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ABSTRACT

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

Aim: We conducted the first GWAS on age-related decline in forced expiratory volume in the first second (FEV1) and in its ratio to forced vital capacity (FVC) stratified *a priori* by asthma status.

Methods: Discovery cohorts included adults of European ancestry (1441 asthmatics, 2677 non-asthmatics; Epidemiological Study on the Genetics and Environment of Asthma (EGEA); Swiss Cohort Study on Air Pollution And Lung And Heart Disease In Adults (SAPALDIA); European Community Respiratory Health Survey (ECRHS)). The associations of FEV1 and FEV1/FVC decline with 2.5 million single nucleotide polymorphisms (SNPs) were estimated. Thirty loci were followed-up by *in silico* replication (1160 asthmatics, 10858 non-asthmatics: Atherosclerosis Risk in Communities (ARIC); Framingham Heart Study (FHS); British 1958 Birth Cohort (B58C); Dutch asthma study).

Results: Main signals identified differed between asthmatics and non-asthmatics. None of the SNPs reached genome-wide significance. The association between the height related gene *DLEU7* and FEV1 decline suggested for non-asthmatics in the discovery phase was replicated (discovery $P=4.8 \times 10^{-6}$; replication $P=0.03$) and additional sensitivity analyses point to a relation to growth. The top ranking signal, *TUSC3*, associated with FEV1/FVC decline in asthmatics ($P=5.3 \times 10^{-8}$) did not replicate. SNPs

previously associated with cross-sectional lung function were not prominently associated with decline.

Conclusions: Genetic heterogeneity of lung function may be extensive. Our results suggest that genetic determinants of longitudinal and cross-sectional lung function differ and vary by asthma status.

Key Messages:

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

Capsule summary:

This meta-analysis provides evidence for genetic heterogeneity of lung function between asthmatics and non-asthmatics; and between cross-sectionally and longitudinally measured lung function. The study adds evidence for the role of height-related genes in lung health.

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Keywords:

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

Abbreviations:

ARIC, Atherosclerosis Risk in Communities Study

ATS, American Thoracic Society

B58C, British 1958 Birth Cohort

chr, chromosome

COPD, chronic obstructive pulmonary disease

ECRHS, European Community Respiratory Health Survey

EGEA, Genetics and Environment of Asthma

FEV1, forced expiratory volume in the first second

FHS, Framingham Heart Study

FVC, forced vital capacity

GWAS, genome-wide association studies

HapMap, Haplotype Map Project

Q-Q, Quantile-quantile

SAPALDIA, Swiss Cohort Study on Air Pollution And Lung And Heart Disease In Adults

SNP, single nucleotide polymorphism

INTRODUCTION

Low lung function is a feature of both asthma and chronic obstructive pulmonary disease (COPD), with twin studies demonstrating strong heritability (0.51 to 0.77) for forced expiratory volume in the first second (FEV1)[1, 2]. The two 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 (GWAS) have identified novel genetic loci for asthma[5-10], COPD[11-14], and lung function[15-18] 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 may 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 GWAS for lung function in the general population[15-18], with the exception of *HHIP* and *FAM13A* being associated with both lung function[15-18] and COPD[11-14]. Several lines of evidence suggest that different genes influence lung function in asthmatics and in non-asthmatics. Genome-scans in family based linkage studies identified some, but overall limited overlap between chromosomal regions linked to lung function in asthmatics[21], COPD patients[22] and in the general population[23] and it has been suggested that genetic variation may be more important for lung function in asthma after adjusting for smoking and body size differences[21, 24, 25].

Here, we present results from the first lung function GWAS conducted separately for asthmatics and non-asthmatics. This current study also focuses on the rate of lung function decline in adults instead of cross-sectional lung function parameters tested in

263 previous GWAS[15-18]. The discovery cohorts included two population-based studies
264 (SAPALDIA and ECRHS) and one asthma family-based study (EGEA), all of European
265 ancestry with highly comparable and standardized assessment of respiratory health
266 parameters including spirometry from two time points ten years apart. These three
267 studies had been included in the GWAS for asthma conducted by the GABRIEL
268 consortium[7]. Replication cohorts included three population-based cohorts (FHS, ARIC,
269 B58C) and one family-based asthma study (the Dutch Asthma Study).

METHODS

- Discovery cohorts and study population: Three large multi-centric cohorts EGEA[26], SAPALDIA[27] and ECRHS[28] constitute the ESE-consortium. Personal factors of relevance to lung function decline were assessed by interview and anthropometric measurements at baseline and follow-up. Participants included in 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 examination 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 measure from both surveys. Cohort study protocols were in agreement with the Declaration of Helsinki and obtained ethical approval from their respective regional and/or national review boards.

- Lung function assessments, asthma status and genotypes: At each visit, a minimum of two acceptable forced expiratory flows, forced vital capacity (FVC) and forced expiratory volume in the first second (FEV1) complying with American Thoracic Society criteria were obtained[26-29]. No bronchodilator was administered. Based on questionnaire data, asthmatics were defined as asthma self-report at any of the completed surveys and family-based studies considered additional clinical asthma criteria (see online repository). 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 Hapmap2 reference panel, investigation of

population stratification and quality control criteria are described in Figure EI and Table EI in the Online Repository.

