

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

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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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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)<sup>1, 2</sup>. 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<sup>3, 4</sup>. Genome-wide  
246 association studies (GWAS) have identified novel genetic loci for asthma<sup>5-10</sup>, COPD<sup>11-14</sup>,  
247 and lung function<sup>15-18</sup> and provide the opportunity to study agnostically their overlap in  
248 genetic background<sup>19</sup>. 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 development<sup>20</sup>. 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 population<sup>15-18</sup>, with the exception of *HHIP* and *FAM13A* being  
253 associated with both lung function<sup>15-18</sup> and COPD<sup>11-14</sup>. 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 asthmatics<sup>21</sup>, COPD  
257 patients<sup>22</sup> and in the general population<sup>23</sup> 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<sup>21, 24, 25</sup>.

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 GWAS<sup>15-18</sup>. 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<sup>7</sup>. 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 EGEA<sup>26</sup>,  
272 SAPALDIA<sup>27</sup> and ECRHS<sup>28</sup> 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 GWAS<sup>7</sup>. Baseline and follow-up examination were roughly 10  
278 years apart. The analysis was restricted to adult participants (age  $\geq 18$  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 obtained<sup>26-29</sup>. 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)<sup>7</sup>. 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, ARIC<sup>30</sup>, FHS<sup>15</sup>; B58C<sup>31</sup>; Dutch asthma study<sup>32</sup> 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  $\geq 100$  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<sup>-8</sup>) 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<sup>-5</sup>. 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<sup>11, 15-18</sup> and a replication study in asthmatics<sup>33</sup> 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<sup>-4</sup>) 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 groups<sup>24</sup>. Agnostic studies investigating genetic determinants of lung function in both,  
473 family-based<sup>21, 22, 34-37</sup> and population-based samples<sup>15-18, 23, 25</sup> produced little overlap in  
474 chromosomal regions. Genome-wide scans on lung function in asthma<sup>21, 38</sup> or COPD<sup>22</sup>  
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<sup>21, 22</sup> 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-transferases<sup>39-42</sup> as well as  
482 *ADAM-33*<sup>43-46</sup> 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<sup>32, 47</sup>. While  
488 ignored in ours as well as previous GWAS, such effect modifications should be  
489 considered in the future<sup>48</sup>.

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 sex<sup>25</sup>.  
494 Consistent with these results, neither asthma, atopy and COPD genes previously  
495 identified in large GWAS<sup>5-9, 11</sup> nor genes related to smoking behavior<sup>49</sup> 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 analyses<sup>50-52</sup>. Three other height genes, *HHIP*, *GPR126* and *PTCH*, were associated  
502 with cross-sectional lung function<sup>15-17</sup>. 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 infancy<sup>51</sup>, 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<sup>20</sup>. 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<sup>53-55</sup>.  
513 Evidence emerges for a role of *DLEU7* in counterbalancing the proliferative impact of  
514 NF- $\kappa$ B on various cell types<sup>56</sup>. The potential role of the gene product of *TUSC3*, a  
515 proposed tumor suppressor gene<sup>57</sup>, in lung physiology is discussed in the Online  
516 Repository.

517 None of the SNPs identified in GWAS of cross-sectional lung function<sup>15-18</sup> 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<sup>15-18</sup>, 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 adulthood<sup>58</sup>. 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 used<sup>59</sup>. 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-  
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642  
643**REFERENCES**

- 644 1. Hankins D, Drage C, Zamel N, Kronenberg R. Pulmonary function in identical twins  
645 raised apart. *Am Rev Respir Dis* 1982; 125:119-21.
- 646 2. Redline S, Tishler PV, Rosner B, Lewitter FI, Vandenburg M, Weiss ST, et al.  
647 Genotypic and phenotypic similarities in pulmonary function among family members of  
648 adult monozygotic and dizygotic twins. *Am J Epidemiol* 1989; 129:827-36.
- 649 3. Guerra S. Asthma and chronic obstructive pulmonary disease. *Curr Opin Allergy Clin*  
650 *Immunol* 2009; 9:409-16.
- 651 4. Postma DS, Boezen HM. Rationale for the Dutch hypothesis. Allergy and airway  
652 hyperresponsiveness as genetic factors and their interaction with environment in the  
653 development of asthma and COPD. *Chest* 2004; 126:96S-104S; discussion 59S-61S.
