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Published in:
Journal of Allergy and Clinical Immunology

DOI:
[10.1016/j.jaci.2012.01.074](https://doi.org/10.1016/j.jaci.2012.01.074)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2012

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Imboden, M., Bouzigon, E., Curjuric, I., Ramasamy, A., Kumar, A., Hancock, D. B., Wilk, J. B., Vonk, J. M., Thun, G. A., Siroux, V., Nadif, R., Monier, F., Gonzalez, J. R., Wjst, M., Heinrich, J., Loefer, L. R., Franceschini, N., North, K. E., Altmueller, J., ... Probst-Hensch, N. M. (2012). Genome-wide association study of lung function decline in adults with and without asthma. *Journal of Allergy and Clinical Immunology*, 129(5), 1218-1228. <https://doi.org/10.1016/j.jaci.2012.01.074>

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Genome-wide association study of lung function decline in adults with and without asthma

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Background: Genome-wide association studies have identified determinants of chronic obstructive pulmonary disease, asthma, and lung function level; however, none have addressed decline in lung function.

Objective: We conducted the first genome-wide association study on the age-related decrease in FEV₁ and its ratio to forced vital capacity (FVC) stratified *a priori* by asthma status.

Methods: Discovery cohorts included adults of European ancestry (1,441 asthmatic and 2,677 nonasthmatic participants: the Epidemiological Study on the Genetics and Environment of Asthma, the Swiss Cohort Study on Air Pollution and Lung and Heart Disease in Adults, and the European Community Respiratory Health Survey). The associations of FEV₁ and FEV₁/FVC ratio decrease with 2.5 million single nucleotide polymorphisms (SNPs) were estimated. Thirty loci were followed up by *in silico* replication (1,160 asthmatic and 10,858

nonasthmatic participants: Atherosclerosis Risk in Communities, the Framingham Heart Study, the British 1958 Birth Cohort, and the Dutch Asthma Study).

Results: Main signals identified differed between asthmatic and nonasthmatic participants. None of the SNPs reached genome-wide significance. The association between the height-related gene *DLEU7* and FEV₁ decrease suggested for nonasthmatic participants in the discovery phase was replicated (discovery, $P = 4.8 \times 10^{-6}$; replication, $P = .03$), and additional sensitivity analyses point to a relation to growth. The top ranking signal, *TUSC3*, which is associated with FEV₁/FVC ratio decrease in asthmatic participants ($P = 5.3 \times 10^{-8}$), did not replicate. SNPs previously associated with cross-sectional lung function were not prominently associated with decline.

Conclusions: Genetic heterogeneity of lung function might be extensive. Our results suggest that genetic determinants of

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Information on support for this research is provided in the acknowledgments section of this article.

Disclosure of potential conflict of interest: J. B. Wilk has received research support from the National Institutes of Health/National Heart, Lung, and Blood Institute and the Flight Attendant Medical Research Institute. G. H. Koppelman has received research support from the Netherlands Asthma Foundation. D. S. Postma is a consultant for Nycomed and has received research support from the Top Institute Pharma and AstraZeneca. F. Kauffmann has received research support from the French Agency of Research, French Agency for Environmental and Occupational Health and Safety, and INSERM–Ministry of Research “Cohortes et Collections.” The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication April 13, 2011; revised October 26, 2011; accepted for publication January 24, 2012.

Available online March 16, 2012.

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0091-6749/\$36.00

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doi:10.1016/j.jaci.2012.01.074

longitudinal and cross-sectional lung function differ and vary by asthma status. (J Allergy Clin Immunol 2012;129:1218-28.)

Key words: Asthma, cohort studies, genome-wide association, lung function decline, heterogeneity

Low lung function is a feature of both asthma and chronic obstructive pulmonary disease (COPD), with twin studies demonstrating strong heritability (0.51-0.77) for FEV₁.^{1,2} The 2 respiratory diseases and lung function itself share predisposing and phenotypic features, including increased airway responsiveness and atopy, as well as exogenous risk factors.^{3,4} Genome-wide association studies (GWASs) have identified novel genetic loci for asthma,⁵⁻¹⁰ COPD,¹¹⁻¹⁴ and lung function¹⁵⁻¹⁸ and provide the opportunity to study agnostically their overlap in genetic background.¹⁹ Some of the implicated genes, such as *PDE4D*, support a link between asthma and COPD, which might be rooted in shared pathways during lung development.²⁰ However, the majority of the genes implicated in asthma or COPD GWAS analyses have not been identified as top association signals in GWASs for lung function in the general population,¹⁵⁻¹⁸ with the exception of *HHIP* and *FAM13A* being associated with both lung function¹⁵⁻¹⁸ and COPD.¹¹⁻¹⁴ Several lines of evidence suggest that different genes influence lung function in asthmatic and nonasthmatic subjects. Genome scans in family-based linkage studies identified some, but overall limited, overlap between chromosomal regions linked to lung function in asthmatic patients,²¹ patients with COPD,²² and the general population,²³ and it has been suggested that genetic variation might be more important for lung function in asthmatic patients after adjusting for smoking and body size differences.^{21,24,25}

Here we present results from the first lung function GWAS conducted separately for asthmatic and nonasthmatic participants. This study also focuses on the rate of lung function decrease in adults instead of cross-sectional lung function parameters tested in previous GWASs.¹⁵⁻¹⁸ The discovery cohorts included 2 population-based studies (the Swiss Cohort Study on Air Pollution and Lung and Heart Disease in Adults [SAPALDIA] and the European Community Respiratory Health Survey [ECRHS]) and 1 asthma family-based study (Genetics and Environment of Asthma [EGEA]), with cohort participants of European ancestry and with highly comparable and standardized assessment of respiratory health parameters, including spirometry from 2 time points 10 years apart. These 3 studies had been included in the GWAS for asthma conducted by the GABRIEL consortium.⁷ Replication cohorts included 3 population-based cohorts (the Framingham Heart Study [FHS], the Atherosclerosis Risk in Communities [ARIC], the British 1958 Birth Cohort [B58C]) and 1 family-based asthma study (the Dutch Asthma Study).

METHODS

Discovery cohorts and study population

Three large multicentric cohorts, EGEA,²⁶ SAPALDIA,²⁷ and ECRHS,²⁸ constitute the ESE consortium. Personal factors of relevance to lung function decrease were assessed by means of interviews and anthropometric measurements at baseline and follow-up. Participants included in the discovery phase were derived from the nested asthma case-control samples (SAPALDIA and ECRHS) or from the entire study population (EGEA) subjected to genome-wide genotyping in the context of the GABRIEL asthma GWAS.⁷ Baseline and follow-up examinations were roughly 10 years apart. The analysis was restricted to adult participants (age ≥18 years at the time of the baseline spirometry) with complete information on age, height, and sex, as well as valid lung function measures from both surveys. Cohort study protocols were in

Abbreviations used

ARIC:	Atherosclerosis Risk in Communities Study
B58C:	British 1958 Birth Cohort
COPD:	Chronic obstructive pulmonary disease
ECRHS:	European Community Respiratory Health Survey
EGEA:	Genetics and Environment of Asthma
FHS:	Framingham Heart Study
FVC:	Forced vital capacity
GWAS:	Genome-wide association study
SAPALDIA:	Swiss Cohort Study on Air Pollution and Lung and Heart Disease in Adults
SNP:	Single nucleotide polymorphism

agreement with the Declaration of Helsinki and obtained ethical approval from the respective regional review boards, national review boards, or both.

Lung function assessments, asthma status, and genotypes

At each visit, measurements of a minimum of 2 acceptable forced expiratory flows, forced vital capacity (FVC) and FEV₁, complying with American Thoracic Society criteria were obtained.²⁶⁻²⁹ No bronchodilator was administered. On the basis of questionnaire data, asthmatic participants were defined by providing an asthma self-report at any of the completed surveys, and family-based studies considered additional clinical asthma criteria (see the **Methods** section in this article's Online Repository at www.jacionline.org). Genotyping for discovery cohorts was centrally performed on the Illumina Human 610quad BeadChip at the Centre National de Génotypage (CNG, Evry, France).⁷ Imputation of genotypes based on the Hapmap2 reference panel, investigation of population stratification, and quality control criteria are described in **Fig E1** and **Table E1** in this article's Online Repository at www.jacionline.org.