-Replication Cohorts: Four cohorts of European ancestry with available genome-wide data, ARIC[30], FHS[15]; B58C[31]; Dutch asthma study[32] were used for replication. Subjects included in the current analysis were older than 24 years, had complete information on covariates (age, height, and sex) and valid lung function measures from at least two time-points. The lung function measurements were conducted at least ten years apart, except three years apart for ARIC (Table I). Distinct genotype data platforms and imputation software were used (Table EII, Online Repository).

- Statistical analysis: Annual decline in FEV1 and FEV1/FVC was calculated as difference between follow-up and baseline spirometric measurements (mL for FEV1 and % for FEV1/FVC) 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 centre in asthmatics and non-asthmatics separately. Comparability between studies of standardized residuals was tested using Wilcoxon-Mann-Whitney test ($P>0.94$). The standardized residuals were used as dependent variable and regressed on genome-wide single nucleotide polymorphisms (SNPs) adjusted for study-specific principal components capturing population ancestry (see online supplement for details). Study-specific SNP effect estimates were combined through meta-analysis using fixed and random effects models. We used a threshold of $P<5x10^{-8}$ (the Bonferroni adjustment for one million independent tests) to declare a pooled effect as genome-wide significant. Selection criteria for replication loci are described in the methods section of the online repository. SNPs with suggestive evidence of association with

315 decline in FEV1 or FEV1/FVC were chosen for *in silico* replication (Table EIII, Online
316 Repository). Study-specific regression models and meta-analyses across replication
317 cohorts were as described for the discovery phase. Replication cohorts with spirometry
318 data from more than two different time points modelled the lung function decline
319 phenotype by fitting a least-squares slope using the available data (FHS, Dutch asthma
320 study). P≤0.05 was considered as statistically significant at the replication level.
321 The results of the main meta-analyses for the top 1000 SNPs are available in the online
322 repository (Table EIV A to D, Online Repository). We also conducted a meta-analysis by
323 combining non-asthmatic and asthmatic samples and tested for heterogeneity between
324 these samples (Table EV, Online Repository). Additional sensitivity analyses were done
325 by: a) restricting the GWAS sample to subjects aged 30 and older for FEV1 decline
326 (Table EIV E and F, Online Repository); b) conducting GWAS analyses on percent
327 change instead of absolute annual decline in lung function (Table EIV G to J, Online
328 Repository); c) investigating smoking stratified joint effects for replications SNPs (Table
329 EVI, Online Repository); d) excluding ARIC, a cohort having substantially shorter follow-
330 up time that the other cohorts (three years instead of ten years) from replication
331 analyses (Table EVII, Online Repository). Methods and results of these additional
332 analyses are described in the online repository.
333

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 subjects with FEV1/FVC below 70% (Table I, Table EVIII, Online Repository). Baseline lung function parameters, but not their annual changes were lower in asthmatics when compared to non-asthmatics in each study. The proportion of never smokers was comparable among asthmatics, but varied among non-asthmatics (ranging from 28.5% in B58C to 46.5% in EGEA). No substantial differences in the smoking prevalence between people with and without asthma were observed within each study. Comparing the discovery cohorts in more detail (Table EVIII, Online Repository), atopy (total IgE ≥100kU/ml) and hay fever were more prevalent in both asthmatics and non-asthmatics from EGEA when compared to 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, in agreement with the study design. Asthmatics from EGEA had a younger age of disease onset due to the mode of recruitment of the proband.

Main findings from meta-analyses of discovery and replication phase

In the discovery phase, GWAS meta-analysis of decline in FEV1 and FEV1/FVC was conducted in 2677 non-asthmatics and in 1441 asthmatics. Genomic inflation factors were low for both lung function parameters (λ<1.047, Table EIX, Online Repository) suggesting minimal unaccounted population stratification. The replication panel included

a total of 10'858 non-asthmatics and 1'138 asthmatics. Thirty lead SNPs belonging to 30 loci ($5x10^{-8}< P_{discovery} <6x10^{-5}$) were chosen for replication.

The four lung function parameter- and asthma-specific meta-analyses identified one association signal that almost reached the genome-wide significance level ($P = 5.3x10^{-8}$) at the locus 8p22 containing the *TUSC3* gene for FEV1/FVC decline in asthmatics while all other signals had $P<5x10^{-7}$ (Figure I), but this signal was not associated with FEV1/FVC decline in asthmatics in the replication sample. The only locus of the selected replication candidate loci that formally replicated was 13q14.3, containing the *DLEU7* gene, associated with decline in FEV1 in the non-asthmatics ($P_{discovery}=4.8x10^{-6}$ and $P_{replication}=0.03$).

In the global *post hoc* analysis combining both asthmatics and non-asthmatics (N=4118), a striking finding was the absence of any pronounced association signals ($P >1x10^{-6}$) despite increased statistical power. This was in agreement with the minimal overlap of association signals observed in asthmatics and non-asthmatics separately. Most signals at $P<10^{-5}$ from the asthma-stratified analysis in the discovery phase exhibited statistically significant heterogeneity of effects between the two groups (Table II). At the replication stage, none of the replication SNPs was associated with lung function decline in asthmatics and non-asthmatics combined.