- 654 5. Moffatt MF, Kabesch M, Liang L, Dixon AL, Strachan D, Heath S, et al. Genetic variants  
655 regulating ORMDL3 expression contribute to the risk of childhood asthma. *Nature* 2007;  
656 448:470-3.
- 657 6. Himes BE, Hunninghake GM, Baurley JW, Rafaels NM, Sleiman P, Strachan DP, et al.  
658 Genome-wide association analysis identifies PDE4D as an asthma-susceptibility gene.  
659 *Am J Hum Genet* 2009; 84:581-93.
- 660 7. Moffatt MF, Gut IG, Demenais F, Strachan DP, Bouzigon E, Heath S, et al. A large-  
661 scale, consortium-based genomewide association study of asthma. *N Engl J Med* 2010;  
662 363:1211-21.
- 663 8. Sleiman PM, Flory J, Imielinski M, Bradfield JP, Annaiah K, Willis-Owen SA, et al.  
664 Variants of DENND1B associated with asthma in children. *N Engl J Med* 2010; 362:36-  
665 44.
- 666 9. DeWan AT, Triche EW, Xu X, Hsu LI, Zhao C, Belanger K, et al. PDE11A associations  
667 with asthma: results of a genome-wide association scan. *J Allergy Clin Immunol* 2010;  
668 126:871-3 e9.
- 669 10. Hancock DB, Romieu I, Shi M, Sienra-Monge JJ, Wu H, Chiu GY, et al. Genome-wide  
670 association study implicates chromosome 9q21.31 as a susceptibility locus for asthma in  
671 mexican children. *PLoS Genet* 2009; 5:e1000623.
- 672 11. Pillai SG, Ge D, Zhu G, Kong X, Shianna KV, Need AC, et al. A genome-wide  
673 association study in chronic obstructive pulmonary disease (COPD): identification of two  
674 major susceptibility loci. *PLoS Genet* 2009; 5:e1000421.
- 675 12. Pillai SG, Kong X, Edwards LD, Cho M, Anderson WH, Coxson HO, et al. Loci  
676 Identified by Genome-wide Association Studies Influence Different Disease-related  
677 Phenotypes in COPD. *Am J Respir Crit Care Med* 2010.
- 678 13. Cho MH, Boutaoui N, Klanderma BJ, Sylvia JS, Ziniti JP, Hersh CP, et al. Variants in  
679 FAM13A are associated with chronic obstructive pulmonary disease. *Nat Genet* 2010;  
680 42:200-2.
- 681 14. Kong X, Cho MH, Anderson W, Coxson HO, Muller N, Washko G, et al. Genome-wide  
682 Association Study Identifies BICD1 as a Susceptibility Gene for Emphysema. *Am J*  
683 *Respir Crit Care Med* 2011; 183:43-9.
- 684 15. Wilk JB, Chen TH, Gottlieb DJ, Walter RE, Nagle MW, Brandler BJ, et al. A genome-  
685 wide association study of pulmonary function measures in the Framingham Heart Study.  
686 *PLoS Genet* 2009; 5:e1000429.

- 687 16. Hancock DB, Eijgelsheim M, Wilk JB, Gharib SA, Loehr LR, Marcianti KD, et al. Meta-  
688 analyses of genome-wide association studies identify multiple loci associated with  
689 pulmonary function. *Nat Genet* 2010; 42:45-52.
- 690 17. Repapi E, Sayers I, Wain LV, Burton PR, Johnson T, Obeidat M, et al. Genome-wide  
691 association study identifies five loci associated with lung function. *Nat Genet* 2010;  
692 42:36-44.
- 693 18. Artigas MS, Loth DW, Wain LV, Gharib SA, Obeidat M, Tang W, et al. Genome-wide  
694 association and large-scale follow up identifies 16 new loci influencing lung function.  
695 *Nat Genet* 2011.
- 696 19. Weiss ST. Lung function and airway diseases. *Nat Genet* 2010; 42:14-6.
- 697 20. Weiss ST. What genes tell us about the pathogenesis of asthma and chronic obstructive  
698 pulmonary disease. *Am J Respir Crit Care Med* 2010; 181:1170-3.
- 699 21. Postma DS, Meyers DA, Jongepier H, Howard TD, Koppelman GH, Bleecker ER.  
