁/FVC, % | 74.1 (7.1) | 80.6 (5.8) | 78.2 (9.2) | 81.6 (6.5) | 70.5 (10.5) | 75.7 (8.0) | 74.7 (7.8) |

| Cohort:       | KORA     | LBC1921  | LBC1936  | PIVUS    | RS       | SAPALDIA | SHIP       |
|---------------|----------|----------|----------|----------|----------|----------|------------|
| No. of participants | 890      | 512      | 1,002    | 818      | 1,321    | 1,401    | 1,760      |
| No. of FEV₁ measurements | 1,597     | 706      | 1,790    | 1,469    | 2,016    | 2,692    | 2,571      |
| No. of FEV₁ per person | 2        | 2        | 2        | 2        | 2        | 2        | 2          |
| Follow-up duration, yr | 3.2       | 8.9      | 4.8      | 5.8      | 8.3      | 10.9     | 7.9        |
| Males, %     | 47.2     | 41.4     | 50.8     | 49.9     | 45.1     | 48       | 49.4       |
| Baseline age, yr | 53.8 (4.5)| 79.1 (0.6)| 69.6 (0.8)| 70.2 (0.2)| 74.4 (5.6)| 41.1 (11.2)| 52.4 (13.8) |
| Baseline height, cm | 169.3 (9.3)| 163.2 (9.4)| 166.5 (8.9)| 169.0 (9.3)| 167.3 (9.1)| 169.4 (9.1)| 169.3 (9.7) |
| Current smokers, % | 20.5      | 7.0      | 12.9     | 10.2     | 11.1     | 26.9     | 32.8       |
| Former smokers, % | 40.9      | 50.4     | 42.6     | 39.6     | 56.7     | 25.8     | 23.8       |
| Baseline pack-years | 11.2 (17.1)| 15.3 (22.3)| 16.9 (25.8)| 14.3 (15.8)| 25.7 (21.3)| 17.4 (18.0)| 11.3 (11.9) |
| Baseline FEV₁, mL | 2880 (792) | 1887 (625) | 2371 (687) | 2452 (682) | 2215 (652) | 3516 (861) | 3238 (876) |
| Baseline FEV₁/FVC, % | 77.5 (6.2) | 79.0 (11.8)| 78.3 (10.2)| 76.0 (10.8)| 74.8 (7.9)| 78.5 (8.2)| 83.1 (6.6) |

**Definition of abbreviations:** ARIC = Atherosclerosis Risk in Communities; BS8C = British 1958 Birth Cohort; BHS = Busselton Health Study; CARDIA = Coronary Artery Risk Development in Young Adults; CHS = Cardiovascular Health Study; FHS = Framingham Heart Study; Health ABC = Health, Aging, and Body Composition; KORA = Cooperative Health Research in the Region of Augsburg; LBC1921 = Lothian Birth Cohort 1921; LBC1936 = Lothian Birth Cohort 1936; PIVUS = Prospective Investigation of the Vasculature in Uppsala Seniors; RS = Rotterdam Study; SAPALDIA = Swiss Study on Air Pollution and Lung Diseases in Adults; SD = standard deviation; SHIP = Study of Health in Pomerania.

*Data are presented as mean (SD) unless otherwise indicated; total no. participants = 27,249, total no. FEV₁ measurements = 62,130.*

*Pack-years are calculated among current and former smokers at study baseline.*

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Table 2. Model estimates for the rate of change in FEV$_1$ in never smokers and effects of other smoking patterns (compared with never smokers) on the rate of change in FEV$_1$ (mL/year).*

