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1 **Genome-wide association study of lung function decline in adults with and**
 2 **without asthma.**

3
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173 **ABSTRACT**

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

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

180 **Methods:** Discovery cohorts included adults of European ancestry (1441 asthmatics,
181 2677 non-asthmatics; Epidemiological Study on the Genetics and Environment of
182 Asthma (EGEA); Swiss Cohort Study on Air Pollution And Lung And Heart Disease In
183 Adults (SAPALDIA); European Community Respiratory Health Survey (ECRHS)). The
184 associations of FEV1 and FEV1/FVC decline with 2.5 million single nucleotide
185 polymorphisms (SNPs) were estimated. Thirty loci were followed-up by *in silico*
186 replication__ (1160 asthmatics, 10858 non-asthmatics: Atherosclerosis Risk in
187 Communities (ARIC); Framingham Heart Study (FHS); British 1958 Birth Cohort
188 (B58C); Dutch asthma study).

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

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

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

200

201

202 **Key Messages:**

203 • Knowledge regarding genes with pleiotropic effects on asthma, chronic
204 obstructive pulmonary disease as well as on lung function level and its
205 longitudinal course is limited.

206 • This first GWAS meta-analysis on lung function decline conducted separately in
207 non-asthmatic and asthmatic cohort participants suggests that genetic
208 determinants of lung function decline are different in the two groups.

209 • The results further suggest that previously identified genetic determinants of
210 cross-sectional lung function are not major determinants of the decline.

211

212

213 **Capsule summary:**

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

218

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220

221 **Keywords:**

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

223

224 **Abbreviations:**

225 ARIC, Atherosclerosis Risk in Communities Study

226 ATS, American Thoracic Society

227 B58C, British 1958 Birth Cohort

228 chr, chromosome

229 COPD, chronic obstructive pulmonary disease

230 ECRHS, European Community Respiratory Health Survey

231 EGEA, Genetics and Environment of Asthma

232 FEV1, forced expiratory volume in the first second

233 FHS, Framingham Heart Study

234 FVC, forced vital capacity

235 GWAS, genome-wide association studies

236 HapMap, Haplotype Map Project

237 Q-Q, Quantile-quantile

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

239 SNP, single nucleotide polymorphism

240 **INTRODUCTION**

241 Low lung function is a feature of both asthma and chronic obstructive pulmonary
242 disease (COPD), with twin studies demonstrating strong heritability (0.51 to 0.77) for
243 forced expiratory volume in the first second (FEV1)^{1, 2}. The two respiratory diseases and
244 lung function itself share predisposing and phenotypic features, including increased
245 airway responsiveness and atopy as well as exogenous risk factors^{3, 4}. Genome-wide
246 association studies (GWAS) have identified novel genetic loci for asthma⁵⁻¹⁰, COPD¹¹⁻¹⁴,
247 and lung function¹⁵⁻¹⁸ and provide the opportunity to study agnostically their overlap in
248 genetic background¹⁹. Some of the implicated genes, such as *PDE4D*, support a link
249 between asthma and COPD which may be rooted in shared pathways during lung
250 development²⁰. However, the majority of the genes implicated in asthma or COPD
251 GWAS analyses have not been identified as top association signals in GWAS for lung
252 function in the general population¹⁵⁻¹⁸, with the exception of *HHIP* and *FAM13A* being
253 associated with both lung function¹⁵⁻¹⁸ and COPD¹¹⁻¹⁴. Several lines of evidence suggest
254 that different genes influence lung function in asthmatics and in non-asthmatics.
255 Genome-scans in family based linkage studies identified some, but overall limited
256 overlap between chromosomal regions linked to lung function in asthmatics²¹, COPD
257 patients²² and in the general population²³ and it has been suggested that genetic
258 variation may be more important for lung function in asthma after adjusting for smoking
259 and body size differences^{21, 24, 25}.

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

263 previous GWAS¹⁵⁻¹⁸. 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 consortium⁷. Replication cohorts included three population-based cohorts (FHS, ARIC,
269 B58C) and one family-based asthma study (the Dutch Asthma Study).