Replication cohorts

Four cohorts of European ancestry with available genome-wide data, ARIC,³⁰ FHS,¹⁵ B58C,³¹ and the Dutch asthma study,³² were used for replication. Subjects included in the current analysis were older than 24 years and had complete information on covariates (age, height, and sex) and valid lung function measures from at least 2 time points. The lung function measurements were conducted at least 10 years apart, except for ARIC, in which measurements were conducted 3 years apart (**Table I**). Distinct genotype data platforms and imputation software were used (see **Table E2** in this article's Online Repository at www.jacionline.org).

Statistical analysis

The annual decrease in FEV₁ and FEV₁/FVC ratio was calculated as the difference between follow-up and baseline spirometric measurements (milliliters for FEV₁ and percentages for FEV₁/FVC ratio) divided by the duration of follow-up in years. Standardized residuals were derived from sex-specific linear regression models adjusted for age, height, and study center in asthmatic and nonasthmatic participants separately. Comparability between studies of standardized residuals was tested by using the Wilcoxon-Mann-Whitney test ($P > .94$). The standardized residuals were used as dependent variables and regressed on genome-wide single nucleotide polymorphisms (SNPs) adjusted for study-specific principal components capturing population ancestry (see the **Methods** section in this article's Online Repository). Study-specific SNP effect estimates were combined through meta-analysis by using fixed and random effects models. We used a threshold P value of less than 5×10^{-8} (the Bonferroni adjustment for 1 million independent tests) to declare a pooled effect as genome-wide significant. Selection criteria for replication loci are described in the **Methods** section in this article's Online Repository. SNPs with suggestive evidence of association with a decrease in FEV₁ or FEV₁/FVC ratio were chosen for *in silico* replication (see **Table E3** in this article's Online Repository at www.jacionline.org). Study-specific regression models and meta-analyses across replication cohorts were as described for the discovery phase. Replication cohorts

TABLE I. Baseline characteristics of discovery and replication cohorts by asthma status

	No.*	Men (%)	Age, mean ± SD	Height, mean ± SD	FEV ₁ , mean ± SD (L)	FEV ₁ /FVC ratio, mean ± SD	Follow-up length, † mean ± SD (y)	Annual decrease in FEV ₁ , mean ± SD (mL/y)	Annual decrease in FEV ₁ /FVC, mean ± SD (%/y)	Never smokers (%)
Nonasthmatic participants										
Discovery (ESE cohorts)										
EGEA	529	45.2	41.4 ± 11.7	1.68 ± 0.08	3.45 ± 0.78	0.83 ± 0.06	11.2 ± 1.0	-28.6 ± 25.7	-0.47 ± 0.53	46.5
SAPALDIA	805	49.2	41.8 ± 11.1	1.70 ± 0.09	3.62 ± 0.81	0.79 ± 0.07	10.9 ± 0.2	-34.0 ± 28.3	-0.40 ± 0.46	43.1
ECRHS	1343	49.7	34.1 ± 7.1	1.70 ± 0.10	3.81 ± 0.83	0.83 ± 0.06	8.9 ± 0.9	-26.3 ± 30.7	-0.30 ± 0.50	40.7
Replication with <i>in silico</i> data										
ARIC	7156	46.3	54.5 ± 5.6	1.69 ± 0.09	3.01 ± 0.75	0.75 ± 0.07	2.9 ± 0.2	-52.0 ± 57.4	-0.19 ± 0.98	40.8
FHS	3232	44.9	52.9 ± 10.2	1.67 ± 0.10	2.89 ± 0.81	0.77 ± 0.08	10.5 ± 3.6	-24.9 ± 23.9	-0.33 ± 0.57	36.1
B58C	470	48.7	35.0 ± 0.2	1.70 ± 0.09	3.68 ± 0.73	0.81 ± 0.06	10.1 ± 0.5	-34.9 ± 31.4	-0.21 ± 0.67	28.5
Asthmatic participants										
Discovery (ESE cohorts)										
EGEA	330	50.6	38.5 ± 12.5	1.70 ± 0.09	3.26 ± 0.91	0.77 ± 0.11	11.6 ± 1.0	-27.6 ± 39.4	-0.44 ± 0.68	44.6
SAPALDIA	540	46.5	40.2 ± 11.3	1.69 ± 0.09	3.36 ± 0.89	0.76 ± 0.95	10.9 ± 0.3	-35.5 ± 33.9	-0.45 ± 0.54	42.4
ECRHS	571	42.7	33.9 ± 7.3	1.69 ± 0.10	3.43 ± 0.81	0.78 ± 0.09	8.8 ± 0.7	-26.7 ± 42.6	-0.20 ± 0.60	42.5
Replication with <i>in silico</i> data										
ARIC	325	50.2	54.2 ± 5.7	1.69 ± 0.10	2.73 ± 0.87	0.68 ± 0.10	2.9 ± 0.2	-43.9 ± 77.2	-0.037 ± 1.25	41.9
FHS	346	41.3	50.1 ± 10.3	1.68 ± 0.09	2.72 ± 0.84	0.73 ± 0.09	10.2 ± 3.8	-29.8 ± 23.7	-0.38 ± 0.51	36.1
B58C	231	44.2	35.0 ± 0.2	1.69 ± 0.10	3.45 ± 0.75	0.78 ± 0.08	10.3 ± 0.5	-34.4 ± 37.6	-0.17 ± 0.89	37.2
Dutch Asthma Study	258	60.9	35.1 ± 7.6	1.75 ± 0.09	3.03 ± 0.95	0.65 ± 0.13	14.6 ± 7.2	-22.8 ± 47.0	-0.14 ± 0.89	40.7

*This column comprises the maximal number of subjects who contributed to at least 1 GWAS analysis (decrease in either FEV₁ or FEV₁/FVC ratio).

†Time spacing between the first and second spirometric assessment.

with spirometric data from more than 2 different time points modeled the lung function decrease phenotype by fitting a least-squares slope using available data (FHS and the Dutch Asthma Study). A *P* value of .05 or less was considered statistically significant at the replication level.

The results of the main meta-analyses for the top 1000 SNPs are available in the online repository (see Table E4, A-D, in this article's Online Repository at www.jacionline.org). We also conducted a meta-analysis by combining nonasthmatic and asthmatic samples and tested for heterogeneity between these samples (see Table E5 in this article's Online Repository at www.jacionline.org). Additional sensitivity analyses were done by (1) restricting the GWAS sample to subjects aged 30 years and older for FEV₁ decrease (see Table E4, E and F); (2) conducting GWAS analyses on percentage change instead of absolute annual decrease in lung function (see Table E4, G-J); (3) investigating smoking-stratified joint effects for replications SNPs (see Table E6 in this article's Online Repository at www.jacionline.org); and (4) excluding ARIC, a cohort with a substantially shorter follow-up time than the other cohorts (3 years instead of 10 years), from replication analyses (see Table E7 in this article's Online Repository at www.jacionline.org). Methods and results of these additional analyses are described in this article's Online Repository.

RESULTS

Characteristics of the study populations

The cohorts included in this study differed by age and type of recruitment and accordingly in lung function and the proportion of participants with FEV₁/FVC ratios of less than 70% (Table I and see Table E8 in this article's Online Repository at www.jacionline.org). Baseline lung function parameters, but not their annual changes, were less in asthmatic participants when compared with those in nonasthmatic participants in each study. The proportion of never smokers was comparable among asthmatic participants but varied among nonasthmatic participants

(range, 28.5% in B58C to 46.5% in EGEA). No substantial differences in the smoking prevalence between participants with and without asthma were observed within each study. Comparing the discovery cohorts in more detail (see Table E8), atopy (total IgE, ≥100 kU/mL) and hay fever were more prevalent in both asthmatic and nonasthmatic participants from EGEA when compared with those from ECRHS and SAPALDIA. Current asthma was more prevalent (84.4%) in EGEA than in SAPALDIA (25.5%) or ECRHS (43.3%), and the prevalence of a positive family history for asthma was also highest in EGEA, which is in agreement with the study design. Asthmatic participants from EGEA had a younger age of disease onset because of the mode of recruitment of the proband.

Main findings from meta-analyses of discovery and replication phases

In the discovery phase GWAS meta-analysis of decrease in FEV₁ and FEV₁/FVC ratio was conducted in 2,677 nonasthmatic and 1,441 asthmatic participants. Genomic inflation factors were low for both lung function parameters ($\lambda < 1.047$, see Table E9 in this article's Online Repository at www.jacionline.org), suggesting minimal unaccounted population stratification. The replication panel included a total of 10,858 nonasthmatic and 1,138 asthmatic participants. Thirty lead SNPs belonging to 30 loci ($5 \times 10^{-8} < P_{\text{discovery}} < 6 \times 10^{-5}$) were chosen for replication.