Association signals for annual decline in FEV1 in non-asthmatics

Of fifteen SNPs associated at $P<10^{-5}$ with decline in FEV1 in non-asthmatics ten were clustered at position 112.3 Mb on chromosome 9, containing genes *TXN*, *MUSK* and *SVEP1*. Two of the 15 SNPs were located at 13q14.3 in a locus containing the *DLEU7*

gene; three SNPs belonged to three distinct loci. The association of lead and proxy SNPs in *DLEU7* (Figure II), but not *TXN/MUSK/SVEP1 (*Figure EII) or the other SNPs (Table II) replicated. The G-allele of SNP rs9316500 near the *DLEU7* gene was positively associated with annual FEV1 decline in the discovery cohorts ($P=4.8x10^{-6}$) and in the replication cohorts (P=0.026). Although heterogeneity between studies was not significant (P=0.61), the combined P value did not reach the genome-wide level ($P=5.7x10^{-5}$).

Association signals for annual decline in FEV1 in asthmatics

Eighteen SNPs in nine distinct chromosomal locations were associated with decline in FEV1 in asthmatics at $P<10^{-5}$. None of the loci selected for *in silico* replication was confirmed (Table II).

Association signals for annual decline in FEV1/FVC in non-asthmatics

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

Association signals for annual decline in FEV1/FVC in asthmatics

Twelve SNPs at the locus 8p22 containing the gene *TUSC3* at 15.68Mb were associated with FEV1/FVC decline at $P<10^{-7}$ in asthmatics (Figure I). Regional locus plot and forest plot are presented in the online repository (Figure EIII). The top association signals in this locus were conferred by distinct SNPs in each cohort, though apparently they were located in the same putative haplotype segment in SAPALDIA and

403 in EGEA (Figure EIV, Online Repository). There was no statistically significant
404 association in ECRHS. Meta-analysis of the discovery samples identified SNP
405 rs4831760 as top signal in *TUSC3* gene, but heterogeneity between discovery studies
406 was borderline significant (P=0.07). The C-allele ($P=5.3x10^{-8}$) was positively associated
407 with annual decline in FEV1/FVC in asthmatics (Beta=0.22 ±0.04 (standard error); Table
408 II). However this association was not replicated (P=0.80). In the meta-analysis
409 combining discovery and replication samples the resulting P-value for rs4831760 was
410 $2.8x10^{-5}$. All but the Dutch asthma study, exhibited effect estimates in the same
411 direction as the discovery panel. Two other candidate loci (*MPP7* and *SYNE2)* also
412 failed replication testing.
413
414 *SNPs previously associated in GWAS meta-analyses on cross-sectional lung function*
415 The associations of top hit SNPs from previous GWAS meta-analyses on cross-
416 sectional lung function[11, 15-18] and a replication study in asthmatics[33] were assessed
417 separately for asthmatics and non-asthmatics in the discovery cohorts. Associations
418 were assessed for both, lung function parameters of decline (annual decline and
419 percent change) and cross-sectional lung function level. Overall, a subset of variants
420 and loci showed replication of association with cross-sectional lung function in either
421 non-asthmatics or asthmatics. Few of the loci showed strong association with decline in
422 lung function. We present associations at P<0.05 in Table III and those at P≥0.05 in
423 Table EX in the online repository.
424 For baseline FEV1, we observed associations for SNPs belonging to 4q24 (*GSTCD,*
425 rs11731417, $P=1.3x10^{-4}$) and 15q23 (*THSD4,* rs1913768, P=0.003). Associations with

baseline FEV1 were mainly restricted to non-asthmatics. For baseline FEV1/FVC, associations of SNPs of *THSD4* were prominent (e.g. rs12899618, $P=3.3x10^{-4}$) and again restricted to non-asthmatics.

For decline phenotypes of FEV1, we observed associations for SNPs in regions 6p21 (*DAAM2*, 0.003<P<0.02) and 4q28 (*HHIP*, 0.02<P<0.05) among asthmatics and in *THSD4* (0.003<P<0.04) among non-asthmatics. The strongest associations observed for decline phenotypes of FEV1/FVC were two SNPs in *MMP15* (16q13, 0.003<P<0.002) in non-asthmatics, only. Association in the combined sample of asthmatics and non-asthmatics did not substantially alter the results.

Summary of findings from sensitivity analyses

We observed in non-asthmatics, aged 30 years and more, that *MUSK* and *DLEU7* were no longer prominently associated with FEV1 decline, but SNPs in other genes remained strongly associated (*ZIC1*, rs6785065, $P=2.3x10^{-5}$; *UBL3*, rs278037, $P=4.8x10^{-5}$).

Results of the GWAS on percent change in lung function showed that the FEV1 association signal for *DLEU7* in the non-asthmatics was no longer significant; however the signals for *MUSK* (rs1889321, $P=2.92x10^{-7}$) and other loci remained unaltered (*ZIC1*, rs6785065, $P=2.0x10^{-5}$; *KIRREL3*, rs11604082, $P=4.1x10^{-6}$; *KIAA2117*, rs10082549, $P=2.7x10^{-6}$). Top signals associated with decline in FEV1/FVC in asthmatics remained unaltered for *TUSC3* (rs4831760, $P=5.2x10^{-8}$) and for *SYNE2* (rs7144584, $P=6.4x10^{-7}$) 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 *SYNE2*, *RORA*, *BCAS1,* or *PLXNA4* genes.

Replication meta-analysis excluding the ARIC data substantially reduced sample size in non-asthmatics and the association of *DLEU7* with decline of FEV1 was no longer significant. Instead two loci for association with decline in FEV1 in asthmatics (*PLXNA4*, rs10808265, $P_{discovery}=1.7x10^{-6}$, $P_{replication}=0.02$ and *SLC45A3*, rs16856186, $P_{discovery}=8.9x10^{-6}$, $P_{replication}=0.04$) and one locus, FLJ25393, for decline in FEV1/FVC in non-asthmatics (rs2658782, $P_{discovery}=4.3x10^{-6}$, $P_{replication}=0.03$) gained statistical significance.