270 **METHODS**

271 - Discovery cohorts and study population: Three large multi-centric cohorts EGEA²⁶,
272 SAPALDIA²⁷ and ECRHS²⁸ constitute the ESE-consortium. Personal factors of
273 relevance to lung function decline were assessed by interview and anthropometric
274 measurements at baseline and follow-up. Participants included in discovery phase were
275 derived from the nested asthma case/control samples (SAPALDIA and ECRHS) or from
276 the entire study population (EGEA) subjected to genome-wide genotyping in the context
277 of the GABRIEL asthma GWAS⁷. Baseline and follow-up examination were roughly 10
278 years apart. The analysis was restricted to adult participants (age ≥ 18 years at the time
279 of the baseline spirometry) with complete information on age, height and sex as well as
280 valid lung function measure from both surveys. Cohort study protocols were in
281 agreement with the Declaration of Helsinki and obtained ethical approval from their
282 respective regional and/or national review boards.

283 - Lung function assessments, asthma status and genotypes: At each visit, a minimum of
284 two acceptable forced expiratory flows, forced vital capacity (FVC) and forced expiratory
285 volume in the first second (FEV1) complying with American Thoracic Society criteria
286 were obtained²⁶⁻²⁹. No bronchodilator was administered. Based on questionnaire data,
287 asthmatics were defined as asthma self-report at any of the completed surveys and
288 family-based studies considered additional clinical asthma criteria (see online
289 repository). Genotyping for discovery cohorts was centrally performed on the Illumina
290 Human 610quad BeadChip at the Centre National de Génotypage (CNG, Evry,
291 France)⁷. Imputation of genotypes based on Hapmap2 reference panel, investigation of

292 population stratification and quality control criteria are described in Figure E1 and Table
293 E1 in the Online Repository.

294 -Replication Cohorts: Four cohorts of European ancestry with available genome-wide
295 data, ARIC³⁰, FHS¹⁵; B58C³¹; Dutch asthma study³² were used for replication. Subjects
296 included in the current analysis were older than 24 years, had complete information on
297 covariates (age, height, and sex) and valid lung function measures from at least two
298 time-points. The lung function measurements were conducted at least ten years apart,
299 except three years apart for ARIC (Table I). Distinct genotype data platforms and
300 imputation software were used (Table E11, Online Repository).

301 - Statistical analysis: Annual decline in FEV1 and FEV1/FVC was calculated as as
302 difference between follow-up and baseline spirometric measurements (mL for FEV1 and
303 % for FEV1/FVC) divided by the duration of follow-up in years. Standardized residuals
304 were derived from sex-specific linear regression models adjusted for age, height and
305 study centre in asthmatics and non-asthmatics separately. Comparability between
306 studies of standardized residuals was tested using Wilcoxon-Mann-Whitney test
307 (P>0.94). The standardized residuals were used as dependent variable and regressed
308 on genome-wide single nucleotide polymorphisms (SNPs) adjusted for study-specific
309 principal components capturing population ancestry (see online supplement for details).
310 Study-specific SNP effect estimates were combined through meta-analysis using fixed
311 and random effects models. We used a threshold of $P < 5 \times 10^{-8}$ (the Bonferroni
312 adjustment for one million independent tests) to declare a pooled effect as genome-
313 wide significant. Selection criteria for replication loci are described in the methods
314 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). $P \leq 0.05$ 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 than 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

334 **RESULTS**

335 *Characteristics of the study populations*

336 The cohorts included in this study differed by age and type of recruitment, and
337 accordingly in lung function and the proportion of subjects with FEV1/FVC below 70%
338 (Table I, Table EVIII, Online Repository). Baseline lung function parameters, but not
339 their annual changes were lower in asthmatics when compared to non-asthmatics in
340 each study. The proportion of never smokers was comparable among asthmatics, but
341 varied among non-asthmatics (ranging from 28.5% in B58C to 46.5% in EGEA). No
342 substantial differences in the smoking prevalence between people with and without
343 asthma were observed within each study. Comparing the discovery cohorts in more
344 detail (Table EVIII, Online Repository), atopy (total IgE ≥ 100 kU/ml) and hay fever were
345 more prevalent in both asthmatics and non-asthmatics from EGEA when compared to
346 ECRHS and SAPALDIA. Current asthma was more prevalent (84.4%) in EGEA than in
347 SAPALDIA (25.5%) or ECRHS (43.3%) and the prevalence of a positive family history
348 for asthma was also highest in EGEA, in agreement with the study design. Asthmatics
349 from EGEA had a younger age of disease onset due to the mode of recruitment of the
350 proband.