| Study          | Annual FEV$_1$ change in never smokers | Additional Effect$^1$ of smoking patterns on annual FEV$_1$ change |
|----------------|----------------------------------------|---------------------------------------------------------------|
|                | (referent group)                        | Persistent smokers                                           |
|                |                                        | Intermittent smokers                                         |
|                |                                        | Former smokers                                               |
|                | $\beta$ | SE  | $\beta$ | SE  | $\beta$ | SE  | $\beta$ | SE  | $\beta$ | SE  |
| ARIC           | −14.0  | 1.3 | −12.4  | 1.7 | −5.5   | 2.1 | −5.3   | 1.4 |
| BSBC           | −29.6  | 1.5 | −9.4   | 2.8 | −2.2   | 3.4 | −3.0   | 3.0 |
| BHS            | −23.0  | 1.0 | −20.0  | 3.0 | −8.0   | 2.0 | −9.0   | 2.0 |
| CARDIA         | −26.4  | 0.5 | −6.7   | 1.3 | −0.2   | 1.0 | −1.0   | 1.2 |
| CHS            | −35.0  | 1.1 | −2.2   | 3.3 | −4.6   | 2.2 | −2.4   | 1.7 |
| FHS            | −26.0  | 0.6 | −8.1   | 1.3 | −2.9   | 1.0 | −1.1   | 0.8 |
| Health ABC     | −39.7  | 1.3 | −12.9  | 6.1 | −6.8   | 4.4 | −2.6   | 1.7 |
| KORA           | −22.1  | 3.7 | −1.1   | 7.2 | −10.4  | 9.3 | −18.8  | 4.9 |
| LBC1921        | −10.0  | 3.6 | −11.6  | 15.7| −2.8   | 14.4| −18.8  | 4.9 |
| LBC1936        | −32.3  | 3.6 | −19.0  | 9.9 | −40.1  | 16.8| −43.3  | 5.3 |
| PIVUS          | −21.1  | 2.5 | −15.9  | 8.2 | −21.7  | 13.4| −3.9   | 3.9 |
| RS             | −27.5  | 3.7 | −1.8   | 9.0 | −9.3   | 8.6 | −4.6   | 4.5 |
| SAPALDIA       | −29.7  | 1.2 | −7.4   | 2.3 | −2.0   | 2.6 | −2.8   | 2.1 |
| SHIP           | −31.8  | 2.8 | −0.4   | 10.9| −0.1   | 3.9 | −15.0  | 7.3 |
| 14-cohort meta- | −26.9  | 0.3 | −8.8   | 0.7 | −2.6   | 0.6 | −2.3   | 0.5 |

Definition of abbreviations: ARIC = Atherosclerosis Risk in Communities; BSBC = British 1958 Birth Cohort; BHS = Busselton Health Study; CARDIA = Coronary Artery Risk Development in Young Adults; CHS = Cardiovascular Health Study; FHS = Framingham Heart Study; Health ABC = Health, Aging, and Body Composition; KORA = Cooperative Health Research in the Region of Augsburg; LBC1921 = Lothian Birth Cohort 1921; LBC1936 = Lothian Birth Cohort 1936; PIVUS = Prospective Investigation of the Vasculature in Uppsala Seniors; RS = Rotterdam Study; SAPALDIA = Swiss Study on Air Pollution and Lung Diseases in Adults; SE = standard error; SHIP = Study of Health in Pomerania.

$^1$Data shown are the effect estimates ($\beta$ and SE) of the time and smoking-by-time interaction terms in the preliminary mixed effects model fully adjusted for all specified variables except the SNP terms. Time represents the rate of change in FEV$_1$ in never smokers and the smoking-by-time interaction term represents the effects of the other three smoking patterns on the rate of change in FEV$_1$, compared with never smokers. Smoking categories are defined as persistent (smoke throughout follow-up), intermittent (stop and/or start smoking during follow-up) and former (smoke only prior to start of follow-up).

Effect estimates in smoking categories are added to estimates in never smokers to compute the actual rate of change in each group (for example, in ARIC, the point estimate of the rate of change in FEV$_1$ in persistent smokers was $−14.0 − 12.4 = −26.4$ mL/year).

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Table 3. Association of the most statistically significant SNPs with the rate of change in FEV₁ (mL/year) in the meta-analysis of 14 cohort studies (n = 27,249)*

| SNP            | Chr Position | Closest Gene(s) | Coded Allele | Frequency | β   | SE  | P Value | P Value (after genomic control) |
|----------------|--------------|-----------------|--------------|-----------|-----|-----|---------|--------------------------------|
| rs12137475     | 1            | ST3GAL3         | T            | 0.11      | −3.5| 0.8 | 3.90 × 10⁻⁶ | 6.50 × 10⁻⁶ |
| rs766488       | 1            | NFIA            | A            | 0.31      | 1.4 | 0.3 | 6.60 × 10⁻⁶ | 2.60 × 10⁻⁶ |
| rs17698444     | 1            | ESRRG/GPATCH2   | C            | 0.89      | −2.2| 0.5 | 2.00 × 10⁻⁶ | 3.90 × 10⁻⁶ |
| rs12692550     | 2            | TMCO3           | T            | 0.17      | −1.7| 0.4 | 5.16 × 10⁻⁶ | 5.16 × 10⁻⁶ |
| rs2260722      | 1             |                | A            | 0.72      | 0.7 | 0.3 | 1.88 × 10⁻⁶ | 1.88 × 10⁻⁶ |
| rs8027498      | 15           | IL16/STARD5/TMC3| C            | 0.10      | 2.3 | 0.5 | 5.71 × 10⁻⁷ | 9.41 × 10⁻⁷ |
| rs4077833      | 15           | SV2B            | C            | 0.85      | 1.7 | 0.3 | 3.59 × 10⁻⁶ | 3.59 × 10⁻⁶ |