DISCUSSION

A main result of this study is the observed genetic heterogeneity of lung function decline between asthmatics and non-asthmatics. When we combined the two 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 significant heterogeneity according to the disease status. In the replication phase, this heterogeneity was also confirmed for the *DLEU7* locus which was associated with FEV1 decline in non-asthmatics only. Finally, many of the SNPs identified by previous GWAS 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 non-asthmatic families previously suggested genetic heterogeneity between these two groups[24]. Agnostic studies investigating genetic determinants of lung function in both, family-based [21, 22, 34-37] and population-based samples[15-18, 23, 25] produced little overlap in chromosomal regions. Genome-wide scans on lung function in asthma[21, 38] or COPD[22] families 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 the forced vital capacity (FEV1/FVC) in families with asthma and COPD[21, 22] suggest that at least some gene(s) could be important in the development of airway obstruction in both diseases.

Furthermore, genetic polymorphisms in glutathione S-transferases[39-42] as well as *ADAM-33*[43-46] were associated with lower lung function at all ages and in different subgroups of the population (general population, patients with COPD and asthma). Gene-lung function associations that are of relevance to several population and patient strata may be determined specifically by complex gene-gene and gene-environment interactions, as suggested for lung function decline and its complex association with estrogen receptor 1 polymorphisms, smoking, steroid use, and gender[32, 47]. While ignored in ours as well as previous GWAS, such effect modifications should be considered in the future[48].

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 GWAS[5-9, 11] nor genes related to smoking behavior[49] were associated with lung function decline in our study. The association of FEV1 decline with a gene related to height, *DLEU7*, was ranking high, but only in subjects without asthma (rs9316500, $P_{discovery}=4.8x\ 10^{-6}$; $P_{replication}=0.03$). *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[50-52]. 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

504 *DLEU7* being associated with peak height velocity in infancy[51], suggests that aspects
505 beyond adult height influence lung function and possibly its response to non-genetic
506 determinants. Several genes implicated in respiratory diseases indicate that early lung
507 development impacts respiratory health later in life[20]. Sensitivity analyses are supportive
508 for a growth-specific role of *DLEU7.* The association of genetic variants in *DLEU7* with
509 decline in FEV1 disappeared in analyses considering baseline lung function or restricted
510 to subjects above age 30 with no remaining physiologic lung growth. There might be a
511 link between physiologic growth and unregulated cell differentiation as the *DLEU7* gene
512 is also a proposed tumor suppressor gene in chronic lymphocytic leukemia[53-55].
513 Evidence emerges for a role of *DLEU7* in counterbalancing the proliferative impact of
514 NF-kB on various cell types[56]. The potential role of the gene product of *TUSC3,* a
515 proposed tumor suppressor gene[57], in lung physiology is discussed in the Online
516 Repository.
517 None of the SNPs identified in GWAS of cross-sectional lung function[15-18] ranked high in
518 this current GWAS on lung function decline. A strong risk factor for accelerated lung
519 function decline in adulthood is cigarette smoking, but our study was too small to assess
520 gene smoking interaction at the GWAS level. We had decided *a priori* against smoking
521 adjustment as it is not a confounder, and any link between genotype and smoking is
522 likely to be, at least in part, in the same causal pathway (e.g. gene products
523 metabolizing tobacco constituents or influencing smoking behavior). Their identification
524 as determinants of lung function decline is of public health importance. Consistent with
525 previous GWAS on cross-sectional lung function[15-18], neither the *TUSC3* (heterogeneity
526 between ever/never smokers P=0.98) nor other top hit signals were modified by

smoking except for SNPs in *SYNE2*, *RORA, BCAS1* and *PLXN4*. Arguments for biologic plausibility are mentioned in the Online Repository.

The strength of the present study is the longitudinal design of all cohorts included. Repeated spirometric assessments within the same subject is thought to capture more precisely exogenous factors and genes leading to accelerated loss of lung function in adulthood[58]. The discovery cohorts shared comparable questionnaire and spirometry protocols and they were specifically designed to investigate environmental and genetic causes of lung function decline and asthma in a standardized way. Each study has two measures of pre-bronchodilator lung function about ten 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 as spirometry results are sensitive to technicians and devices used[59]. The pre-bronchodilation lung function measurements in our and previous lung function GWAS do not allow to differentiate reversible from non-reversible obstruction to airflow. Populations included in this study differed by age which is also reflected by the diverging proportion of subjects with FEV1/FVC <0.7 at follow-up between the discovery cohorts. Discovery and replication populations also differ by time spacing between the spirometry assessments. We can only speculate of on the overall impact 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 decline, 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. As 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 sub-phenotypes or the impact of asthma medication intake. It is also likely that a substantial part of complex disease may be explained by rare mutations not considered by current GWAS. 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 GWAS, 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 sub-phenotypes (including treatment) to decrease phenotypic heterogeneity and therefore increase statistical power to detect true association candidate loci[60].

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818

819 **FIGURE LEGENDS:**

820 **Figure I:** Manhattan plots of association results for decline in lung function. A) FEV1
821 decline in non-asthmatics. B) FEV1 decline in asthmatics. C) FEV1/FVC decline in non-
822 asthmatics. D) FEV1/FVC decline in asthmatics.