351

352 *Main findings from meta-analyses of discovery and replication phase*

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

357 a total of 10'858 non-asthmatics and 1'138 asthmatics. Thirty lead SNPs belonging to
358 30 loci ($5 \times 10^{-8} < P_{\text{discovery}} < 6 \times 10^{-5}$) were chosen for replication.

359 The four lung function parameter- and asthma-specific meta-analyses identified one
360 association signal that almost reached the genome-wide significance level ($P = 5.3 \times 10^{-8}$)
361 at the locus 8p22 containing the *TUSC3* gene for FEV1/FVC decline in asthmatics
362 while all other signals had $P < 5 \times 10^{-7}$ (Figure I), but this signal was not associated with
363 FEV1/FVC decline in asthmatics in the replication sample. The only locus of the
364 selected replication candidate loci that formally replicated was 13q14.3, containing the
365 *DLEU7* gene, associated with decline in FEV1 in the non-asthmatics ($P_{\text{discovery}} = 4.8 \times 10^{-6}$
366 and $P_{\text{replication}} = 0.03$).

367 In the global *post hoc* analysis combining both asthmatics and non-asthmatics
368 ($N = 4118$), a striking finding was the absence of any pronounced association signals (P
369 $> 1 \times 10^{-6}$) despite increased statistical power. This was in agreement with the minimal
370 overlap of association signals observed in asthmatics and non-asthmatics separately.
371 Most signals at $P < 10^{-5}$ from the asthma-stratified analysis in the discovery phase
372 exhibited statistically significant heterogeneity of effects between the two groups (Table
373 II). At the replication stage, none of the replication SNPs was associated with lung
374 function decline in asthmatics and non-asthmatics combined.

375

376 *Association signals for annual decline in FEV1 in non-asthmatics*

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

380 gene; three SNPs belonged to three distinct loci. The association of lead and proxy
381 SNPs in *DLEU7* (Figure II), but not *TXN/MUSK/SVEP1* (Figure EII) or the other SNPs
382 (Table II) replicated. The G-allele of SNP rs9316500 near the *DLEU7* gene was
383 positively associated with annual FEV1 decline in the discovery cohorts ($P=4.8 \times 10^{-6}$)
384 and in the replication cohorts ($P=0.026$). Although heterogeneity between studies was
385 not significant ($P=0.61$), the combined P value did not reach the genome-wide level
386 ($P=5.7 \times 10^{-5}$).

387

388 *Association signals for annual decline in FEV1 in asthmatics*

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

392

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

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

396

397 *Association signals for annual decline in FEV1/FVC in asthmatics*

398 Twelve SNPs at the locus 8p22 containing the gene *TUSC3* at 15.68Mb were
399 associated with FEV1/FVC decline at $P < 10^{-7}$ in asthmatics (Figure I). Regional locus
400 plot and forest plot are presented in the online repository (Figure EIII). The top
401 association signals in this locus were conferred by distinct SNPs in each cohort, though
402 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 asthmatics³³ 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

426 baseline FEV1 were mainly restricted to non-asthmatics. For baseline FEV1/FVC,
427 associations of SNPs of THSD4 were prominent (e.g. rs12899618, $P=3.3 \times 10^{-4}$) and
428 again restricted to non-asthmatics.

429 For decline phenotypes of FEV1, we observed associations for SNPs in regions 6p21
430 (DAAM2, $0.003 < P < 0.02$) and 4q28 (HHIP, $0.02 < P < 0.05$) among asthmatics and in
431 THSD4 ($0.003 < P < 0.04$) among non-asthmatics. The strongest associations observed
432 for decline phenotypes of FEV1/FVC were two SNPs in MMP15 (16q13,
433 $0.003 < P < 0.002$) in non-asthmatics, only. Association in the combined sample of
434 asthmatics and non-asthmatics did not substantially alter the results.

435

436 Summary of findings from sensitivity analyses

437 We observed in non-asthmatics, aged 30 years and more, that MUSK and DLEU7 were
438 no longer prominently associated with FEV1 decline, but SNPs in other genes remained
439 strongly associated (ZIC1, rs6785065, $P=2.3 \times 10^{-5}$; UBL3, rs278037, $P=4.8 \times 10^{-5}$).