700 Genomewide screen for pulmonary function in 200 families ascertained for asthma. *Am J*  
701 *Respir Crit Care Med* 2005; 172:446-52.
- 702 22. Silverman EK, Palmer LJ, Mosley JD, Barth M, Senter JM, Brown A, et al. Genomewide  
703 linkage analysis of quantitative spirometric phenotypes in severe early-onset chronic  
704 obstructive pulmonary disease. *Am J Hum Genet* 2002; 70:1229-39.
- 705 23. Wilk JB, DeStefano AL, Arnett DK, Rich SS, Djousse L, Crapo RO, et al. A genome-  
706 wide scan of pulmonary function measures in the National Heart, Lung, and Blood  
707 Institute Family Heart Study. *Am J Respir Crit Care Med* 2003; 167:1528-33.
- 708 24. Holberg CJ, Morgan WJ, Wright AL, Martinez FD. Differences in familial segregation of  
709 FEV1 between asthmatic and nonasthmatic families. Role of a maternal component. *Am*  
710 *J Respir Crit Care Med* 1998; 158:162-9.
- 711 25. Palmer LJ, Knuiaman MW, Divitini ML, Burton PR, James AL, Bartholomew HC, et al.  
712 Familial aggregation and heritability of adult lung function: results from the Busselton  
713 Health Study. *Eur Respir J* 2001; 17:696-702.
- 714 26. Kauffmann F, Dizier MH, Pin I, Paty E, Gormand F, Vervloet D, et al. Epidemiological  
715 study of the genetics and environment of asthma, bronchial hyperresponsiveness, and  
716 atopy: phenotype issues. *Am J Respir Crit Care Med* 1997; 156:S123-9.
- 717 27. Downs SH, Schindler C, Liu LJ, Keidel D, Bayer-Oglesby L, Brutsche MH, et al.  
718 Reduced exposure to PM10 and attenuated age-related decline in lung function. *N Engl J*  
719 *Med* 2007; 357:2338-47.
- 720 28. The European Community Respiratory Health Survey II. *Eur Respir J* 2002; 20:1071-9.
- 721 29. Standardization of Spirometry, 1994 Update. American Thoracic Society. *Am J Respir*  
722 *Crit Care Med* 1995; 152:1107-36.
- 723 30. The Atherosclerosis Risk in Communities (ARIC) Study: design and objectives. The  
724 ARIC investigators. *Am J Epidemiol* 1989; 129:687-702.
- 725 31. Marossy AE, Strachan DP, Rudnicka AR, Anderson HR. Childhood chest illness and the  
726 rate of decline of adult lung function between ages 35 and 45 years. *Am J Respir Crit*  
727 *Care Med* 2007; 175:355-9.
- 728 32. Dijkstra A, Howard TD, Vonk JM, Ampleford EJ, Lange LA, Bleecker ER, et al.  
729 Estrogen receptor 1 polymorphisms are associated with airway hyperresponsiveness and  
730 lung function decline, particularly in female subjects with asthma. *J Allergy Clin*  
731 *Immunol* 2006; 117:604-11.

- 732 33. Li X, Howard TD, Moore WC, Ampleford EJ, Li H, Busse WW, et al. Importance of  
733 hedgehog interacting protein and other lung function genes in asthma. *J Allergy Clin*  
734 *Immunol* 2011; 127:1457-65.
- 735 34. Malhotra A, Peiffer AP, Ryujin DT, Elsner T, Kanner RE, Leppert MF, et al. Further  
736 evidence for the role of genes on chromosome 2 and chromosome 5 in the inheritance of  
737 pulmonary function. *Am J Respir Crit Care Med* 2003; 168:556-61.
- 738 35. Ober C, Abney M, McPeck MS. The genetic dissection of complex traits in a founder  
739 population. *Am J Hum Genet* 2001; 69:1068-79.
- 740 36. Bouzigon E, Dizier MH, Krahenbuhl C, Lemainque A, Annesi-Maesano I, Betard C, et  
741 al. Clustering patterns of LOD scores for asthma-related phenotypes revealed by a  
742 genome-wide screen in 295 French EGEA families. *Hum Mol Genet* 2004; 13:3103-13.
- 743 37. Barton SJ, Koppelman GH, Vonk JM, Browning CA, Nolte IM, Stewart CE, et al.  