823

824 **Figure II:** Association of the *DLEU7* locus with decline in FEV1 in non-asthmatics. A)
825 Regional association plot, discovery phase. B) Forest plot for rs9316500. A:
826 Chromosome position (NCBI build 36.3) and recombination rate (hg18 build). The
827 sentinel SNP is represented as a diamond and r2 for SNPs to the sentinel SNP
828 (HapMap CEU phase II). B: The size of the square of each study reflects the
829 contributing weight to the meta-analysis, details in Table EXI.

FOOTNOTES

Footnotes to Table I:

* N comprises the maximal number of subjects who contributed to at least one GWAS analysis (either decline in FEV1 or in FEV1/FVC).

†Time spacing between the first and the second spirometry assessment.

Footnote to Table II:

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

Footnote to Table III:

* Associations of SNPs previously associated in cross-sectional lung function in GWAS studies, (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 (MAF) 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; *MECOM*: 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; *TGFB2*: rs993925; *THSD4*: rs12899618; *THSD4*: rs1568010, rs1913768; *TNS1*: rs918949, rs1035672, rs929937; *ZKSCAN3*: rs6903823. Non-significant associations reported in online repository.

† Baseline cross-sectional lung function was calculated using Quanjer formula[61].

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

TABLES

Table I: Baseline characteristics of discovery and replication cohorts, by asthma status.

Non-asthmatics	N*	% Men	mean ± SD Age	mean ± SD Height	mean ± SD (L) FEV1	mean ± SD FEV1/FVC	mean ± SD (y) Follow-up length†	mean ± SD (mL/y) annual decline FEV1	mean ± SD (%/y) annual decline FEV1/FVC	% Never smokers
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 in silico 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
Asthmatics										
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 in silico 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	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

Table II: Association of (lead) SNPs subjected to replication with A) decline in FEV1 and B) decline in FEV1/FVC; stratified by asthma status.

A - decline in FEV1					**Discovery phase**				**Replication phase**			
dbSNP ID	**chr**	**position (build 36.3)**	**gene nearby**	**Maximal frequency of coding allele**	**Estimate of joint analysis**	**P for joint analysis**	**P for heterogeneity between studies**	**P for heterogeneity between asthmatics and non-asthmatics**	**Estimate of joint analysis in replication cohorts**	**P for joint analysis**	**P for heterogeneity between between studies**	**P for heterogeneity between asthmatics and non-asthmatics**
Non-Asthmatics												
rs1889321	9	112340656	MUSK*	0.287	-0.150	6.95E-07	0.814	**0.0187**	-0.011	0.480	0.713	0.053
rs9316500	**13**	**49992115**	**DLEU7**	**0.336**	**0.135**	**4.81E-06**	**0.613**	**0.0255**	**0.033**	**0.026**	**0.124**	**0.075**
rs6785065	3	149016533	ZIC1	0.274	-0.136	0.00001	0.234	0.1700	-0.006	0.686	0.525	0.55
rs278037	13	29322627	UBL3	0.178	-0.151	0.00002	0.364	**0.0058**	-0.006	0.734	0.231	0.50
rs7641198	3	117396577	LSAMP	0.147	0.164	0.00003	0.669	0.1997	-0.002	0.939	0.690	0.15
rs421847	21	19269950	PRSS7	0.281	0.128	0.00003	0.831	**0.0350**	-0.016	0.310	0.247	0.86
rs496809	18	74857661	SALL3	0.078	-0.236	0.00004	0.412	**0.0041**	0.022	0.443	0.373	0.60
rs10933964	3	110021881	TRAT1	0.499	-0.117	0.00006	0.345	**0.0022**	-0.015	0.265	0.869	**0.041**
Asthmatics												
rs10808265	7	131840229	PLXNA4B	0.484	-0.175	1.66E-06	0.844	**0.0020**	0.069	0.105	0.258	0.16
rs1902618	15	58951491	RORA	0.234	-0.220	1.72E-06	0.449	**0.0043**	0.029	0.590	0.777	0.58
rs3843306	1	91060718	BARHL2	0.460	0.176	5.11E-06	0.042	**8.33E-06**	0.047	0.270	0.883	0.24
rs7006290	8	41734295	ANK1	0.319	0.185	5.19E-06	0.058	**0.0003**	0.038	0.456	0.574	0.45
rs12436689	14	84723772	FLRT2	0.244	-0.212	6.87E-06	0.420	**0.0010**	-0.017	0.759	0.051	0.95
rs12615721	2	81710037	CTNNA2	0.104	-0.303	7.65E-06	0.853	**0.0020**	-0.127	0.129	0.824	0.08
rs10516809	4	89640109	HERC5	0.101	0.306	8.67E-06	0.790	**3.60E-05**	-0.060	0.446	0.200	0.41
rs16856186	1	203944749	SLC45A3	0.098	0.268	8.92E-06	0.510	**0.0034**	-0.079	0.350	0.094	0.46
rs158536	20	52148709	BCAS1	0.408	0.162	0.00002	0.948	**0.0001**	0.075	0.100	0.917	0.09
rs477725	19	42066106	ZNF345	0.158	0.223	0.00003	0.821	**0.0031**	-0.069	0.273	0.255	0.14
rs9662589	1	230344234	DISC1	0.221	0.188	0.00005	0.868	**0.0002**	-0.020	0.706	0.153	0.76