440 Results of the GWAS on percent change in lung function showed that the FEV1
441 association signal for DLEU7 in the non-asthmatics was no longer significant; however
442 the signals for MUSK (rs1889321, $P=2.92 \times 10^{-7}$) and other loci remained unaltered
443 (ZIC1, rs6785065, $P=2.0 \times 10^{-5}$; KIRREL3, rs11604082, $P=4.1 \times 10^{-6}$; KIAA2117,
444 rs10082549, $P=2.7 \times 10^{-6}$). Top signals associated with decline in FEV1/FVC in
445 asthmatics remained unaltered for TUSC3 (rs4831760, $P=5.2 \times 10^{-8}$) and for SYNE2
446 (rs7144584, $P=6.4 \times 10^{-7}$) after taking baseline lung function into account.

447 Smoking stratified analyses of the replication SNPs revealed no substantial difference in
448 association between ever and never smokers except for a few SNPs belonging to loci
449 containing *SYNE2*, *RORA*, *BCAS1*, or *PLXNA4* genes.
450 Replication meta-analysis excluding the ARIC data substantially reduced sample size in
451 non-asthmatics and the association of *DLEU7* with decline of FEV1 was no longer
452 significant. Instead two loci for association with decline in FEV1 in asthmatics (*PLXNA4*,
453 rs10808265, $P_{\text{discovery}}=1.7 \times 10^{-6}$, $P_{\text{replication}}=0.02$ and *SLC45A3*, rs16856186,
454 $P_{\text{discovery}}=8.9 \times 10^{-6}$, $P_{\text{replication}}=0.04$) and one locus, FLJ25393, for decline in FEV1/FVC in
455 non-asthmatics (rs2658782, $P_{\text{discovery}}=4.3 \times 10^{-6}$, $P_{\text{replication}}=0.03$) gained statistical
456 significance.
457

458 **DISCUSSION**

459 A main result of this study is the observed genetic heterogeneity of lung function decline
460 between asthmatics and non-asthmatics. When we combined the two groups in the
461 discovery phase we observed no genome-wide significant association signal despite
462 larger sample size. All top hit association signals detected by the asthma stratified
463 analysis showed significant heterogeneity according to the disease status. In the
464 replication phase, this heterogeneity was also confirmed for the *DLEU7* locus which was
465 associated with FEV1 decline in non-asthmatics only. Finally, many of the SNPs
466 identified by previous GWAS on lung function exhibited associations specific to asthma
467 status.

468

469 The finding of genetic heterogeneity in lung function reported here is consistent with
470 available evidence. Differences in familial segregation of FEV1 in asthmatic and non-
471 asthmatic families previously suggested genetic heterogeneity between these two
472 groups²⁴. Agnostic studies investigating genetic determinants of lung function in both,
473 family-based^{21, 22, 34-37} and population-based samples^{15-18, 23, 25} produced little overlap in
474 chromosomal regions. Genome-wide scans on lung function in asthma^{21, 38} or COPD²²
475 families also suggested a heterogeneous genetic architecture of lung function.

476

477 Nevertheless, some previously reported overlapping linkage regions for the ratio of
478 FEV1 over vital capacity (FEV1/VC) and FEV1 over the forced vital capacity
479 (FEV1/FVC) in families with asthma and COPD^{21, 22} suggest that at least some gene(s)
480 could be important in the development of airway obstruction in both diseases.

481 Furthermore, genetic polymorphisms in glutathione S-transferases³⁹⁻⁴² as well as
482 *ADAM-33*⁴³⁻⁴⁶ were associated with lower lung function at all ages and in different
483 subgroups of the population (general population, patients with COPD and asthma).
484 Gene-lung function associations that are of relevance to several population and patient
485 strata may be determined specifically by complex gene-gene and gene-environment
486 interactions, as suggested for lung function decline and its complex association with
487 estrogen receptor 1 polymorphisms, smoking, steroid use, and gender^{32, 47}. While
488 ignored in ours as well as previous GWAS, such effect modifications should be
489 considered in the future⁴⁸.