744 PLAUR polymorphisms are associated with asthma, PLAUR levels, and lung function  
745 decline. *J Allergy Clin Immunol* 2009; 123:1391-400 e17.
- 746 38. Xu X, Fang Z, Wang B, Chen C, Guang W, Jin Y, et al. A genomewide search for  
747 quantitative-trait loci underlying asthma. *Am J Hum Genet* 2001; 69:1271-7.
- 748 39. Gilliland FD, Gauderman WJ, Vora H, Rappaport E, Dubeau L. Effects of glutathione-S-  
749 transferase M1, T1, and P1 on childhood lung function growth. *Am J Respir Crit Care*  
750 *Med* 2002; 166:710-6.
- 751 40. Flamant C, Henrion-Caude A, Boelle PY, Bremont F, Brouard J, Delaisi B, et al.  
752 Glutathione-S-transferase M1, M3, P1 and T1 polymorphisms and severity of lung  
753 disease in children with cystic fibrosis. *Pharmacogenetics* 2004; 14:295-301.
- 754 41. Romieu I, Sienra-Monge JJ, Ramirez-Aguilar M, Moreno-Macias H, Reyes-Ruiz NI,  
755 Estela del Rio-Navarro B, et al. Genetic polymorphism of GSTM1 and antioxidant  
756 supplementation influence lung function in relation to ozone exposure in asthmatic  
757 children in Mexico City. *Thorax* 2004; 59:8-10.
- 758 42. Imboden M, Downs SH, Senn O, Matyas G, Brandli O, Russi EW, et al. Glutathione S-  
759 transferase genotypes modify lung function decline in the general population:  
760 SAPALDIA cohort study. *Respir Res* 2007; 8:2.
- 761 43. Jongepier H, Boezen HM, Dijkstra A, Howard TD, Vonk JM, Koppelman GH, et al.  
762 Polymorphisms of the ADAM33 gene are associated with accelerated lung function  
763 decline in asthma. *Clin Exp Allergy* 2004; 34:757-60.
- 764 44. Simpson A, Maniatis N, Jury F, Cakebread JA, Lowe LA, Holgate ST, et al.  
765 Polymorphisms in a disintegrin and metalloprotease 33 (ADAM33) predict impaired  
766 early-life lung function. *Am J Respir Crit Care Med* 2005; 172:55-60.
- 767 45. Sadeghnejad A, Ohar JA, Zheng SL, Sterling DA, Hawkins GA, Meyers DA, et al.  
768 Adam33 polymorphisms are associated with COPD and lung function in long-term  
769 tobacco smokers. *Respir Res* 2009; 10:21.
- 770 46. van Diemen CC, Postma DS, Vonk JM, Bruinenberg M, Schouten JP, Boezen HM. A  
771 disintegrin and metalloprotease 33 polymorphisms and lung function decline in the  
772 general population. *Am J Respir Crit Care Med* 2005; 172:329-33.
- 773 47. Dijkstra A, Vonk JM, Jongepier H, Koppelman GH, Schouten JP, ten Hacken NH, et al.  
774 Lung function decline in asthma: association with inhaled corticosteroids, smoking and  
775 sex. *Thorax* 2006; 61:105-10.
- 776 48. Cornelis MC, Agrawal A, Cole JW, Hansel NN, Barnes KC, Beaty TH, et al. The Gene,  
777 Environment Association Studies consortium (GENEVA): maximizing the knowledge

- 778 obtained from GWAS by collaboration across studies of multiple conditions. *Genet*  
779 *Epidemiol* 2010; 34:364-72.
- 780 49. Caporaso N, Gu F, Chatterjee N, Sheng-Chih J, Yu K, Yeager M, et al. Genome-wide  
781 and candidate gene association study of cigarette smoking behaviors. *PLoS One* 2009;  
782 4:e4653.
- 783 50. Weedon MN, Lango H, Lindgren CM, Wallace C, Evans DM, Mangino M, et al.  
784 Genome-wide association analysis identifies 20 loci that influence adult height. *Nat*  
785 *Genet* 2008; 40:575-83.
- 786 51. Sovio U, Bennett AJ, Millwood IY, Molitor J, O'Reilly PF, Timpson NJ, et al. Genetic  
787 determinants of height growth assessed longitudinally from infancy to adulthood in the  
788 northern Finland birth cohort 1966. *PLoS Genet* 2009; 5:e1000409.