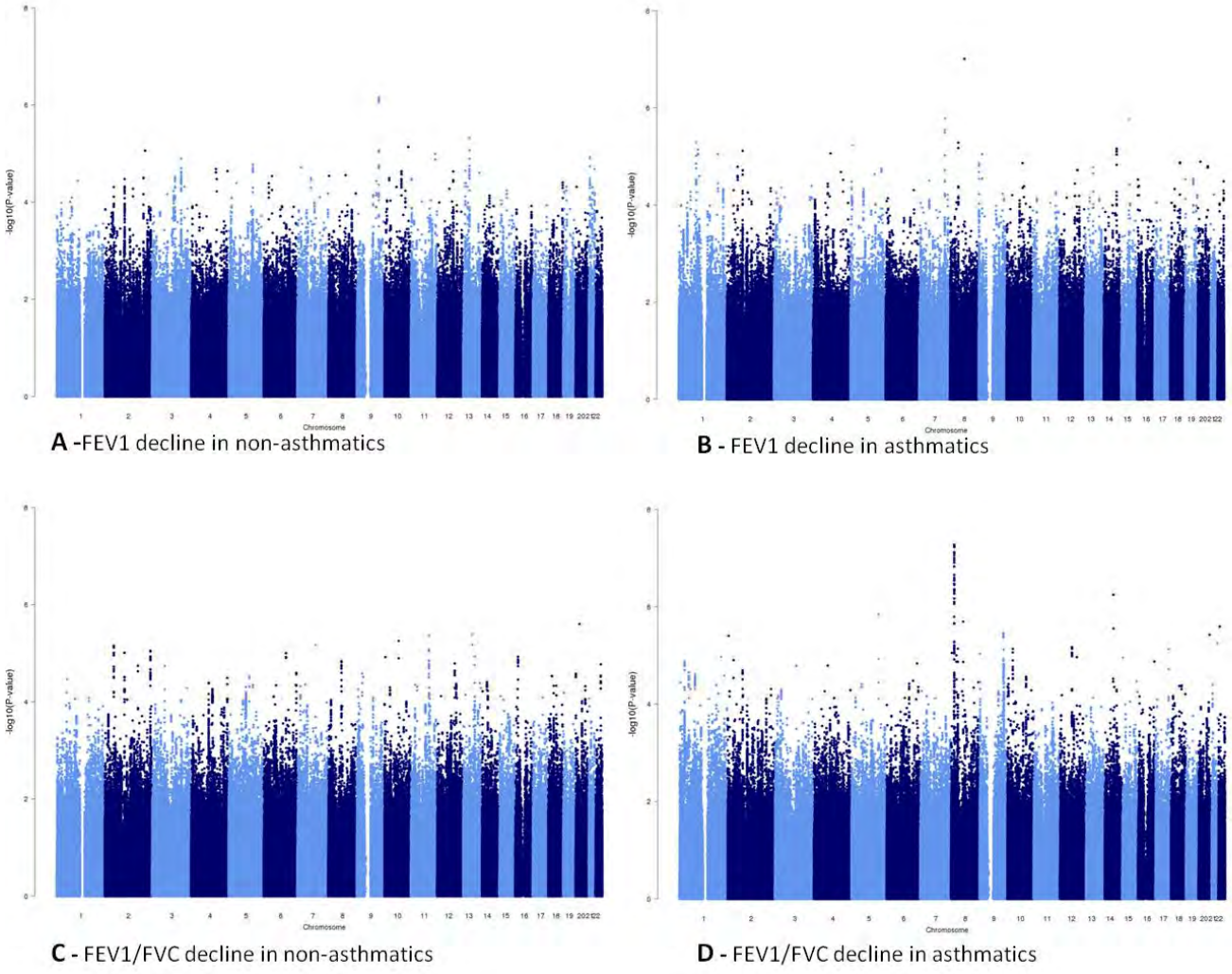
rs777433	2	128084705	LIMS2	0.407	0.151	0.00010	0.811	0.1223	-0.018	0.691	0.564	0.52
B - decline in FEV1/FVC					Discovery phase				Replication phase			
dbSNP ID	**chr**	**position (build 36.3)**	**gene nearby**	**Maximal frequency of coding allele**	**Estimate of joint analysis**	**P for joint analysis**	**P for heterogeneity between studies**	**P for heterogeneity between asthmatics and non-asthmatics**	**estimate of joint analysis in replication cohorts**	**P for joint analysis**	**P for heterogeneity between between studies**	**P for heterogeneity between asthmatics and non-asthmatics**
Non-Asthmatics												
rs2658782	11	92806379	FLJ25393	0.166	0.186	4.33E-06	0.362	**0.0041**	0.031	0.135	0.242	0.91
rs1867982	10	73197053	C10orf54	0.109	0.202	5.56E-06	0.839	**0.0034**	-0.008	0.745	0.412	0.24
rs12712969	2	46185673	PRKCE	0.268	-0.147	7.08E-06	0.687	**0.0116**	0.012	0.448	0.916	0.76
rs10187654	2	234478798	TRPM8	0.205	0.151	8.87E-06	0.797	**0.0049**	-0.015	0.382	0.676	0.15
rs356642	2	100903870	NPAS2	0.189	0.158	9.79E-06	0.162	**0.0014**	-0.010	0.565	0.282	0.28
rs890515	8	67534388	ADHFE1	0.497	0.119	0.00001	0.580	**0.0257**	0.003	0.847	0.443	0.58
rs10738890	9	32448081	DDX58	0.391	-0.118	0.00003	0.832	0.5847	-0.009	0.567	0.032	0.73
Asthmatics												
rs4831760	8	15576956	TUSC3	0.326	0.222	5.27E-08	0.066	**7.74E-08**	0.011	0.799	0.541	0.73
rs7144584	14	63345565	SYNE2	0.116	-0.318	5.62E-07	0.616	**0.0010**	0.089	0.272	0.752	0.43
rs1148186	10	28657641	MPP7	0.194	0.219	7.28E-06	0.760	**0.0035**	-0.033	0.602	0.967	0.60