490

491 Results from the Busselton Health Study on familial aggregation and heritability of adult
492 lung function previously suggested the existence of genetic determinants of adult lung
493 function independent of asthma, atopy, cigarette smoking, height, age or sex²⁵.
494 Consistent with these results, neither asthma, atopy and COPD genes previously
495 identified in large GWAS^{5-9, 11} nor genes related to smoking behavior⁴⁹ were associated
496 with lung function decline in our study. The association of FEV1 decline with a gene
497 related to height, *DLEU7*, was ranking high, but only in subjects without asthma
498 (rs9316500, $P_{\text{discovery}}=4.8 \times 10^{-6}$; $P_{\text{replication}}=0.03$). *DLEU7* gene product and expression
499 remain poorly characterized, but its mRNA has been detected in the lung. The *DLEU7*
500 locus was identified as a determinant of adult height in previous GWAS meta-
501 analyses⁵⁰⁻⁵². Three other height genes, *HHIP*, *GPR126* and *PTCH*, were associated
502 with cross-sectional lung function¹⁵⁻¹⁷. All of these lung function models including ours
503 were adjusted for adult height. The observed association, related to both *HHIP* and

504 *DLEU7* being associated with peak height velocity in infancy⁵¹, 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 life²⁰. 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 leukemia⁵³⁻⁵⁵.
513 Evidence emerges for a role of *DLEU7* in counterbalancing the proliferative impact of
514 NF- κ B on various cell types⁵⁶. The potential role of the gene product of *TUSC3*, a
515 proposed tumor suppressor gene⁵⁷, in lung physiology is discussed in the Online
516 Repository.

517 None of the SNPs identified in GWAS of cross-sectional lung function¹⁵⁻¹⁸ 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 function¹⁵⁻¹⁸, neither the *TUSC3* (heterogeneity
526 between ever/never smokers $P=0.98$) nor other top hit signals were modified by

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

529 The strength of the present study is the longitudinal design of all cohorts included.
530 Repeated spirometric assessments within the same subject is thought to capture more
531 precisely exogenous factors and genes leading to accelerated loss of lung function in
532 adulthood⁵⁸. The discovery cohorts shared comparable questionnaire and spirometry
533 protocols and they were specifically designed to investigate environmental and genetic
534 causes of lung function decline and asthma in a standardized way. Each study has two
535 measures of pre-bronchodilator lung function about ten years apart, but clearly our
536 findings would be more robust if further lung function measures were available over an
537 even longer period of follow-up. All discovery cohorts have used the same genotyping
538 platform and stringent quality control criteria have been applied.

539 Sample size is a limitation of this study, and remains a general challenge in lung
540 function studies with a need for high phenotypic comparability as spirometry results are
541 sensitive to technicians and devices used⁵⁹. The pre-bronchodilation lung function
542 measurements in our and previous lung function GWAS do not allow to differentiate
543 reversible from non-reversible obstruction to airflow. Populations included in this study
544 differed by age which is also reflected by the diverging proportion of subjects with
545 FEV1/FVC <0.7 at follow-up between the discovery cohorts. Discovery and replication
546 populations also differ by time spacing between the spirometry assessments. We can
547 only speculate of on the overall impact of such differences. We do note that replication
548 results were sensitive to the exclusion of ARIC data (the study with highest mean age,
549 largest annual decline, and shortest follow-up time).

550 Other limitations are shared with any GWAS meta-analyses investigating complex
551 phenotypes such as lack in power for investigating gene-environment interactions or
552 studying subgroups of diseases. As the sample size of our study was comparatively
553 small, especially for the asthmatic sample in the replication phase, we had limited ability
554 to address differences in asthma sub-phenotypes or the impact of asthma medication
555 intake. It is also likely that a substantial part of complex disease may be explained by
556 rare mutations not considered by current GWAS. Finally, assessing the joint effect of
557 SNPs having small effects individually and potentially interacting with each other
558 remains another challenge.

559

560 In conclusion, this first GWAS meta-analysis on lung function decline provides
561 suggestive evidence for genetic heterogeneity between persons with and without
562 asthma and between cross-sectionally and longitudinally measured lung function.
563 Consistent with cross-sectional GWAS, our results are also suggestive of height related
564 genes playing a role. Further studies in this area would be enhanced by greater
565 comparability of age range, spacing of lung function assessments, and asthma sub-
566 phenotypes (including treatment) to decrease phenotypic heterogeneity and therefore
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642
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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 r^2 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.