*Data reported are the meta-analysis results of the SNP-by-time interaction term from the GWAS mixed effects model. A positive β-coefficient indicates an attenuation of FEV₁ decline and a negative β FEV₁ decline.

Definition of abbreviations: Chr = chromosome; SE = standard error; SNP = single-nucleotide polymorphism.

We sought corroborative evidence for SNPs with \( P < 1 \times 10^{-5} \) in the AGES-Reykjavik cohort study \( n = 1,494 \), and in LHS \( n = 4,048 \), a clinical cohort study of smokers with mild COPD, in which a longitudinal GWAS was recently reported.[13]

Gene expression analyses

Expression profiles of genes at the novel loci were evaluated in human lung tissues and primary cell samples using RT-PCR (Table S7 in File S1). Using publicly available data from the Lung Genomics Research Consortium (LGRC), expression profiles of these genes were compared in lung specimens of 219 COPD patients and 137 controls, and sentinel (most associated) SNPs at the novel loci were also searched against an eQTL database of lymphoblastoid cell lines.[16]

This manuscript follows the PRISMA statement and a checklist is available online (Checklist S1).

Results

Population characteristics

The majority of the 14 cohort studies had FEV₁ at two times, but five studies (BHS, CARDIA, CHS, FHS, Health ABC) had ≥ 3 FEV₁ measurements per participant. The maximum length of follow-up ranged from 4 to 29 years. Studies with older participants generally had fewer current smokers and more former smokers, and had lower mean baseline FEV₁.

Smoking patterns and rate of decline in FEV₁

All 14 studies implemented a preliminary mixed model adjusted for all specified variables except the SNP terms and reported the estimated rate of change in FEV₁ by smoking pattern (Table 2). The rate of decline in FEV₁ in never smokers ranged from 10.0 to 39.7 mL/year, and was generally steeper in studies with older participants, as expected.[4] Across all 14 studies, the meta-analyzed rate of change in FEV₁ was a decline of 26.9 ± 0.3 mL/year in never smokers, and was 8.8 ± 0.7, 2.6 ± 0.6, and 2.3 ± 0.5 mL/year steeper in persistent, intermittent, and former smokers, respectively (Table 2). We repeated the meta-analyses in the five cohort studies with ≥ 5 FEV₁ measurements per participant, and found similar, although less statistically significant results.

Discovery meta-analyses

Study-specific genomic inflation factors (\( \hat{\lambda}_{gc} \)) were calculated for the SNP-by-time interaction term and used for study-level genomic control prior to the meta-analyses. Study-specific \( \hat{\lambda}_{gc} \) values ranged from 0.96 to 1.11 (Table S1 in File S1) and the meta-analysis \( \hat{\lambda}_{gc} \) was 1.01 for both the 14-study and five-study meta-analyses. Figures S1 and S2 in File S1 present the Manhattan and quantile-quantile (QQ) plots.