The 4 lung function parameter- and asthma-specific meta-analyses identified 1 association signal that almost reached the genome-wide significance level ($P = 5.3 \times 10^{-8}$) at locus 8p22 containing the *TUSC3* gene for FEV₁/FVC ratio decrease in asthmatic participants whereas all other signals had a *P* value of less

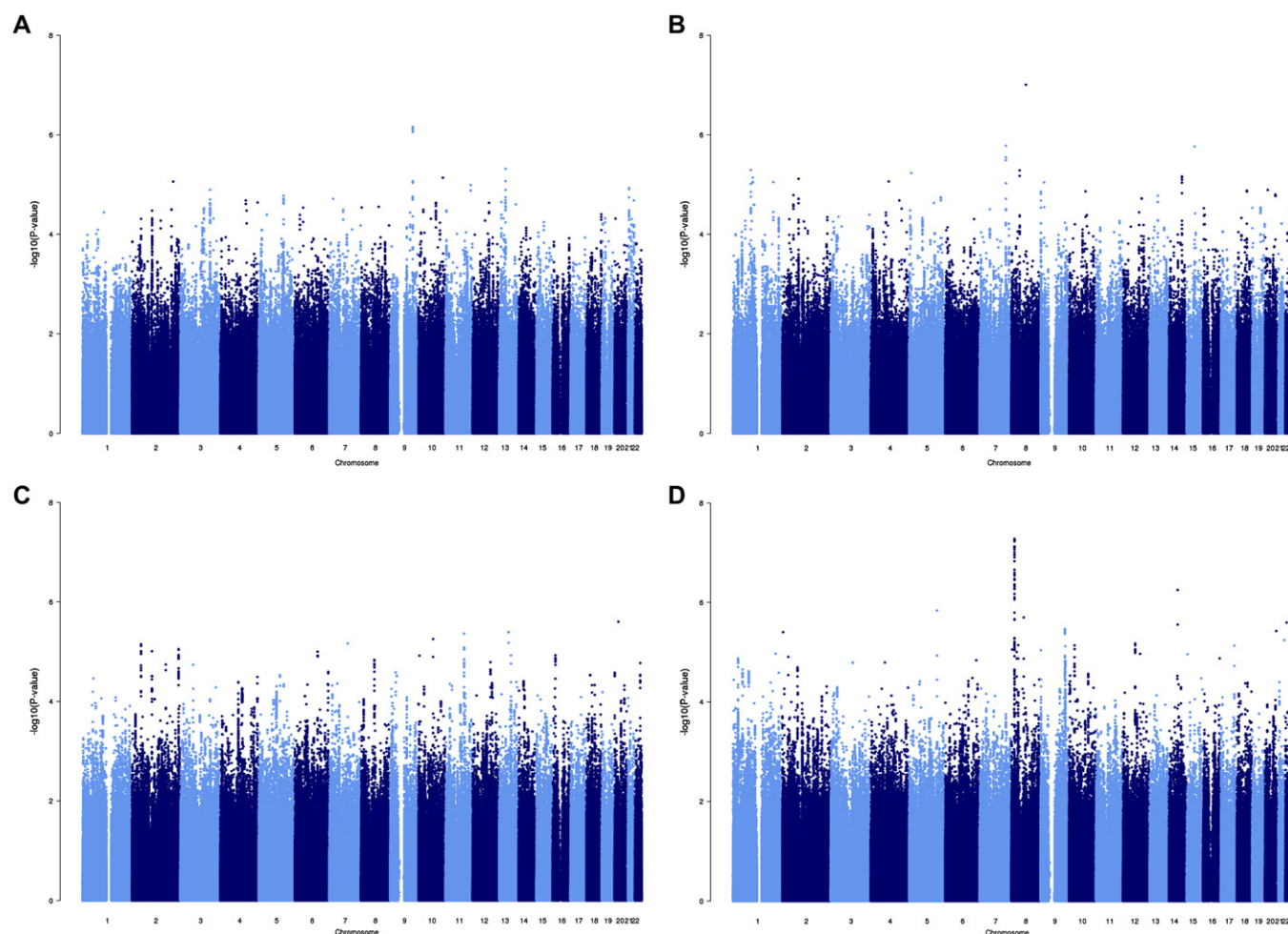


FIG 1. Manhattan plots of association results for lung function decline. **A**, FEV₁ decrease in nonasthmatic participants. **B**, FEV₁ decrease in asthmatic participants. **C**, FEV₁/FVC ratio decrease in nonasthmatic participants. **D**, FEV₁/FVC ratio decrease in asthmatic participants.

than 5×10^{-7} (Fig 1), but this signal was not associated with FEV₁/FVC ratio decrease in asthmatic participants in the replication sample. The only locus of the selected replication candidate loci that formally replicated was 13q14.3, containing the *DLEU7* gene, which was associated with decrease in FEV₁ in the nonasthmatic participants ($P_{\text{discovery}} = 4.8 \times 10^{-6}$ and $P_{\text{replication}} = .03$).

In the global *post hoc* analysis combining both asthmatic and nonasthmatic participants ($n = 4118$), a striking finding was the absence of any pronounced association signals ($P > 1 \times 10^{-6}$) despite increased statistical power. This was in agreement with the minimal overlap of association signals observed in asthmatic and nonasthmatic participants separately. Most signals at a P value of less than 10^{-5} from the asthma-stratified analysis in the discovery phase exhibited statistically significant heterogeneity of effects between the 2 groups (Table II). At the replication stage, none of the replication SNPs were associated with lung function decrease in asthmatic and nonasthmatic participants combined.

Association signals for annual decrease in FEV₁ in nonasthmatic participants

Of 15 SNPs associated at a P value of less than 10^{-5} with a decrease in FEV₁ in nonasthmatic participants, 10 were clustered at position 112.3 Mb on chromosome 9 containing the genes *TXN*, *MUSK*, and *SVEP1*. Two of the 15 SNPs were located at

13q14.3 in a locus containing the *DLEU7* gene; 3 SNPs belonged to 3 distinct loci. The association of lead and proxy SNPs in *DLEU7* (Fig 2) but not *TXN/MUSK/SVEP1* (see Fig E2 in this article's Online Repository at www.jacionline.org) or the other SNPs (Table II) was replicated. The G allele of SNP rs9316500 near the *DLEU7* gene was positively associated with annual FEV₁ decrease in both the discovery ($P = 4.8 \times 10^{-6}$) and replication ($P = .026$) cohorts. Although heterogeneity between studies was not significant ($P = .61$), the combined P value did not reach the genome-wide level ($P = 5.7 \times 10^{-5}$).

Association signals for annual decrease in FEV₁ in asthmatic participants

Eighteen SNPs in 9 distinct chromosomal locations were associated with a decrease in FEV₁ in asthmatic participants at a P value of less than 10^{-5} . None of the loci selected for *in silico* replication were confirmed (Table II).

Association signals for annual decrease in FEV₁/FVC ratio in nonasthmatic participants

Seven loci showed association with FEV₁/FVC ratio decrease in nonasthmatic participants ($10^{-6} < P < 10^{-5}$), but no locus selected for replication was confirmed (Table II).

TABLE II. Association of (lead) SNPs subjected to replication with decrease in FEV₁ and decrease in FEV₁/FVC ratio stratified by asthma status