830

831 **FOOTNOTES**832 **Footnotes to Table I:**

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

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

836

837 **Footnote to Table II:**

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

839

840 **Footnote to Table III:**

841 * Associations of SNPs previously associated in cross-sectional lung function in GWAS
842 studies, (1) Framingham¹⁵, (2) CHARGE¹⁷, (3) Spirometa¹⁶, (4) Asthmatics³³ and (5)
843 CHARGE-Spirometa¹⁸ were assessed in the discovery cohorts only if minor allele
844 frequency (MAF) was at least 5%. SNPs tested for associations: *ADAM19*: rs2277027,
845 rs1422795, rs6890282; *ADCY2*: rs7710510, rs6555465; *ARMC2*: rs2798641; *C10orf11*:
846 rs11001819; *CCDC38*: rs1036429; *CDC123*: rs7068966; *CFDP1*: rs2865531; *DAAM2*:
847 rs3008798, rs1318002, rs2395730; *FAM13A1*: rs6830970, rs2869967; *GPR126*:
848 rs9496346, rs6570507, rs11155242, rs7753012, rs3748069, rs171891, rs263178;
849 *HDAC4*: rs12477314; *HHIP*: rs1032295, rs1512285, rs720485, rs1828591, rs13118928,
850 rs1512288, rs6817273; *HTR4*: rs3995090, rs1833710; *INTS12-GSTCD-NPNT*:
851 rs3960769, rs17035917, rs17035960, rs11727735, rs10516526, rs11731417; *KCEN2*:
852 rs9978142; *LRP1*: rs11172113; *MECOM*: rs1344555; *MFAP2*: rs2284746; *MMP15*:

853 rs2304488, rs12447804; *MTMR3*: rs17646919; *NCR3*: rs2857595; *NOTCH4*: rs206015;
 854 *ONECUT1*: rs2456526; *PID1*: rs1435867, rs1358443, rs3845823; *PTCH1*: rs10512249,
 855 rs576594; *RARB*: rs1529672; *SPATA9*: rs153916; *TGFB2*: rs993925; *THSD4*:
 856 rs12899618; *THSD4*: rs1568010, rs1913768; *TNS1*: rs918949, rs1035672, rs929937;
 857 *ZKSCAN3*: rs6903823. Non-significant associations reported in online repository.
 858 † Baseline cross-sectional lung function was calculated using Quanjer formula⁶¹.
 859 ‡Proxies tested for cross-sectional association (r^2 , D'): for rs12447804 - rs2304488
 860 (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 \pm SD Age	mean \pm SD Height	mean \pm SD (L) FEV1	mean \pm SD FEV1/FVC	mean \pm SD (y) Follow-up length†	mean \pm SD (mL/y) annual decline FEV1	mean \pm SD (%/y) annual decline FEV1/FVC	% Never smokers
Discovery (ESE-cohorts)										
EGEA	529	45.2	41.4 \pm 11.7	1.68 \pm 0.08	3.45 \pm 0.78	0.83 \pm 0.06	11.2 \pm 1.0	-28.6 \pm 25.7	-0.47 \pm 0.53	46.5
SAPALDIA	805	49.2	41.8 \pm 11.1	1.70 \pm 0.09	3.62 \pm 0.81	0.79 \pm 0.07	10.9 \pm 0.2	-34.0 \pm 28.3	-0.40 \pm 0.46	43.1
ECRHS	1343	49.7	34.1 \pm 7.1	1.70 \pm 0.10	3.81 \pm 0.83	0.83 \pm 0.06	8.9 \pm 0.9	-26.3 \pm 30.7	-0.30 \pm 0.50	40.7
Replication with in silico data										
ARIC	7156	46.3	54.5 \pm 5.6	1.69 \pm 0.09	3.01 \pm 0.75	0.75 \pm 0.07	2.9 \pm 0.2	-52.0 \pm 57.4	-0.19 \pm 0.98	40.8
FHS	3232	44.9	52.9 \pm 10.2	1.67 \pm 0.10	2.89 \pm 0.81	0.77 \pm 0.08	10.5 \pm 3.6	-24.9 \pm 23.9	-0.33 \pm 0.57	36.1
B58C	470	48.7	35.0 \pm 0.2	1.70 \pm 0.09	3.68 \pm 0.73	0.81 \pm 0.06	10.1 \pm 0.5	-34.9 \pm 31.4	-0.21 \pm 0.67	28.5
Asthmatics										
Discovery (ESE-cohorts)										
EGEA	330	50.6	38.5 \pm 12.5	1.70 \pm 0.09	3.26 \pm 0.91	0.77 \pm 0.11	11.6 \pm 1.0	-27.6 \pm 39.4	-0.44 \pm 0.68	44.6
SAPALDIA	540	46.5	40.2 \pm 11.3	1.69 \pm 0.09	3.36 \pm 0.89	0.76 \pm 0.95	10.9 \pm 0.3	-35.5 \pm 33.9	-0.45 \pm 0.54	42.4
ECRHS	571	42.7	33.9 \pm 7.3	1.69 \pm 0.10	3.43 \pm 0.81	0.78 \pm 0.09	8.8 \pm 0.7	-26.7 \pm 42.6	-0.20 \pm 0.60	42.5
Replication with in silico data										
ARIC	325	50.2	54.2 \pm 5.7	1.69 \pm 0.10	2.73 \pm 0.87	0.68 \pm 0.10	2.9 \pm 0.2	-43.9 \pm 77.2	-0.037 \pm 1.25	41.9
FHS	346	41.3	50.1 \pm 10.3	1.68 \pm 0.09	2.72 \pm 0.84	0.73 \pm 0.09	10.2 \pm 3.8	-29.8 \pm 23.7	-0.38 \pm 0.51	36.1
B58C	231	44.2	35.0 \pm 0.2	1.69 \pm 0.10	3.45 \pm 0.75	0.78 \pm 0.08	10.3 \pm 0.5	-34.4 \pm 37.6	-0.17 \pm 0.89	37.2
Dutch Asthma	258	60.9	35.1 \pm 7.6	1.75 \pm 0.09	3.03 \pm 0.95	0.65 \pm 0.13	14.6 \pm 7.2	-22.8 \pm 47.0	-0.14 \pm 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 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