Table III : Association* of SNPs previously identified in GWAS on cross-sectional lung function with percent predicted lung function at baseline, as well as percent change and annual decline in lung function for A) FEV1 and B) FEV1/FVC in ESE-discovery cohorts by asthma status.

A						Non-Asthmatics			Asthmatics		
						FEV1 percent predicted	FEV1 percent change	FEV1 decline [%/y]	FEV1 percent predicted	FEV1 percent change	FEV1 decline [%/y]
dbSNP ID	chr	position (build 36.3)	refs	gene nearby	Maximal frequency of coding allele	P-value	P-value	P-value	P-value	P-value	P-value
rs1435867	2	229219173	2	**PID1**	0.065	**0.021**	0.845	0.418	0.824	0.321	0.377
rs17035917	4	106740191	2,3	**INTS12-GSTCD-NPNT**	0.071	**0.006**	0.135	0.077	0.073	0.056	0.061
rs17035960	4	106751295	2,3	**INTS12-GSTCD-NPNT**	0.071	**0.004**	0.093	0.054	0.067	0.056	0.063
rs11727735	4	106851319	2,3	**INTS12-GSTCD-NPNT**	0.076	**2.14E-04**	0.361	0.198	0.057	0.114	0.074
rs10516526	4	106908353	2,3	**INTS12-GSTCD-NPNT**	0.072	**1.96E-04**	0.327	0.177	0.062	0.120	0.078
rs11731417	4	106965461	2,3	**INTS12-GSTCD-NPNT**	0.073	**1.32E-04**	0.335	0.177	**0.048**	0.146	0.090
rs1032295	4	145654034	2	**HHIP**	0.397	0.173	0.096	0.306	0.274	**0.042**	**0.033**
rs1512285	4	145670409		**HHIP**	0.462	**0.032**	**0.029**	0.141	0.152	**0.033**	**0.024**
rs720485	4	145682038	2,3	**HHIP**	0.391	0.159	0.510	0.786	0.943	**0.044**	0.058
rs1512288	4	145710731	2,3	**HHIP**	0.401	0.188	0.533	0.813	0.781	**0.046**	0.057
rs6817273	4	145711453	2,3	**HHIP**	0.400	0.179	0.535	0.816	0.866	**0.046**	0.057
rs3008798	6	39887840	3	**DAAM2**	0.464	0.326	0.960	0.850	0.755	**0.009**	**0.017**
rs1318002	6	39892112	3	**DAAM2**	0.480	0.649	0.725	0.902	0.782	**0.015**	**0.023**
rs2395730	6	39892343	3	**DAAM2**	0.442	0.522	0.716	0.513	0.619	**0.003**	**0.007**
rs12899618	15	69432174	3	**THSD4**	0.158	**0.003**	**0.003**	**0.014**	0.424	0.137	0.131
rs1913768	15	69436598	3	**THSD4**	0.159	**0.003**	**0.002**	**0.011**	0.393	0.162	0.152
rs1568010	15	69455566	4	**THSD4**	0.372	0.535	**0.042**	0.067	0.413	0.241	0.111
rs2304488	16	56631711	1	**MMP15**	0.186	**0.033**	0.101	0.147	0.112	0.344	0.506
rs12447804‡	16	56632783	5	**MMP15**	0.179	**0.033**	0.111	0.161	0.112	0.382	0.482