In the meta-analysis including all 14 cohort studies, 15 SNPs at nine independent loci were associated with the rate of change in FEV₁ at \( P < 1 \times 10^{-5} \), and none reached the genome-wide significance threshold of \( P < 5 \times 10^{-8} \). The association results for the sentinel SNPs at these nine loci are presented in Table 3, and more detailed results for all 15 SNPs are included in Table S2 in File S1. The most statistically significant association, and the only one that reached \( P < 1 \times 10^{-10} \), was for rs4077833, an intronic SNP located in the novel IL16/STARD5/TMC3 gene region on chromosome 15 (\( P = 5.71 \times 10^{-7} \); Figures 1A and 1B). The C allele of rs4077833, with a frequency of 10%, was associated with
an attenuation of the rate of decline in FEV1 by 2.3 mL/year in comparison to the G allele. For estimation of longitudinal trajectory in lung function, having more than two measurements over time provides greater precision.[4] We performed a further meta-analysis with the five cohort studies (BHS, CHS, CARDIA, FHS, Health ABC) having ≥3 FEV1 measurements per participant, with a combined sample size of 10,476 participants and 32,054 FEV1 measurements (Methods S1 in File S1 for further details). A novel region on chromosome 11 had a genome-wide significant association (P < 5 × 10⁻⁸) with the rate of change in FEV1 (Table 4). The most statistically significant finding at this locus was for rs507211, an intronic SNP located in ME3 (Figures 2A and 2B). Six other SNPs, which are in linkage disequilibrium (LD) with rs507211 and are located in ME3, were identified at P < 1 × 10⁻⁶ (Table S3 in File S1). The rs507211 A allele, with a frequency of 25%, was associated with an attenuation of the rate of decline in FEV1 by 2.09 mL/year in comparison to the G allele (P = 2.18 × 10⁻⁶). Besides the ME3 locus, 17 SNPs from four other chromosomal regions had P values between 5 × 10⁻⁸ and 1 × 10⁻⁵ for associations with the rate of change in FEV1 (Tables 4 and Table S3 in File S1).

Additional analyses

Corroborative evidence was sought for the sentinel SNP at each of the 14 loci associated at P < 1 × 10⁻⁵ (from both the 14-study and five-study meta-analyses) in 1,494 adults from the AGES-Reykjavik population-based cohort study (Table S4 in File S1). A P value of 0.004, representing the Bonferroni correction for 14 tests at the α = 0.05 level, was selected as a priori as the threshold for statistical significance. No SNPs achieved this threshold. The lowest P value was for rs740577 in CACNG4 (P = 0.08), which showed consistent effect direction and magnitude with the original meta-analysis.

These same 14 SNPs were further examined in LHS, a clinical cohort study of 4,048 smokers with mild COPD for evidence of consistent association between healthy and diseased individuals.[17] None of the 14 SNPs were associated with the rate of change in FEV1 in LHS at P < 0.004 (Table S4 in File S1).

Previous meta-analyses in the CHARGE and SpiroMeta consortia identified 26 novel loci associated with cross-sectional lung function change over time, we performed a large-scale GWAS of the rate of change in FEV1 in 27,249 participants from 14 population-based cohort studies. We identified a novel locus (IL16/STARD5/TMC3) on chromosome 15 with suggestive evidence for association with the rate of change in FEV1. Given the greater precision to estimate longitudinal trends with more measurements, a meta-analysis of the five cohort studies with ≥3 FEV1 measurements per participant was performed, and it identified a second novel locus (ME3) on chromosome 11 at genome-wide statistical significance. For both loci, the minor allele was protective, and the magnitude of the association with the rate of change in FEV1 was similar to that of being an intermittent or former smoker versus a never-smoker.

The sentinel SNP at the novel chromosome 15 locus is located in TMC3, although two neighboring genes, IL16 and STARD5 both harbor SNPs that are in modest LD with the sentinel SNP (Figure 1A). TMC3, a member of the transmembrane channel-like gene family, likely functions as an ion channel, transporter, or modifier,[19] and has been associated with deafness and skin cancer.[20,21] IL16 is a pleiotropic immunomodulatory cytokine that acts as a chemoattractant for CD4⁺ cells and contributes to their recruitment and activation in response to inflammation.[22] Notably, asthma was the first disease where increased IL16 expression was observed.[23] Subsequent studies confirmed that in the non-diseased state IL16 is almost exclusively expressed by T lymphocytes in lymphatic tissue, whereas in asthmatic patients IL16 is also synthesized by airway epithelial cells to inhibit airway inflammation.[24-26] A promoter polymorphism (T-295C) in IL16 was associated with asthma in a Caucasian population in England,[27] although this finding was not confirmed in an Australian study.[28] STARD5 belongs to the steroidogenic acute gene expression analyses

Three genes (IL16, STARD5, and TMC3) at the novel chromosome 15 locus and ME3 at the novel chromosome 11 locus were selected for follow-up mRNA expression profiling in human lung tissue, and primary cultures of human bronchial epithelial and airway smooth muscle cells, together with control tissues (peripheral blood mononuclear cells and brain). Transcripts of STARD5 and ME3 were found in all lung-derived tissues, transcripts of IL16 were found in lung tissue and smooth muscle cells, but not in epithelial cells, and TMC3 was not expressed in any of the lung-derived tissues (Table S6 in File S1).