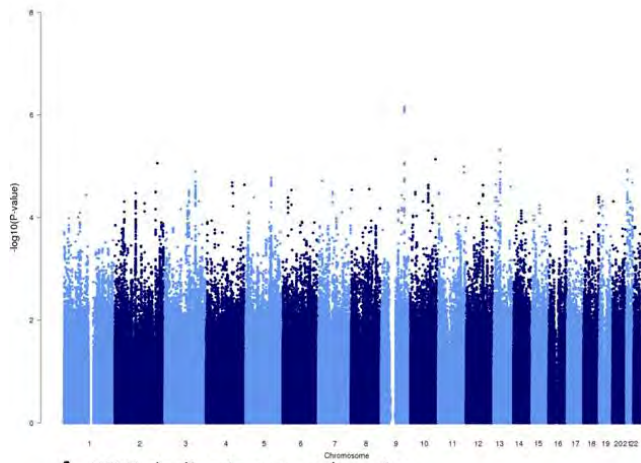
rs777433	2	128084705	LIMS2	0.407	0.151	0.00010	0.811	0.1223	-0.018	0.691	0.564	0.52
B - decline in FEV1/FVC					Discovery phase				Replication phase			
dbSNP ID	chr	position (build 36.3)	gene nearby	Maximal frequency of coding allele	Estimate of joint analysis	P for joint analysis	P for heterogeneity 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 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.

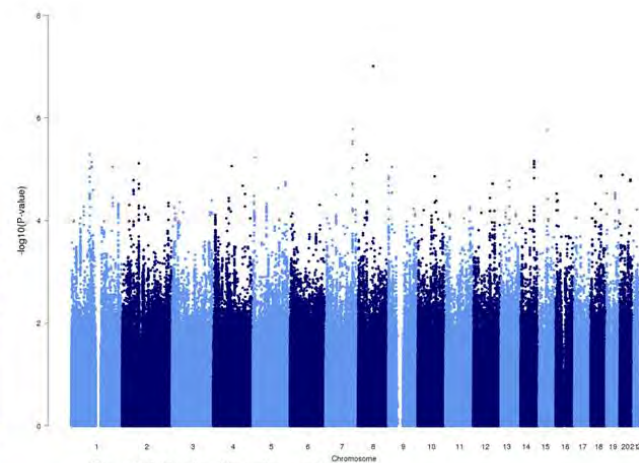
A	dbSNP ID	chr	position (build 36.3)	refs	gene nearby	Maximal frequency of coding allele	Non-Asthmatics			Asthmatics		
							FEV1 percent predicted	FEV1 percent change	FEV1 decline [%/y]	FEV1 percent predicted	FEV1 percent change	FEV1 decline [%/y]
							P-value	P-value	P-value	P-value	P-value	P-value
	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	dbSNP ID	chr	position (build 36.3)	refs	gene nearby	Maximal frequency of coding allele	Non-Asthmatics			Asthmatics		
							FEV1 percent predicted	FEV1 percent change	FEV1 decline [%/y]	FEV1 percent predicted	FEV1 percent change	FEV1 decline [%/y]
							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