dbSNP ID	Chromosome	Position (build 36.3)	Gene nearby	Maximal frequency of coding allele	Discovery phase				Replication phase			
					Estimate of joint analysis	P value for joint analysis	P value for heterogeneity between studies	P value for heterogeneity between asthmatic and nonasthmatic participants	Estimate of joint analysis in replication cohorts	P value for joint analysis	P value for heterogeneity between studies	P value for heterogeneity between asthmatic and nonasthmatic participants
Decrease in FEV₁												
Nonasthmatic participants												
rs1889321	9	112340656	<i>MUSK*</i>	0.287	-0.150	6.95E-07	.814	.0187	-0.011	.480	.713	.053
rs9316500	13	49992115	<i>DLEU7</i>	0.336	0.135	4.81E-06	.613	.0255	0.033	.026	.124	.075
rs6785065	3	149016533	<i>ZIC1</i>	0.274	-0.136	.00001	.234	.1700	-0.006	.686	.525	.55
rs278037	13	29322627	<i>UBL3</i>	0.178	-0.151	.00002	.364	.0058	-0.006	.734	.231	.50
rs7641198	3	117396577	<i>LSAMP</i>	0.147	0.164	.00003	.669	.1997	-0.002	.939	.690	.15
rs421847	21	19269950	<i>PRSS7</i>	0.281	0.128	.00003	.831	.0350	-0.016	.310	.247	.86
rs496809	18	74857661	<i>SALL3</i>	0.078	-0.236	.00004	.412	.0041	0.022	.443	.373	.60
rs10933964	3	110021881	<i>TRAT1</i>	0.499	-0.117	.00006	.345	.0022	-0.015	.265	.869	.041
Asthmatic participants												
rs10808265	7	131840229	<i>PLXNA4B</i>	0.484	-0.175	1.66E-06	.844	.0020	0.069	.105	.258	.16
rs1902618	15	58951491	<i>RORA</i>	0.234	-0.220	1.72E-06	.449	.0043	0.029	.590	.777	.58
rs3843306	1	91060718	<i>BARHL2</i>	0.460	0.176	5.11E-06	.042	8.33E-06	0.047	.270	.883	.24
rs7006290	8	41734295	<i>ANK1</i>	0.319	0.185	5.19E-06	.058	.0003	0.038	.456	.574	.45
rs12436689	14	84723772	<i>FLRT2</i>	0.244	-0.212	6.87E-06	.420	.0010	-0.017	.759	.051	.95
rs12615721	2	81710037	<i>CTNNA2</i>	0.104	-0.303	7.65E-06	.853	.0020	-0.127	.129	.824	.08
rs10516809	4	89640109	<i>HERC5</i>	0.101	0.306	8.67E-06	.790	3.60E-05	-0.060	.446	.200	.41
rs16856186	1	203944749	<i>SLC45A3</i>	0.098	0.268	8.92E-06	.510	.0034	-0.079	.350	.094	.46
rs158536	20	52148709	<i>BCAS1</i>	0.408	0.162	.00002	.948	.0001	0.075	.100	.917	.09
rs477725	19	42066106	<i>ZNF345</i>	0.158	0.223	.00003	.821	.0031	-0.069	.273	.255	.14
rs9662589	1	230344234	<i>DISC1</i>	0.221	0.188	.00005	.868	.0002	-0.020	.706	.153	.76
rs777433	2	128084705	<i>LIMS2</i>	0.407	0.151	.00010	.811	.1223	-0.018	.691	.564	.52
Decrease in FEV₁/FVC ratio												
Nonasthmatic participants												
rs2658782	11	92806379	<i>FLJ25393</i>	0.166	0.186	4.33E-06	.362	.0041	0.031	.135	.242	.91
rs1867982	10	73197053	<i>C10orf54</i>	0.109	0.202	5.56E-06	.839	.0034	-0.008	.745	.412	.24
rs12712969	2	46185673	<i>PRKCE</i>	0.268	-0.147	7.08E-06	.687	.0116	0.012	.448	.916	.76
rs10187654	2	234478798	<i>TRPM8</i>	0.205	0.151	8.87E-06	.797	.0049	-0.015	.382	.676	.15
rs356642	2	100903870	<i>NPAS2</i>	0.189	0.158	9.79E-06	.162	.0014	-0.010	.565	.282	.28
rs890515	8	67534388	<i>ADHFE1</i>	0.497	0.119	.00001	.580	.0257	0.003	.847	.443	.58
rs10738890	9	32448081	<i>DDX58</i>	0.391	-0.118	.00003	.832	.5847	-0.009	.567	.032	.73
Asthmatic participants												
rs4831760	8	15576956	<i>TUSC3</i>	0.326	0.222	5.27E-08	.066	7.74E-08	0.011	.799	.541	.73
rs7144584	14	63345565	<i>SYNE2</i>	0.116	-0.318	5.62E-07	.616	.0010	0.089	.272	.752	.43
rs1148186	10	28657641	<i>MPP7</i>	0.194	0.219	7.28E-06	.760	.0035	-0.033	.602	.967	.60

**MUSK* refers to the *TXN/MUSK/SVEP1* locus.

Association signals for annual decrease in FEV₁/FVC ratio in asthmatic participants

Twelve SNPs at locus 8p22 containing the gene *TUSC3* at 15.68 Mb were associated with FEV₁/FVC ratio decrease at a *P* value of less than 10⁻⁷ in asthmatic participants (Fig 1). Regional locus and forest plots are presented in Fig E3 in this article's Online Repository at www.jacionline.org. The top association signals in this locus were conferred by distinct SNPs in each cohort, although apparently they were located in the same putative haplotype segment in SAPALDIA and in EGEA (see Fig E4 in this article's Online Repository at www.jacionline.org). There was no statistically significant association in ECRHS. Meta-analysis of the discovery samples identified SNP rs4831760 as the top signal in *TUSC3* gene, but heterogeneity between discovery studies was borderline significant (*P* = .07). The C allele (*P* = 5.3 × 10⁻⁸) was positively associated with annual decrease in FEV₁/FVC ratio in asthmatic participants (β = 0.22 ± 0.04 [SE], Table II). However, this

association was not replicated (*P* = .80). In the meta-analysis combining discovery and replication samples, the resulting *P* value for rs4831760 was 2.8 × 10⁻⁵. All but the Dutch Asthma Study exhibited effect estimates in the same direction as the discovery panel. Two other candidate loci (*MPP7* and *SYNE2*) also failed replication testing.

SNPs previously associated in GWAS meta-analyses on cross-sectional lung function

The associations of top-hit SNPs from previous GWAS meta-analyses on cross-sectional lung function^{11,15-18} and a replication study in asthmatic patients³³ were assessed separately for asthmatic and nonasthmatic participants in the discovery cohorts. Associations were assessed for both lung function parameters of decrease (annual decrease and percentage change) and cross-sectional lung function levels. Overall, a subset of variants and loci showed replication of association with cross-sectional lung

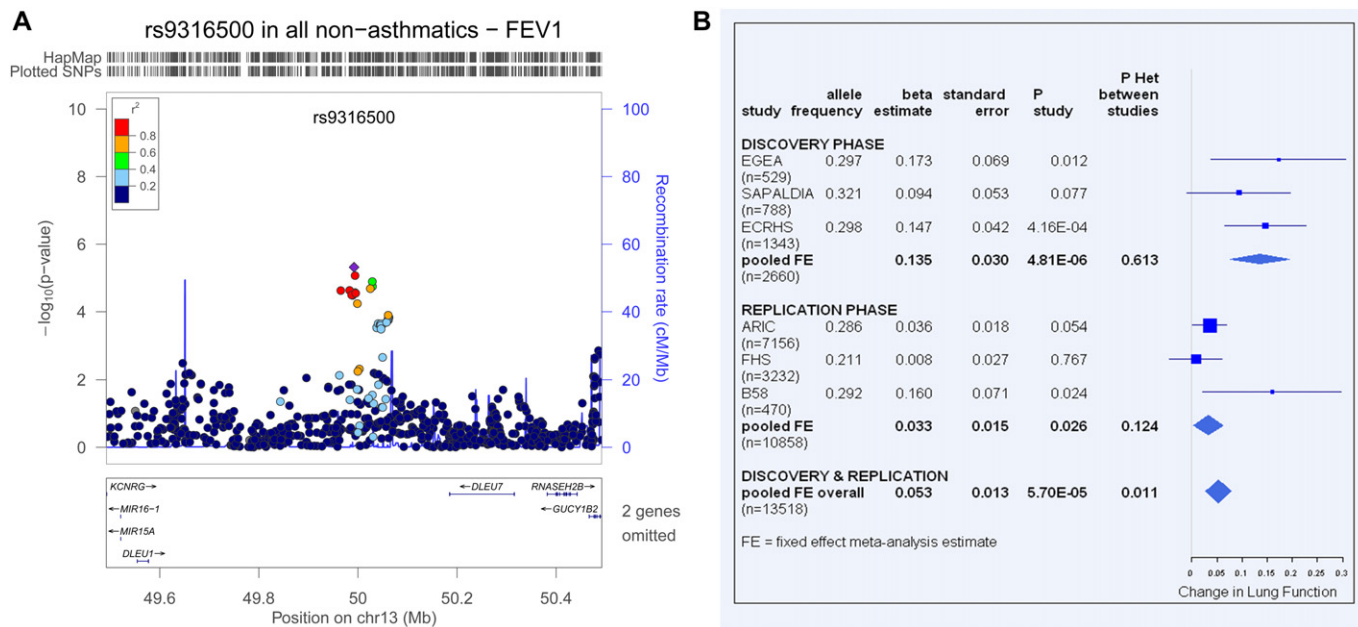


FIG 2. Association of the *DLEU7* locus with decrease in FEV₁ in nonasthmatic participants. **A**, Regional association plot, discovery phase. **B**, Forest plot for rs9316500. **A**, Chromosome position (National Center for Biotechnology Information build 36.3) and recombination rate (hg18 build). The sentinel SNP is represented as a diamond and *r*² value for SNPs to the sentinel SNP (HapMap CEU phase II). **B**, The size of the square of each study reflects the contributing weight to the meta-analysis. Details are shown in Table E11 in this article's Online Repository at www.jacionline.org.

function in either nonasthmatic or asthmatic participants. Few of the loci showed strong association with decrease in lung function. We present associations at a *P* value of less than .05 in Table III^{15-18,33,34} and those at a *P* value of .05 or greater in Table E10 in this article's Online Repository at www.jacionline.org.