We performed additional follow-up analysis of the sentinel SNPs at the two novel loci using an eQTL database of lymphoblastoid cell lines (Table S8 in File S1). Trans-eQTL associations were observed between rs4077833 at the IL16/STARD5/TMC3 locus and a nuclear receptor, NR1I2 (chromosome 3; P = 6.84 × 10⁻⁵) and between rs507211 at the ME3 locus and KIAA11109 (chromosome 4; P = 5.20 × 10⁻⁴), which is part of a gene cluster (KIAA11109-TENR-IL2-IL21) that encodes two interleukins (IL2 and IL21).[18]
Table 4. Association of the most statistically significant SNPs with the rate of change in FEV1 (mL/year) in the meta-analysis of the five cohort studies with ≥3 FEV1 measurements per participant (n = 10,476).

| SNP       | Chr | Position  | Closest Gene(s)       | Coded Allele | Frequency | SE   | P Value |
|-----------|-----|-----------|-----------------------|--------------|-----------|------|---------|
| rs10209501| 6   | 2         | FOSL2/PLB1             | A            | 0.33      | 1.6  | 7.09 × 10⁻⁶ |
| rs10764053| 6   | 5         | BAZ2B                  | T            | 0.18      | 2.0  | 2.02 × 10⁻⁶ |
| rs1729588 | 6   | 2         | FLJ25363/MIR4445       | A            | 0.30      | 1.6  | 8.38 × 10⁻⁶ |
| rs11298688| 6   | 5         | C10orf112              | A            | 0.37      | 1.5  | 4.15 × 10⁻⁶ |
| rs507211  | 6   | 11        | ME3                    | A            | 0.25      | 2.1  | 2.18 × 10⁻⁶ |

* SE = standard error; SNP = single-nucleotide polymorphism.

Data reported are the meta-analysis results of the SNP-by-time interaction term from the GWAS mixed effects model. A positive β-coefficient indicates an attenuation of FEV1 decline and a negative β-coefficient an acceleration of FEV1 decline.

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There is phenotypic variation among the 14 cohort studies in aspects such as baseline age and cigarette smoking, and in factors that are of special importance to this longitudinal GWAS, such as the number of FEV1 measurements per participant and follow-up duration. Phenotypic heterogeneity represents a general challenge in genetic epidemiology, particularly in the investigation of longitudinal phenotypes. Thus, we performed a meta-analysis using the subset of cohort studies with ≥3 FEV1 measurements per participant, given that longitudinal trajectories are best estimated over longer time periods and with more measurements. There was little overlap between the top loci identified in the two meta-analyses at \( P < 1 \times 10^{-5} \), suggesting that phenotypic heterogeneity affected the association results. Future meta-studies of lung function decline should aim to increase sample size while maintaining high phenotypic comparability among participating studies. In addition, the trajectory of lung function change, especially over a long period of time, is known to be nonlinear, regulatory lipid transfer domain protein superfamily, and is involved in the trafficking of cholesterol and other lipids between intracellular membranes.[29] Recent in vitro studies showed increased STARD5 expression and protein redistribution as a protective mechanism in response to induced endoplasmic reticulum (ER) stress and consequent over-accumulation of intracellular free cholesterol.[30] We confirmed the expression of STARD5 in all human lung tissues examined and of IL16 in human lung smooth muscle cells, but not epithelial cells, in line with previous observations. In contrast, no expression of TMC3 was detected in any of the tested human lung tissues. We also found significantly lower levels of IL16 in whole lung samples from COPD patients compared with controls, in contrast to its increased expression in asthma, and significantly higher levels of STARD5 in COPD patients compared with controls. Taken together, these results suggest IL16 as the most likely candidate accounting for the observed association, but further investigation is needed to elucidate underlying mechanisms.

The sentinel SNP at the novel chromosome 11 locus is located in ME3, whose protein product is a mitochondrial NADP(+) dependent malic enzyme that catalyzes the oxidative decarboxylation of malate to pyruvate using NADP+ as a cofactor.[31] Mitochondrial malic enzymes play a role in the energy metabolism in tumors, and are considered potential therapeutic targets in cancer.[32,33] We performed independent expression profiling of ME3 and confirmed its expression in all human lung tissues examined, and found significantly higher levels of ME3 in lung samples from COPD patients compared with controls. In addition, we looked up the sentinel SNP in ME3 in a recent GWAS of airway obstruction and found a \( P \) value of 0.049.[34] Taken together, these results support ME3 as a biologically plausible candidate in the regulation of lung function and pathogenesis of COPD.