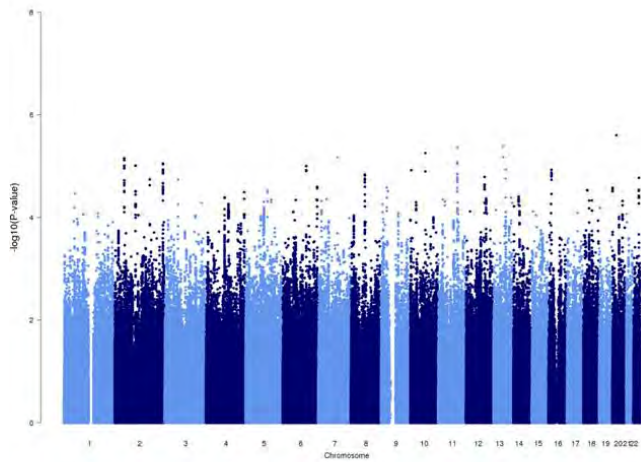
Figure 1



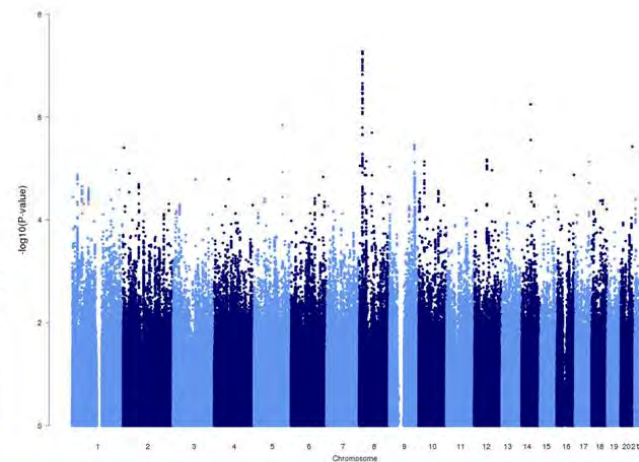
A - FEV1 decline in non-asthmatics



B - FEV1 decline in asthmatics



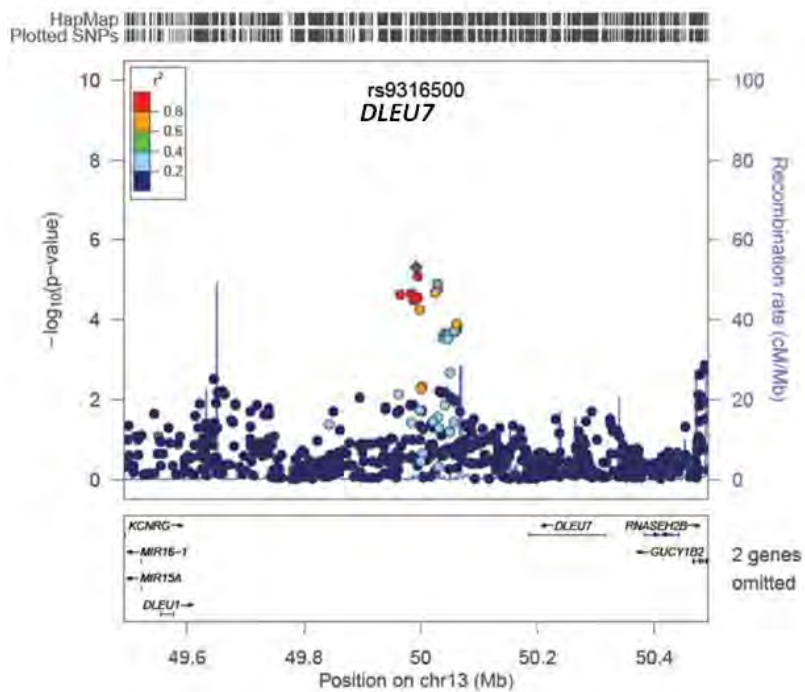
C - FEV1/FVC decline in non-asthmatics



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	0.089	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=2860)		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