- 789 52. Kang SJ, Chiang CW, Palmer CD, Tayo BO, Lettre G, Butler JL, et al. Genome-wide  
790 association of anthropometric traits in African- and African-derived populations. *Hum*  
791 *Mol Genet* 2010; 19:2725-38.
- 792 53. Palamarchuk A, Efanov A, Nazaryan N, Santanam U, Alder H, Rassenti L, et al. 13q14  
793 deletions in CLL involve cooperating tumor suppressors. *Blood*; 115:3916-22.
- 794 54. Hammarsund M, Corcoran MM, Wilson W, Zhu C, Einhorn S, Sangfelt O, et al.  
795 Characterization of a novel B-CLL candidate gene--DLEU7--located in the 13q14 tumor  
796 suppressor locus. *FEBS Lett* 2004; 556:75-80.
- 797 55. Rahmatpanah FB, Carstens S, Hooshmand SI, Welsh EC, Sjahputera O, Taylor KH, et al.  
798 Large-scale analysis of DNA methylation in chronic lymphocytic leukemia. *Epigenomics*  
799 2009; 1:39-61.
- 800 56. Pekarsky Y, Zanesi N, Croce CM. Molecular basis of CLL. *Semin Cancer Biol* 2010.
- 801 57. Cooke SL, Pole JC, Chin SF, Ellis IO, Caldas C, Edwards PA. High-resolution array  
802 CGH clarifies events occurring on 8p in carcinogenesis. *BMC Cancer* 2008; 8:288.
- 803 58. Wang X, Dockery DW, Wypij D, Fay ME, Ferris BG, Jr. Pulmonary function between 6  
804 and 18 years of age. *Pediatr Pulmonol* 1993; 15:75-88.
- 805 59. Kunzli N, Ackermann-Lieblich U, Keller R, Perruchoud AP, Schindler C. Variability of  
806 FVC and FEV1 due to technician, team, device and subject in an eight centre study: three  
807 quality control studies in SAPALDIA. Swiss Study on Air Pollution and Lung Disease in  
808 Adults. *Eur Respir J* 1995; 8:371-6.
- 809 60. Vercelli D, Martinez FD. The Faustian bargain of genetic association studies: bigger  
810 might not be better, or at least it might not be good enough. *J Allergy Clin Immunol*  
811 2006; 117:1303-5.
- 812 61. Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung  
813 volumes and forced ventilatory flows. Report Working Party Standardization of Lung  
814 Function Tests, European Community for Steel and Coal. Official Statement of the  
815 European Respiratory Society. *Eur Respir J Suppl* 1993; 16:5-40.
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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) Framingham<sup>15</sup>, (2) CHARGE<sup>17</sup>, (3) Spirometa<sup>16</sup>, (4) Asthmatics<sup>33</sup> and (5)  
843 CHARGE-Spirometa<sup>18</sup> 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 formula<sup>61</sup>.  
 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 ± 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
<b>Discovery (ESE-cohorts)</b>										
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
<b>Replication with in silico data</b>										
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
<b>Asthmatics</b>										
<b>Discovery (ESE-cohorts)</b>										
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
<b>Replication with in silico data</b>										