The identification of trans-eQTL associations for the sentinel SNPs at both the IL16/STARD5/TMC3 and ME3 loci is interesting, and while the interpretation of trans-eQTL associations is ambiguous,[35] the regions these SNPs regulate merit further study.

Besides the GWAS meta-analyses, the assembly of 14 longitudinal cohort studies allowed us to meta-analyze the association of cumulative smoking patterns with the rate of change in FEV1 in the general population. The meta-analyzed estimate for the rate of decline in FEV1 in never smokers was 26.9 mL/year, and the general population. The meta-analyzed estimate for the rate of change in FEV1 in participants, given that longitudinal trajectories are best estimated over longer time periods and with more measurements. There was little overlap between the top loci identified in the two meta-analyses at \( P < 1 \times 10^{-5} \), suggesting that phenotypic heterogeneity affected the association results. Future meta-studies of lung function decline should aim to increase sample size while maintaining high phenotypic comparability among participating studies. In addition, the trajectory of lung function change, especially over a long period of time, is known to be nonlinear,

...
GWAS of Longitudinal Change in Adult Lung Function

A

B

Cohorts

- BHS
- CARDIA
- CHS
- FHS
- HABC

Summary

Effects (mL/yr)
which may require the use of nonlinear time effects in the statistical model. In this study, given that over half of the included cohort studies have FEV\textsubscript{1} measurements at only two time points, our consideration was limited to a linear time effect. Further, the outcome studied, the rate of change in lung function, represents one of many ways to describe lung function change. Additional studies of other aspects of lung function change, such as reduced growth and premature decline, would be of interest.

We sought corroborative evidence in a single cohort study of 1,494 participants. This sample size is much smaller and arguably insufficient compared with replications applied to previous studies of cross-sectional lung function phenotypes. Thus, despite the lack of corroboration for the two novel loci identified in the meta-analyses, results from the complementary gene expression analyses provide compelling evidence for biologically plausible roles of the implicated genes in the longitudinal change in lung function.

None of the 14 sentinel SNPs were associated with the rate of change in FEV\textsubscript{1} in the COPD patient-based LHS cohort. Similarly, a previous population-based GWAS of lung function decline noted a high degree of heterogeneity in findings when analyses were stratified by presence/absence of asthma.\cite{12} The observed discrepancy of association results suggests that the genetic determination of lung function decline may be different in healthy individuals compared with COPD patients, may contribute differentially in a pre-diseased vs. post-diseased state in which medications may influence the rates of decline, or that LHS was underpowered for confirming our findings.

In this study, statistical models included a comprehensive list of confounders that are commonly adjusted for when modeling lung function phenotypes. Given the study’s meta-analysis design and the objective to carry out the same statistical model in all cohort studies, additional covariates that were not available in all cohort studies could not be included. In addition, the adjustment of certain confounders, such as smoking, is challenging in a longitudinal study, and although we accounted for the two most important aspects of smoking, cumulative pattern and dosage, residual confounding due to smoking cannot be excluded.

In summary, we performed GWAS of the longitudinal change in lung function and subsequent meta-analyses, using harmonized data from more than 27,000 participants of European ancestry to identify genetic loci influencing the rate of change in FEV\textsubscript{1}. We identified the novel ME3 locus on chromosome 11 at genome-wide significance and found suggestive evidence for association at the novel IL16/STARD5/TMC3 locus on chromosome 15. Additional expression analyses confirmed the expression of ME3, IL16, and STARD5 in multiple lung tissues, and found differential expression profiles of these three genes in the lungs of COPD patients compared to non-COPD controls. These results support the involvement of these implicated genes in the longitudinal change in lung function in adults of European ancestry. Additional studies with larger sample size and in populations of other races/ethnicities are warranted.