For baseline FEV₁, we observed associations for SNPs belonging to 4q24 (*GSTCD*, rs11731417, *P* = 1.3 × 10⁻⁴) and 15q23 (*THSD4*, rs1913768, *P* = .003). Associations with baseline FEV₁ were mainly restricted to nonasthmatic participants. For baseline FEV₁/FVC ratios, associations of SNPs of *THSD4* were prominent (eg, rs12899618, *P* = 3.3 × 10⁻⁴) and again restricted to nonasthmatic participants.

For decrease in phenotypes of FEV₁, we observed associations for SNPs in regions 6p21 (*DAAM2*, .003 < *P* < .02) and 4q28 (*HHIP*, .02 < *P* < .05) among asthmatic participants and in *THSD4* (.003 < *P* < .04) among nonasthmatic participants. The strongest associations observed for decrease in phenotypes of FEV₁/FVC ratio were 2 SNPs in *MMP15* (16q13, .003 < *P* < .002) in nonasthmatic participants only. Association in the combined sample of asthmatic and nonasthmatic participants did not substantially alter the results.

Summary of findings from sensitivity analyses

We observed in nonasthmatic participants aged 30 years and older that *MUSK* and *DLEU7* were no longer prominently associated with FEV₁ decrease, but SNPs in other genes remained strongly associated (*ZIC1*, rs6785065, *P* = 2.3 × 10⁻⁵; *UBL3*, rs278037, *P* = 4.8 × 10⁻⁵).

Results of the GWASs on percentage change in lung function showed that the FEV₁ association signal for *DLEU7* in the

nonasthmatic participants was no longer significant; however, the signals for *MUSK* (rs1889321, *P* = 2.92 × 10⁻⁷) and other loci remained unaltered (*ZIC1*, rs6785065, *P* = 2.0 × 10⁻⁵; *KIRREL3*, rs11604082, *P* = 4.1 × 10⁻⁶; *KIAA2117*, rs10082549, *P* = 2.7 × 10⁻⁶). Top signals associated with decrease in FEV₁/FVC ratio in asthmatic participants remained unaltered for *TUSC3* (rs4831760, *P* = 5.2 × 10⁻⁸) and *SYNE2* (rs7144584, *P* = 6.4 × 10⁻⁷) after taking baseline lung function into account.

Smoking-stratified analyses of the replication SNPs revealed no substantial difference in association between ever and never smokers except for a few SNPs belonging to loci containing the genes *SYNE2*, *RORA*, *BCAS1*, or *PLXNA4*.

Replication meta-analysis excluding the ARIC data substantially reduced sample size in nonasthmatic participants, and the association of *DLEU7* with decrease in FEV₁ was no longer significant. Instead, 2 loci for association with decrease in FEV₁ in asthmatic participants (*PLXNA4*, rs10808265, *P*_{discovery} = 1.7 × 10⁻⁶, *P*_{replication} = .02 and *SLC45A3*, rs16856186, *P*_{discovery} = 8.9 × 10⁻⁶, *P*_{replication} = .04) and 1 locus, *FLJ25393*, for a decrease in FEV₁/FVC ratio in nonasthmatic participants (rs2658782, *P*_{discovery} = 4.3 × 10⁻⁶, *P*_{replication} = .03) gained statistical significance.

DISCUSSION

A main result of this study is the observed genetic heterogeneity of lung function decrease between asthmatic and nonasthmatic subjects. When we combined the 2 groups in the discovery phase, we observed no genome-wide significant association signal despite larger sample size. All top-hit association signals detected by the asthma-stratified analysis showed

TABLE III. Association* of SNPs previously identified in GWASs on cross-sectional lung function with percent predicted† lung function at baseline, as well as percentage change and annual decrease in lung function for FEV₁ and FEV₁/FVC ratio in ESE discovery cohorts by asthma status

dbSNP ID	Chromosome	Position (build 36.3)	References	Gene nearby	Maximal frequency of coding allele	Nonasthmatic participants			Asthmatic participants		
						FEV ₁ % predicted	FEV ₁ % change	FEV ₁ decrease (%/y)	FEV ₁ % predicted	FEV ₁ % change	FEV ₁ decrease (%/y)
						P value	P value	P value	P value	P value	P value
FEV₁											
rs1435867	2	229219173	2	<i>PID1</i>	0.065	.021	.845	.418	.824	.321	.377
rs17035917	4	106740191	2, 3	<i>INTS12-GSTCD-NPNT</i>	0.071	.006	.135	.077	.073	.056	.061
rs17035960	4	106751295	2, 3	<i>INTS12-GSTCD-NPNT</i>	0.071	.004	.093	.054	.067	.056	.063
rs11727735	4	106851319	2, 3	<i>INTS12-GSTCD-NPNT</i>	0.076	2.14E-04	.361	.198	.057	.114	.074
rs10516526	4	106908353	2, 3	<i>INTS12-GSTCD-NPNT</i>	0.072	1.96E-04	.327	.177	.062	.120	.078
rs11731417	4	106965461	2, 3	<i>INTS12-GSTCD-NPNT</i>	0.073	1.32E-04	.335	.177	.048	.146	.090
rs1032295	4	145654034	2	<i>HHIP</i>	0.397	.173	.096	.306	.274	.042	.033
rs1512285	4	145670409		<i>HHIP</i>	0.462	.032	.029	.141	.152	.033	.024
rs720485	4	145682038	2, 3	<i>HHIP</i>	0.391	.159	.510	.786	.943	.044	.058
rs1512288	4	145710731	2, 3	<i>HHIP</i>	0.401	.188	.533	.813	.781	.046	.057
rs6817273	4	145711453	2, 3	<i>HHIP</i>	0.400	.179	.535	.816	.866	.046	.057
rs3008798	6	39887840	3	<i>DAAM2</i>	0.464	.326	.960	.850	.755	.009	.017
rs1318002	6	39892112	3	<i>DAAM2</i>	0.480	.649	.725	.902	.782	.015	.023
rs2395730	6	39892343	3	<i>DAAM2</i>	0.442	.522	.716	.513	.619	.003	.007
rs12899618	15	69432174	3	<i>THSD4</i>	0.158	.003	.003	.014	.424	.137	.131
rs1913768	15	69436598	3	<i>THSD4</i>	0.159	.003	.002	.011	.393	.162	.152
rs1568010	15	69455566	4	<i>THSD4</i>	0.372	.535	.042	.067	.413	.241	.111
rs2304488	16	56631711	1	<i>MMP15</i>	0.186	.033	.101	.147	.112	.344	.506
rs12447804‡	16	56632783	5	<i>MMP15</i>	0.179	.033	.111	.161	.112	.382	.482
FEV₁/FVC ratio											
rs918949	2	218382942	2, 3	<i>TNSI</i>	0.384	.010	.133	.089	.076	.241	.256
rs1035672	2	218383444	2, 3	<i>TNSI</i>	0.384	.010	.133	.089	.093	.243	.258
rs929937	2	218417460	2, 4	<i>TNSI</i>	0.386	.623	.017	.016	.004	.915	.888
rs3845823	2	229611365	4	<i>PID1</i>	0.432	.039	.963	.852	.393	.997	.987
rs12477314‡	2	239542085	5	<i>HDAC4</i>	0.215	.023	.727	.655	.125	.361	.278
rs1529672	3	25495586	5	<i>RARB</i>	0.159	.012	.329	.337	.605	.716	.860
rs1828591	4	145700230	2, 3	<i>HHIP</i>	0.394	.031	.470	.345	.254	.138	.139
rs13118928	4	145705839	2, 3	<i>HHIP</i>	0.393	.043	.500	.371	.271	.132	.132
rs3995090	5	147826008	2, 3	<i>HTR4</i>	0.394	.011	.785	.699	.029	.649	.456
rs2395730	6	39892343	3	<i>DAAM2</i>	0.442	.277	.554	.685	.979	.036	.039
rs2798641	6	109374743	5	<i>ARMC2</i>	0.209	.315	.444	.530	.006	.188	.158
rs9496346	6	142711031	2	<i>GPR126</i>	0.316	.053	.378	.368	.098	.777	.788
rs6570507	6	142721265	2	<i>GPR126</i>	0.314	.035	.356	.342	.080	.804	.821
rs11155242	6	142733242	2	<i>GPR126</i>	0.210	.008	.785	.670	.268	.857	.807
rs7753012	6	142787576	2	<i>GPR126</i>	0.327	.051	.477	.487	.065	.566	.637
rs3748069	6	142809326	2	<i>GPR126</i>	0.319	.043	.407	.401	.134	.604	.628
rs171891	6	142892305	2, 4	<i>GPR126</i>	0.198	.013	.884	.741	.129	.830	.815
rs10512249	9	97296130	2	<i>PTCH1</i>	0.089	.435	.922	.999	.807	.032	.028
rs11172113	12	55813550	5	<i>LRP1</i>	0.384	.005	.602	.530	.809	.114	.125
rs1036429	12	94795559	5	<i>CCDC38</i>	0.217	.765	.322	.356	.295	.047	.031
rs2456526	15	50876734	1	<i>ONECUT1</i>	0.136	.011	.524	.500	.451	.230	.250
rs12899618	15	69432174	3	<i>THSD4</i>	0.158	3.25E-04	.253	.390	.328	.596	.668
rs1913768	15	69436598	3, 4	<i>THSD4</i>	0.159	4.78E-04	.221	.344	.365	.617	.695
rs2304488	16	56631711	1	<i>MMP15</i>	0.186	.121	.002	.002	.085	.760	.515
rs12447804‡	16	56632783	5	<i>MMP15</i>	0.179	.121	.003	.003	.085	.719	.487
rs2865531‡	16	73947817	5	<i>CFDP1</i>	0.428	.035	.621	.736	.377	.840	.603