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 studies	P for heterogeneity between asthmatics and non-asthmatics
<b>Non-Asthmatics</b>												
rs1889321	9	112340656	MUSK*	0.287	-0.150	6.95E-07	0.814	<b>0.0187</b>	-0.011	0.480	0.713	0.053
<b>rs9316500</b>	<b>13</b>	<b>49992115</b>	<b>DLEU7</b>	<b>0.336</b>	<b>0.135</b>	<b>4.81E-06</b>	<b>0.613</b>	<b>0.0255</b>	<b>0.033</b>	<b>0.026</b>	<b>0.124</b>	<b>0.075</b>
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	<b>0.0058</b>	-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	<b>0.0350</b>	-0.016	0.310	0.247	0.86
rs496809	18	74857661	SALL3	0.078	-0.236	0.00004	0.412	<b>0.0041</b>	0.022	0.443	0.373	0.60
rs10933964	3	110021881	TRAT1	0.499	-0.117	0.00006	0.345	<b>0.0022</b>	-0.015	0.265	0.869	<b>0.041</b>
<b>Asthmatics</b>												
rs10808265	7	131840229	PLXNA4B	0.484	-0.175	1.66E-06	0.844	<b>0.0020</b>	0.069	0.105	0.258	0.16
rs1902618	15	58951491	RORA	0.234	-0.220	1.72E-06	0.449	<b>0.0043</b>	0.029	0.590	0.777	0.58
rs3843306	1	91060718	BARHL2	0.460	0.176	5.11E-06	0.042	<b>8.33E-06</b>	0.047	0.270	0.883	0.24
rs7006290	8	41734295	ANK1	0.319	0.185	5.19E-06	0.058	<b>0.0003</b>	0.038	0.456	0.574	0.45
rs12436689	14	84723772	FLRT2	0.244	-0.212	6.87E-06	0.420	<b>0.0010</b>	-0.017	0.759	0.051	0.95
rs12615721	2	81710037	CTNNA2	0.104	-0.303	7.65E-06	0.853	<b>0.0020</b>	-0.127	0.129	0.824	0.08
rs10516809	4	89640109	HERC5	0.101	0.306	8.67E-06	0.790	<b>3.60E-05</b>	-0.060	0.446	0.200	0.41
rs16856186	1	203944749	SLC45A3	0.098	0.268	8.92E-06	0.510	<b>0.0034</b>	-0.079	0.350	0.094	0.46
rs158536	20	52148709	BCAS1	0.408	0.162	0.00002	0.948	<b>0.0001</b>	0.075	0.100	0.917	0.09
rs477725	19	42066106	ZNF345	0.158	0.223	0.00003	0.821	<b>0.0031</b>	-0.069	0.273	0.255	0.14
rs9662589	1	230344234	DISC1	0.221	0.188	0.00005	0.868	<b>0.0002</b>	-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>B - decline in FEV1/FVC</b>					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
<b>Non-Asthmatics</b>												
rs2658782	11	92806379	FLJ25393	0.166	0.186	4.33E-06	0.362	<b>0.0041</b>	0.031	0.135	0.242	0.91
rs1867982	10	73197053	C10orf54	0.109	0.202	5.56E-06	0.839	<b>0.0034</b>	-0.008	0.745	0.412	0.24
rs12712969	2	46185673	PRKCE	0.268	-0.147	7.08E-06	0.687	<b>0.0116</b>	0.012	0.448	0.916	0.76
rs10187654	2	234478798	TRPM8	0.205	0.151	8.87E-06	0.797	<b>0.0049</b>	-0.015	0.382	0.676	0.15
rs356642	2	100903870	NPAS2	0.189	0.158	9.79E-06	0.162	<b>0.0014</b>	-0.010	0.565	0.282	0.28
rs890515	8	67534388	ADHFE1	0.497	0.119	0.00001	0.580	<b>0.0257</b>	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
<b>Asthmatics</b>												
rs4831760	8	15576956	TUSC3	0.326	0.222	5.27E-08	0.066	<b>7.74E-08</b>	0.011	0.799	0.541	0.73
rs7144584	14	63345565	SYNE2	0.116	-0.318	5.62E-07	0.616	<b>0.0010</b>	0.089	0.272	0.752	0.43
rs1148186	10	28657641	MPP7	0.194	0.219	7.28E-06	0.760	<b>0.0035</b>	-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.