B						Non-Asthmatics			Asthmatics		
						FEV1 percent predicted	FEV1 percent change	FEV1 decline [%/y]	FEV1 percent predicted	FEV1 percent change	FEV1 decline [%/y]
dbSNP ID	chr	position (build 36.3)	refs	gene nearby	Maximal frequency of coding allele	P-value	P-value	P-value	P-value	P-value	P-value
rs918949	2	218382942	2,3	**TNS1**	0.384	**0.010**	0.133	0.089	0.076	0.241	0.256
rs1035672	2	218383444	2,3	**TNS1**	0.384	**0.010**	0.133	0.089	0.093	0.243	0.258
rs929937	2	218417460	2,4	**TNS1**	0.386	0.623	**0.017**	**0.016**	**0.004**	0.915	0.888
rs3845823	2	229611365	4	**PID1**	0.432	**0.039**	0.963	0.852	0.393	0.997	0.987
rs12477314‡	2	239542085	5	**HDAC4**	0.215	**0.023**	0.727	0.655	0.125	0.361	0.278
rs1529672	3	25495586	5	**RARB**	0.159	**0.012**	0.329	0.337	0.605	0.716	0.860
rs1828591	4	145700230	2,3	**HHIP**	0.394	**0.031**	0.470	0.345	0.254	0.138	0.139
rs13118928	4	145705839	2,3	**HHIP**	0.393	**0.043**	0.500	0.371	0.271	0.132	0.132
rs3995090	5	147826008	2,3	**HTR4**	0.394	**0.011**	0.785	0.699	**0.029**	0.649	0.456
rs2395730	6	39892343	3	**DAAM2**	0.442	0.277	0.554	0.685	0.979	**0.036**	**0.039**
rs2798641	6	109374743	5	**ARMC2**	0.209	0.315	0.444	0.530	**0.006**	0.188	0.158
rs9496346	6	142711031	2	**GPR126**	0.316	**0.053**	0.378	0.368	0.098	0.777	0.788
rs6570507	6	142721265	2	**GPR126**	0.314	**0.035**	0.356	0.342	0.080	0.804	0.821
rs11155242	6	142733242	2	**GPR126**	0.210	**0.008**	0.785	0.670	0.268	0.857	0.807
rs7753012	6	142787576	2	**GPR126**	0.337	**0.051**	0.477	0.487	0.065	0.566	0.637
rs3748069	6	142809326	2	**GPR126**	0.319	**0.043**	0.407	0.401	0.134	0.604	0.628
rs171891	6	142892305	2,4	**GPR126**	0.198	**0.013**	0.884	0.741	0.129	0.830	0.815
rs10512249	9	97296130	2	**PTCH1**	0.089	0.435	0.922	0.999	0.807	**0.032**	**0.028**
rs11172113	12	55813550	5	**LRP1**	0.384	**0.005**	0.602	0.530	0.809	0.114	0.125
rs1036429	12	94795559	5	**CCDC38**	0.217	0.765	0.322	0.356	0.295	**0.047**	**0.031**
rs2456526	15	50876734	1	**ONECUT1**	0.136	**0.011**	0.524	0.500	0.451	0.230	0.250
rs12899618	15	69432174	3	**THSD4**	0.158	**3.25E-04**	0.253	0.390	0.328	0.596	0.668
rs1913768	15	69436598	3,4	**THSD4**	0.159	**4.78E-04**	0.221	0.344	0.365	0.617	0.695
rs2304488	16	56631711	1	**MMP15**	0.186	0.121	**0.002**	**0.002**	0.085	0.760	0.515
rs12447804‡	16	56632783	5	**MMP15**	0.179	0.121	**0.003**	**0.003**	0.085	0.719	0.487
rs2865531‡	16	73947817	5	**CFDP1**	0.428	**0.035**	0.621	0.736	0.377	0.840	0.603

Figure 1



A -FEV1 decline in non-asthmatics

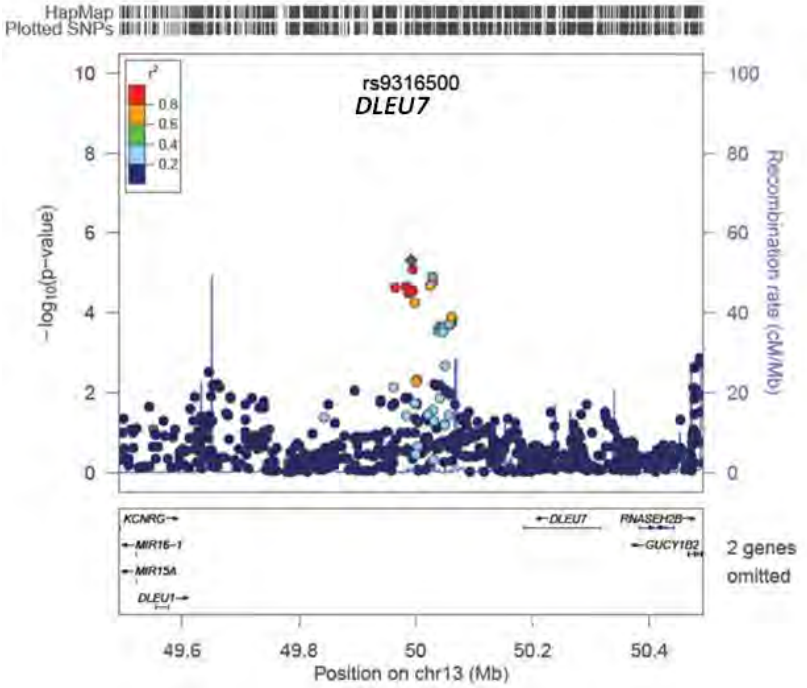
B - FEV1 decline in asthmatics

C - FEV1/FVC decline in non-asthmatics

D - FEV1/FVC decline in asthmatics

Figure 2

A



B

rs9316500, *DLEU7*

study	allele frequency	beta estimate	standard error	P study	P Het between studies
DISCOVERY PHASE					
EGEA (n=529)	0.297	0.173	0.069	0.012	
SAPALDIA (n=788)	0.321	0.094	0.053	0.077	
ECRHS (n=1343)	0.298	0.147	0.042	4.16E-04	
pooled FE (n=2660)		**0.135**	**0.030**	**4.81E-06**	**0.613**
REPLICATION PHASE					
ARIC (n=7156)	0.286	0.036	0.018	0.054	
FHS (n=3232)	0.211	0.008	0.027	0.787	
B58 (n=470)	0.292	0.160	0.071	0.024	
pooled FE (n=10858)		**0.033**	**0.015**	**0.026**	**0.124**
DISCOVERY & REPLICATION					
pooled FE overall (n=13518)		**0.053**	**0.013**	**5.70E-05**	**0.011**

FE = fixed effect meta-analysis estimate