*Associations of SNPs previously associated in cross-sectional lung function in GWASs, (1) Framingham,¹⁵ (2) CHARGE,¹⁷ (3) Spirometa,¹⁶ (4) Asthmatics,³³ and (5) CHARGE-Spirometa,¹⁸ were assessed in the discovery cohorts only if minor allele frequency was at least 5%. SNPs tested for associations—*ADAM19*: rs2277027, rs1422795, rs6890282; *ADCY2*: rs7710510, rs6555465; *ARMC2*: rs2798641; *C10orf11*: rs11001819; *CCDC38*: rs1036429; *CDC123*: rs7068966; *CFDP1*: rs2865531; *DAAM2*: rs3008798, rs1318002, rs2395730; *FAM13A1*: rs6830970, rs2869967; *GPR126*: rs9496346, rs6570507, rs11155242, rs7753012, rs3748069, rs171891, rs263178; *HDAC4*: rs12477314; *HHIP*: rs1032295, rs1512285, rs720485, rs1828591, rs13118928, rs1512288, rs6817273; *HTR4*: rs3995090, rs1833710; *INTS12-GSTCD-NPNT*: rs3960769, rs17035917, rs17035960, rs11727735, rs10516526, rs11731417; *KCEN2*: rs9978142; *LRP1*: rs11172113; *MCOM*: rs1344555; *MFAP2*: rs2284746; *MMP15*: rs2304488, rs12447804; *MTMR3*: rs17646919; *NCR3*: rs2857595; *NOTCH4*: rs206015; *ONECUT1*: rs2456526; *PID1*: rs1435867, rs1358443, rs3845823; *PTCH1*: rs10512249, rs576594; *RARB*: rs1529672; *SPATA9*: rs153916; *TGFβ2*: rs993925; *THSD4*: rs12899618; *THSD4*: rs1568010, rs1913768; *TNSI*: rs918949, rs1035672, rs929937; *ZKSCAN3*: rs6903823. Nonsignificant associations are reported in this article's Online Repository.

†Baseline cross-sectional lung function was calculated by using the Quanjer formula.³⁴

‡Proxies tested for cross-sectional association: for rs12447804-rs2304488 (r^2 , 0.87; D', 1); for rs12477314-rs4521068 (r^2 , 1; D', 1); and for rs2865531-rs12917651 (r^2 , 1; D', 1).

significant heterogeneity according to disease status. In the replication phase this heterogeneity was also confirmed for the *DLEU7* locus that was associated with FEV₁ decrease in nonasthmatic participants only. Finally, many of the SNPs identified by previous GWASs on lung function exhibited associations specific to asthma status.

The finding of genetic heterogeneity in lung function reported here is consistent with available evidence. Differences in familial segregation of FEV₁ in asthmatic and nonasthmatic families previously suggested genetic heterogeneity between these 2 groups.²⁴ Agnostic studies investigating genetic determinants of lung function in both family-based^{21,22,35-38} and population-based^{15-18,23,25} samples produced little overlap in chromosomal regions. Genome-wide scans on lung function in families with asthma^{21,39} or COPD²² also suggested a heterogeneous genetic architecture of lung function.

Nevertheless, some previously reported overlapping linkage regions for the ratio of FEV₁ over vital capacity (FEV₁/VC) and FEV₁ over FVC (FEV₁/FVC) in families with asthma and COPD^{21,22} suggest that at least some gene or genes could be important in the development of airway obstruction in both diseases. Furthermore, genetic polymorphisms in glutathione S-transferases,⁴⁰⁻⁴³ as well as *ADAM33*,⁴⁴⁻⁴⁷ were associated with lower lung function at all ages and in different subgroups of the population (general population, patients with COPD, and asthma patients). Gene-lung function associations that are of relevance to several population and patient strata might be determined specifically by complex gene-gene and gene-environment interactions, as suggested for lung function decrease and its complex association with estrogen receptor 1 polymorphisms, smoking, steroid use, and sex.^{32,48} Although ignored in both ours and previous GWASs, such effect modifications should be considered in the future.⁴⁹

Results from the Busselton Health Study on familial aggregation and heritability of adult lung function previously suggested the existence of genetic determinants of adult lung function independent of asthma, atopy, cigarette smoking, height, age, or sex.²⁵ Consistent with these results, neither asthma, atopy, and COPD genes previously identified in large GWASs^{5-9,11} nor genes related to smoking behavior⁵⁰ were associated with lung function decrease in our study. The association of FEV₁ decrease with a gene related to height, *DLEU7*, ranked high but only in subjects without asthma ($rs9316500$, $P_{\text{discovery}} = 4.8 \times 10^{-6}$; $P_{\text{replication}} = .03$). *DLEU7* gene product and expression remain poorly characterized, but its mRNA has been detected in the lung. The *DLEU7* locus was identified as a determinant of adult height in previous GWAS meta-analyses.⁵¹⁻⁵³ Three other height genes, *HHIP*, *GPR126*, and *PTCH*, were associated with cross-sectional lung function.¹⁵⁻¹⁷ All of these lung function models, including ours, were adjusted for adult height. The observed association, related to both *HHIP* and *DLEU7* being associated with peak height velocity in infancy,⁵² suggests that aspects beyond adult height influence lung function and possibly its response to nongenetic determinants. Several genes implicated in respiratory diseases indicate that early lung development affects respiratory health later in life.²⁰ Sensitivity analyses are supportive for a growth-specific role of *DLEU7*. The association of genetic variants in *DLEU7* with decrease in FEV₁ disappeared in analyses considering baseline lung function or restricted to subjects older than 30 years with no remaining physiologic lung growth. There might be a link between physiologic growth and unregulated cell differentiation because the *DLEU7* gene is also a proposed tumor

suppressor gene in patients with chronic lymphocytic leukemia.⁵⁴⁻⁵⁶ Evidence emerges for a role of *DLEU7* in counterbalancing the proliferative effect of nuclear factor κ B on various cell types.⁵⁷ The potential role of the gene product of *TUSC3*, a proposed tumor suppressor gene,⁵⁸ in lung physiology is discussed in this article's Online Repository.

None of the SNPs identified in GWASs of cross-sectional lung function¹⁵⁻¹⁸ ranked high in this current GWAS on lung function decline. A strong risk factor for accelerated lung function decrease in adulthood is cigarette smoking, but our study was too small to assess gene-smoking interaction at the GWAS level. We had decided *a priori* against smoking adjustment because it is not a confounder, and any link between genotype and smoking is likely to be, at least in part, in the same causal pathway (eg, gene products metabolizing tobacco constituents or influencing smoking behavior). Their identification as determinants of lung function decrease is of public health importance. Consistent with previous GWASs on cross-sectional lung function,¹⁵⁻¹⁸ neither the *TUSC3* (heterogeneity between ever/never smokers, $P = .98$) nor other top-hit signals were modified by smoking except for SNPs in *SYNE2*, *RORA*, *BCAS1*, and *PLXN4*.