**Supporting Information**

File S1  This is a single file that contains all supporting information for the paper. Briefly, File S1 contains the following items: Methods S1, which describes further details of the cohort studies and the statistical methodology; Table S1, Details of SNP genotyping, quality control (QC), imputation, and statistical analysis across the 14 cohort studies; Table S2, Regression results for single nucleotide polymorphisms associated with the rate of change in FEV\textsubscript{1} (mL/year) at $P < 1 \times 10^{-5}$ in the meta-analysis of 14 cohort studies (N = 27,249); Table S3, Regression results for single nucleotide polymorphisms associated with the rate of change in FEV\textsubscript{1} (mL/year) at $P < 1 \times 10^{-5}$ in the meta-analysis of the five cohort studies with three or more FEV\textsubscript{1} measurements per participant (N = 10,476); Table S4, Association of the 14 sentinel SNPs from the meta-analyses in the AGES-Reykjavik study (AGES) and the Lung Health Study (LHS) for the rate of change in FEV\textsubscript{1} (mL/year); Table S5, Association of previously reported loci in GWAS of cross-sectional lung function with the rate of change in FEV\textsubscript{1} (mL/year) in the meta-analysis of 14 cohort studies (N = 27,249); Table S6, mRNA expression profiling of the implicated genes at the two novel loci in human lung and control tissues; Table S7, Primers for mRNA expression profiling; Table S8, Summary of eQTL look-up for the most significant SNPs at the novel chromosome 11 and 15 loci; Figure S1, Manhattan and QQ plots for the meta-analysis of the rate of change in FEV\textsubscript{1} in 14 cohort studies; Figure S2, Manhattan and QQ plots for the meta-analysis of the rate of change in FEV\textsubscript{1} in the five cohort studies with three or more FEV\textsubscript{1} measurements per participant; Figure S3, mRNA expression profiling in human lung samples from 219 COPD patients and 137 controls for A) IL16, B) STARD5, and C) ME3, using publicly available microarray data from the Lung Genomics Research Consortium site (http://www.lung-genomics.org/). The y-axes reflect the probe intensities of each gene transcript in the binary logarithm form, with the red dots indicating the average probe intensities and the red bars indicating standard deviation. The P values were calculated using the two-sample t-test.

(DOCX)

**Checklist S1  PRISMA Checklist.**

(DOCX)

**Author Contributions**

Wrote the paper: All authors. Drafted the manuscript: WT PAC. AGES Study concept, design: TBH VG LJI. ARIC Study concept, design: DJC NF DBH BRJ SJL ACM KEN. B5iC Study concept and design: DPS. BHS Study concept, design: AJ B. Musk. CARDIA Study concept, design: M. Fornage AS. CHS Study concept, design: SRH SAG BMP. FHS Study concept, design: JD GTO JBW. Health ABC Study concept, design: PAC SBK WT. KORA Study concept, design: HH HS. LBC Study concept, design: JJD JMS. LHS Study concept, design: KCB NNH RAM. RS Study concept, design: GGB AH FR BHS AGU. SAPALDIA Study concept, design: MI NMP H. SHIP Study concept, design: BK SG HV. SpiroMeta Study concept, design: IPP MJD. AGES Genotype data/QC: AVS. ARIC Phenotype data/QC: DJC. Genotype data/QC: ACM KEN. B5iC Phenotype data/QC: DPS. Genotype data/QC: WLh. BHS Phenotype data/QC: AJ B. Musk. Genotype data/QC: AJ B. Musk LJI. CARDIA Phenotype data/QC: IJS AS ODW. Genotype data/QC: M. Fornage M. Foy XG. CHS Phenotype data/QC: BMP. Genotype data/QC: TL BMP JIR. FHS Phenotype data/QC: GTO. Genotype data/QC: GTO. Health ABC Phenotype data/QC: PAC SBK B. Mvilohm WT. Genotype data/QC: SBK YL KL. KORA Phenotype data/QC: JK SK HS. LBC Phenotype data/QC: JJD JMS. Genotype data/QC: GD. LHS Genotype

Figure 2. Association of the chromosome 11 locus with the rate of change in FEV\textsubscript{1} in the meta-analysis of the five cohort studies with $\geq 3$ FEV\textsubscript{1} measurements per participant. A) Regional association plot, where the X-axis is Megabase (Mb) position, and the Y-axes are the negative log of the $P$ value on the left and recombination rate on the right. The sentinel SNP is colored in purple and linkage disequilibrium to the sentinel SNP is depicted by degree of color according to the legend. B) Forest plot for rs507211, where the size of the square for each study represents its contributing weight to the meta-analysis.

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