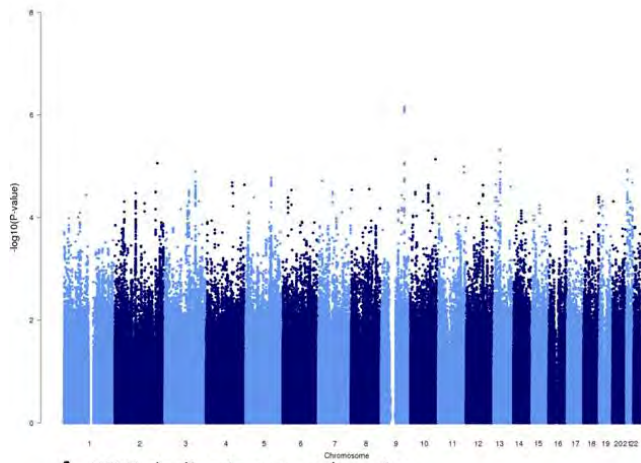
A	dbSNP ID	chr	position (build 36.3)	refs	gene nearby	Maximal frequency of coding allele	Non-Asthmatics			Asthmatics		
							FEV1 percent predicted	FEV1 percent change	FEV1 decline [%/y]	FEV1 percent predicted	FEV1 percent change	FEV1 decline [%/y]
							P-value	P-value	P-value	P-value	P-value	P-value
	rs1435867	2	229219173	2	PID1	0.065	<b>0.021</b>	0.845	0.418	0.824	0.321	0.377
	rs17035917	4	106740191	2,3	INTS12-GSTCD-NPNT	0.071	<b>0.006</b>	0.135	0.077	0.073	0.056	0.061
	rs17035960	4	106751295	2,3	INTS12-GSTCD-NPNT	0.071	<b>0.004</b>	0.093	0.054	0.067	0.056	0.063
	rs11727735	4	106851319	2,3	INTS12-GSTCD-NPNT	0.076	<b>2.14E-04</b>	0.361	0.198	0.057	0.114	0.074
	rs10516526	4	106908353	2,3	INTS12-GSTCD-NPNT	0.072	<b>1.96E-04</b>	0.327	0.177	0.062	0.120	0.078
	rs11731417	4	106965461	2,3	INTS12-GSTCD-NPNT	0.073	<b>1.32E-04</b>	0.335	0.177	<b>0.048</b>	0.146	0.090
	rs1032295	4	145654034	2	HHIP	0.397	0.173	0.096	0.306	0.274	<b>0.042</b>	<b>0.033</b>
	rs1512285	4	145670409		HHIP	0.462	<b>0.032</b>	<b>0.029</b>	0.141	0.152	<b>0.033</b>	<b>0.024</b>
	rs720485	4	145682038	2,3	HHIP	0.391	0.159	0.510	0.786	0.943	<b>0.044</b>	0.058
	rs1512288	4	145710731	2,3	HHIP	0.401	0.188	0.533	0.813	0.781	<b>0.046</b>	0.057
	rs6817273	4	145711453	2,3	HHIP	0.400	0.179	0.535	0.816	0.866	<b>0.046</b>	0.057
	rs3008798	6	39887840	3	DAAM2	0.464	0.326	0.960	0.850	0.755	<b>0.009</b>	<b>0.017</b>
	rs1318002	6	39892112	3	DAAM2	0.480	0.649	0.725	0.902	0.782	<b>0.015</b>	<b>0.023</b>
	rs2395730	6	39892343	3	DAAM2	0.442	0.522	0.716	0.513	0.619	<b>0.003</b>	<b>0.007</b>
	rs12899618	15	69432174	3	THSD4	0.158	<b>0.003</b>	<b>0.003</b>	<b>0.014</b>	0.424	0.137	0.131
	rs1913768	15	69436598	3	THSD4	0.159	<b>0.003</b>	<b>0.002</b>	<b>0.011</b>	0.393	0.162	0.152
	rs1568010	15	69455566	4	THSD4	0.372	0.535	<b>0.042</b>	0.067	0.413	0.241	0.111
	rs2304488	16	56631711	1	MMP15	0.186	<b>0.033</b>	0.101	0.147	0.112	0.344	0.506
	rs12447804‡	16	56632783	5	MMP15	0.179	<b>0.033</b>	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	<b>0.010</b>	0.133	0.089	0.076	0.241	0.256