Arguments for biological plausibility are mentioned in this article's Online Repository.

The strength of the present study is the longitudinal design of all cohorts included. Repeated spirometric assessments within the same subject are thought to capture more precisely exogenous factors and genes leading to accelerated loss of lung function in adulthood.⁵⁹ The discovery cohorts shared comparable questionnaire and spirometric protocols, and they were specifically designed to investigate environmental and genetic causes of lung function decrease and asthma in a standardized way. Each study has 2 measures of prebronchodilator lung function about 10 years apart, but clearly, our findings would be more robust if further lung function measures were available over an even longer period of follow-up. All discovery cohorts have used the same genotyping platform, and stringent quality control criteria have been applied.

Sample size is a limitation of this study and remains a general challenge in lung function studies with a need for high phenotypic comparability because spirometric results are sensitive to technicians and devices used.⁶⁰ The prebronchodilation lung function measurements in our and previous lung function GWASs do not allow one to differentiate reversible from nonreversible obstruction to airflow. Populations included in this study differed by age, which is also reflected by the diverging proportion of subjects with FEV₁/FVC ratios of less than 0.7 at follow-up between the discovery cohorts. Discovery and replication populations also differ by time spacing between the spirometric assessments. We can only speculate on the overall effect of such differences. We do note that replication results were sensitive to the exclusion of ARIC data (the study with highest mean age, largest annual decrease, and shortest follow-up time).

Other limitations are shared with any GWAS meta-analyses investigating complex phenotypes, such as lack in power for investigating gene-environment interactions or studying subgroups of diseases. Because the sample size of our study was comparatively small, especially for the asthmatic sample in the replication phase, we had limited ability to address differences in asthma subphenotypes or the effect of asthma medication intake. It is also likely that a substantial part of complex disease might be explained by rare mutations not considered by current GWASs. Finally, assessing the joint effect of SNPs having small effects

individually and potentially interacting with each other remains another challenge.

In conclusion, this first GWAS meta-analysis on lung function decline provides suggestive evidence for genetic heterogeneity between persons with and without asthma and between cross-sectionally and longitudinally measured lung function. Consistent with cross-sectional GWASs, our results are also suggestive of height-related genes playing a role. Further studies in this area would be enhanced by greater comparability of age range, spacing of lung function assessments, and asthma subphenotypes (including treatment) to decrease phenotypic heterogeneity and therefore increase statistical power to detect true association candidate loci.⁶¹

EGEA: We thank the EGEA cooperative group: *Coordination:* F. Kauffmann; F. Demeais (genetics); I. Pin (clinical aspects). *Respiratory epidemiology:* Inserm U 700, Paris M. Korobaef (Egea1), F. Neukirch (Egea1); Inserm U707, Paris: I. Annesi-Maesano; Inserm CESP/U 1018, Villejuif: F. Kauffmann, N. LeMoual, R. Nadif, M. P. Oryszczyn; Inserm U 823, Grenoble: V. Siroux *Genetics:* Inserm U 393, Paris: J. Feingold; Inserm U 946, Paris: E. Bouzigon, F. Demeais, M. H. Dizier; CNG, Evry: I. Gut, M. Lathrop. *Clinical centers:* Grenoble: I. Pin, C. Pison; Lyon: D. Ecochard (Egea1), F. Gormand, Y. Pacheco; Marseille: D. Charpin (Egea1), D. Vervloet; Montpellier: J. Bousquet; Paris Cochin: A. Lockhart (Egea1), R. Matran (now in Lille); Paris Necker: E. Paty, P. Scheinmann; Paris-Trousseau: A. Grimfeld, J. Just. *Data and quality management:* Inserm ex-U155 (Egea1): J. Hochez; Inserm CESP/U 1018, Villejuif: N. Le Moual, Inserm ex-U780: C. Ravault; Inserm ex-U794: N. Chateigner; Grenoble: J. Ferran.

The authors thank all those who participated in the setting of the study and on the various aspects of the examinations involved: interviewers, technicians for lung function testing, coders, those involved in quality control and data management, and all those who supervised the study in all centers. The authors are grateful to the 3 CIC-Inserm of Necker, Grenoble and Marseille who supported the study and in which subjects were examined. They are indebted to all the subjects who participated, without whom that study would not have been possible.

SAPALDIA: The study could not have been done without the help of the study participants, technical and administrative support, and medical teams and field workers at the local study sites. *Local fieldworkers:* Aarau: M. Broglie, M. Bünter, D. Gashi, Basel: R. Armbruster, T. Damm, U. Egermann, M. Gut, L. Maier, A. Vögelin, L. Walter, Davos: D. Jud, N. Lutz, Geneva: M. Ares, M. Bennour, B. Galobardes, E. Namer, Lugano: B. Baumberger, S. Boccia Soldati, E. Gehrig-Van Essen, S. Ronchetto, Montana: C. Bonvin, C. Burrus, Payerne: S. Blanc, A. V. Ebinger, M. L. Fragnière, J. Jordan, Wald: R. Gimmi, N. Kourkoulos, U. Schafroth. *Administrative staff:* N. Bauer, D. Baehler, C. Gabriel, R. Gutknecht. **SAPALDIA Team:** *Study directorate:* T. Rochat, J. M. Gaspoz, N. Künzli, L. J. S. Liu, N. M. Probst Hensch, C. Schindler. *Scientific team:* J. C. Barthélémy, W. Berger, R. Bettschart, A. Bircher, G. Bolognini, O. Brändli, C. Brombach, M. Brutsche, L. Burdet, M. Frey, U. Frey, M. W. Gerbase, D. Gold, E. de Groot, W. Karrer, R. Keller, B. Knöpfli, B. Martin, D. Miedinger, U. Neu, L. Nicod, M. Pons, F. Roche, T. Rothe, E. Russi, P. Schmid-Grendelmeyer, A. Schmidt-Trucksäss, A. Turk, J. Schwartz, D. Stolz, P. Straehl, J. M. Tschopp, A. von Eckardstein, E. Zemp Stutz. *Scientific team at coordinating centers:* M. Adam, E. Boes, P. O. Bridevaux, D. Carballo, E. Corradi, I. Curjuric, J. Dratva, A. Di Pasquale, L. Grize, D. Keidel, S. Kriemler, A. Kumar, M. Imboden, N. Maire, A. Mehta, F. Meier, H. Phuleria, E. Schaffner, G. A. Thun, A. Ineichen, M. Ragetti, M. Ritter, T. Schikowski, G. Stern, M. Tarantino, M. Tsai, M. Wanner.

ECRHS: The European Community Respiratory Health Survey is a collaboration of European research groups, many of whom also agreed to provide blood samples for genotyping as part of the GABRIEL initiative. Investigators in the collaborating centers are Debbie Jarvis, Matthias Wjst, Manolis Kogevinas, Rain Jogi, Christer Janson, Karl Franklin, Ernst Omenaas, Benedicte Leynaert, Isabelle Pin, Joachim Heinrich, Nino Kuenzli, Nicole M. Probst-Hensch, Josep M. Anto, Jordi Sunyer, Jose-Antonio Maldonado, Jesus

Martinez-Moratalla, Isabel Urritia, and Felix Payo. EGEA, SAPPALDIA, and ECRHS were part of the GABRIEL Consortium, a European 6th Framework Research project on asthma genetics, which allowed us to obtain the genotype information used in this analysis.

ARIC: The authors thank the staff and participants of the ARIC study for their important contributions. Grace Chiu at Westat Inc (Research Triangle Park, NC); Shuangshuang Dai at the National Institute of Environmental Health Sciences; and Richard Howard at the University of North Carolina School of Public Health provided data management and programming assistance. FHS research was conducted by using data and resources from the FHS of the National Heart, Lung, and Blood Institute of the National Institutes of Health and Boston University School of Medicine. The analyses reflect intellectual input and resource development from the FHS investigators participating in the SNP Health Association Resource (SHARe) project.

B58C: We acknowledge use of phenotype and genotype data from the British 1958 Birth Cohort DNA collection funded by the Medical Research Council grant G0000934 and the Wellcome Trust grant 068545/Z/02. (<http://www.b58cgenegs.gul.ac.uk/>). Genotyping for the B58C-WTCCC subset was funded by the Wellcome Trust grant 076113/B/04/Z. The B58C-TIDGC genotyping used resources provided by the Type 1 Diabetes Genetics Consortium, a collaborative clinical study sponsored by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institute of Allergy and Infectious Diseases (NIAID), National Human Genome Research Institute (NHGRI), National Institute of Child Health and Human Development (NICHD), and Juvenile Diabetes Research Foundation International (JDRF) and supported by U01 DK062418. B58C-TIDGC GWAS data were deposited by the Diabetes and Inflammation Laboratory, Cambridge Institute for Medical Research (CIMR), University of Cambridge, which is funded by Juvenile Diabetes Research Foundation International, the Wellcome Trust and the National Institute for Health Research Cambridge Biomedical Research Centre; the CIMR is in receipt of a Wellcome Trust Strategic Award (079895). The B58C-GABRIEL genotyping was supported by a contract from the European Commission Framework Programme 6 (018996) and grants from the French Ministry of Research.

Sources of support:

Discovery cohorts: ESE (EGEA-SAPALDIA-ECRHS)

EGEA: INSERM–Ministry of Research ‘Cohortes et Collections’ grant (4CH06G), French Ministry of Higher Education and Research, University Paris Diderot-Paris 7, grants from the French Agency for Environmental and Occupational Health Safety (grant AFSSETAPR SE-2004), the French National Agency for Research (grants ANR 05-SEST-020-02/05-9-97 and ANR 06-CEBS), PHRC-Paris, Merck Sharp & Dohme [MSD]).

SAPALDIA: Swiss National Science Foundation (grants 4026-28099, 3347CO-108796, 3247BO-104283, 3247BO-104288, 3247BO-104284, 32-65896.01, 32-59302.99, 32-52720.97, 32-4253.94); the Federal Office for Forest, Environment, and Landscape; the Federal Office of Public Health; the Federal Office of Roads and Transport; the canton’s government of Aargau, Basel-Stadt, Basel-Land, Geneva, Luzern, Ticino, Zurich; the Swiss Lung League; the canton’s Lung League of Basel Stadt/Basel Landschaft, Geneva, Ticino and Zurich; Freie Akademische Gesellschaft (FAG); UBS Wealth Foundation.

ECRHS: The coordination of ECRHS II was supported by the European Commission, as part of their Quality of Life programme. The following bodies funded the local studies in ECRHS II: **Albacete:** Fondo de Investigaciones Santarias (FIS) (grants 97/0035-01, 99/0034-01, and 99/0034-02), Hospital Universitario de Albacete, Consejería de Sanidad; **Barcelona:** SEPAR, Public Health Service (grant R01 HL62633-01), Fondo de Investigaciones Santarias (FIS) (grants 97/0035-01, 99/0034-01, and 99/0034-02) CIRIT (grant 1999SGR 00241) Red Respira ISCII; CIBER Epidemiología y Salud Pública (CIBERESP), Spain **Basel:** Swiss National Science Foundation, Swiss Federal Office for Education & Science, Swiss National Accident Insurance Fund (SUVA), USC NIEHS Center grant 5P30 ES07048; **Bergen:** Norwegian Research Council, Norwegian Asthma & Allergy Association (NAAF), Glaxo Wellcome AS, Norway Research Fund; **Erfurt:** GSF-National Research Centre for Environment & Health, Deutsche Forschungsgemeinschaft (DFG) (grant code FR 1526/1-1); **Galdakao:** Basque Health Dept; **Grenoble:** Programme Hospitalier de Recherche

Clinique-DRC de Grenoble 2000 no. 2610, Ministry of Health, Direction de la Recherche Clinique, CHU de Grenoble, Ministère de l'Emploi et de la Solidarité, Direction Générale de la Santé, Comité des Maladies Respiratoires de l'Isère; **Hamburg:** GSF-National Research Centre for Environment & Health, Deutsche Forschungsgemeinschaft (DFG) (grant code MA 711/4-1); **Ipswich and Norwich:** Asthma UK (formerly known as National Asthma Campaign); **Huelva:** Fondo de Investigaciones Sanitarias (FIS) (grant code: 97/0035-01, 99/0034-01 and 99/0034-02); **Oviedo:** Fondo de Investigaciones Sanitarias (FIS) (grant code: 97/0035-01, 99/0034-01 and 99/0034-02); **Paris:** Ministère de l'Emploi et de la Solidarité, Direction Générale de la Santé, UCB-Pharma (France), Aventis (France), Glaxo France, Programme Hospitalier de Recherche Clinique-DRC de Grenoble 2000 no. 2610, Ministry of Health, Direction de la Recherche Clinique, CHU de Grenoble; **Tartu:** Estonian Science Foundation; **Umeå:** Swedish Heart Lung Foundation, Swedish Foundation for Health Care Sciences & Allergy Research, Swedish Asthma & Allergy Foundation, Swedish Cancer & Allergy Foundation; **Uppsala:** Swedish Heart Lung Foundation, Swedish Foundation for Health Care Sciences & Allergy Research, Swedish Asthma & Allergy Foundation, Swedish Cancer & Allergy Foundation; *Financial support for ECRHS I for centers in ECRHS II was provided by:* Ministère de la Santé, Glaxo France, Institut Pneumologique d'Aquitaine, Contrat de Plan Etat-Région Languedoc-Rousillon, CNMATS, CNMRT (90MR/10, 91AF/6), Ministre délégué de la santé, RNSP, France; GSF, and the Bundesminister für Forschung und Technologie, Bonn, Germany; Norwegian Research Council project no. 101422/310; Ministerio Sanidad y Consumo FIS (grants #91/0016060/00E-05E and #93/0393), and grants from Hospital General de Albacete, Hospital General Juan Ramón Jiménez, Consejería de Sanidad Principado de Asturias, Spain; The Swedish Medical Research Council, the Swedish Heart Lung Foundation, the Swedish Association against Asthma and Allergy; Swiss National Science Foundation grant 4026-28099; National Asthma Campaign, British Lung Foundation, Department of Health, South Thames Regional Health Authority, UK. A.R. was supported by the Department of Health, UK and the European Commission as part of GABRIEL contract number 018996 under the Integrated Program LSH-2004-1.2.5-1.

Genotyping of the discovery cohort and part of B58C was funded by the GABRIEL asthma genetic consortium supported by a contract from the European Commission (018996) and grants from the French Ministry of Research, the Wellcome Trust (WT084703MA), and Asthma UK.

Replication cohorts:

ARIC: The Atherosclerosis Risk in Communities Study is carried out as a collaborative study supported by National Heart, Lung, and Blood Institute contracts (HHSN268201100005C, HHSN268201100006C, HHSN268201100007C, HHSN268201100008C, HHSN268201100009C, HHSN268201100010C, HHSN268201100011C, and HHSN268201100012C), R01HL087641, R01HL59367 and R01HL086694; National Human Genome Research Institute contract U01HG004402; and National Institutes of Health contract HHSN268200625226C. Infrastructure was partly supported by Grant Number UL1RR025005, a component of the National Institutes of Health and NIH Roadmap for Medical Research. Work for this manuscript was supported, in part, by the Intramural Research Program of the National Institutes of Health (NIH), National Institute of Environmental Health Sciences (NIEHS, Z01ES043012).

FHS: National Heart, Lung and Blood Institute's FHS (Contract No. N01-HC-25195) and its contract with Affymetrix, Inc for genotyping services (Contract No. N02-HL-6-4278). Dr. Wilk by a Young Clinical Scientist Award from the Flight Attendant Medical Research Institute (FAMRI). A portion of this research utilized the Linux Cluster for Genetic Analysis (LinGA-II) funded by the Robert Dawson Evans Endowment of the Department of Medicine at Boston University School of Medicine and Boston Medical Center.

B58C: British 1958 Birth Cohort was funded by the Medical Research Council grant G0000934 and the Wellcome Trust grant 068545/Z/02 (<http://www.b58c.gene.sgu.ac.uk/>). Genotyping was funded by the Wellcome Trust grant 076113/B/04/Z, by the United States National Institutes of Health and the Juvenile Diabetes Research Foundation U01 DK062418 and by the European Commission Framework Programme 6 (018996).

Dutch Asthma Study: The Dutch Asthma study has been funded by the Netherlands Asthma Foundation grants AF 3.2.07.015; and AF 98.48 and a grant from the University Medical Center Groningen.

Key messages

- Knowledge regarding genes with pleiotropic effects on asthma, COPD, and lung function level and longitudinal course is limited.
- This first GWAS meta-analysis on lung function decline conducted separately in nonasthmatic and asthmatic cohort participants suggests that genetic determinants of lung function decline are different in the 2 groups.
- The results further suggest that previously identified genetic determinants of cross-sectional lung function are not major determinants of the decline.

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