rs1035672	2	218383444	2,3	TNS1	0.384	<b>0.010</b>	0.133	0.089	0.093	0.243	0.258
rs929937	2	218417460	2,4	TNS1	0.386	0.623	<b>0.017</b>	<b>0.016</b>	<b>0.004</b>	0.915	0.888
rs3845823	2	229611365	4	PID1	0.432	<b>0.039</b>	0.963	0.852	0.393	0.997	0.987
rs12477314‡	2	239542085	5	HDAC4	0.215	<b>0.023</b>	0.727	0.655	0.125	0.361	0.278
rs1529672	3	25495586	5	RARB	0.159	<b>0.012</b>	0.329	0.337	0.605	0.716	0.860
rs1828591	4	145700230	2,3	HHIP	0.394	<b>0.031</b>	0.470	0.345	0.254	0.138	0.139
rs13118928	4	145705839	2,3	HHIP	0.393	<b>0.043</b>	0.500	0.371	0.271	0.132	0.132
rs3995090	5	147826008	2,3	HTR4	0.394	<b>0.011</b>	0.785	0.699	<b>0.029</b>	0.649	0.456
rs2395730	6	39892343	3	DAAM2	0.442	0.277	0.554	0.685	0.979	<b>0.036</b>	<b>0.039</b>
rs2798641	6	109374743	5	ARMC2	0.209	0.315	0.444	0.530	<b>0.006</b>	0.188	0.158
rs9496346	6	142711031	2	GPR126	0.316	<b>0.053</b>	0.378	0.368	0.098	0.777	0.788
rs6570507	6	142721265	2	GPR126	0.314	<b>0.035</b>	0.356	0.342	0.080	0.804	0.821
rs11155242	6	142733242	2	GPR126	0.210	<b>0.008</b>	0.785	0.670	0.268	0.857	0.807
rs7753012	6	142787576	2	GPR126	0.337	<b>0.051</b>	0.477	0.487	0.065	0.566	0.637
rs3748069	6	142809326	2	GPR126	0.319	<b>0.043</b>	0.407	0.401	0.134	0.604	0.628
rs171891	6	142892305	2,4	GPR126	0.198	<b>0.013</b>	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	<b>0.032</b>	<b>0.028</b>
rs11172113	12	55813550	5	LRP1	0.384	<b>0.005</b>	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	<b>0.047</b>	<b>0.031</b>
rs2456526	15	50876734	1	ONECUT1	0.136	<b>0.011</b>	0.524	0.500	0.451	0.230	0.250
rs12899618	15	69432174	3	THSD4	0.158	<b>3.25E-04</b>	0.253	0.390	0.328	0.596	0.668
rs1913768	15	69436598	3,4	THSD4	0.159	<b>4.78E-04</b>	0.221	0.344	0.365	0.617	0.695
rs2304488	16	56631711	1	MMP15	0.186	0.121	<b>0.002</b>	<b>0.002</b>	0.085	0.760	0.515
rs12447804‡	16	56632783	5	MMP15	0.179	0.121	<b>0.003</b>	<b>0.003</b>	0.085	0.719	0.487
rs2865531‡	16	73947817	5	CFDP1	0.428	<b>0.035</b>	0.621	0.736	0.377	0.840	0.603

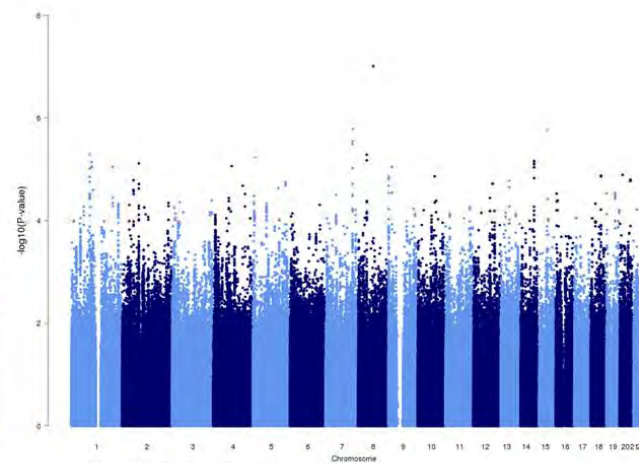




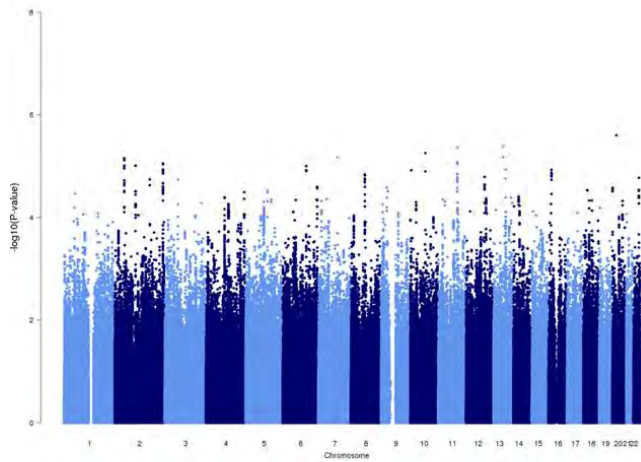
**Figure 1**



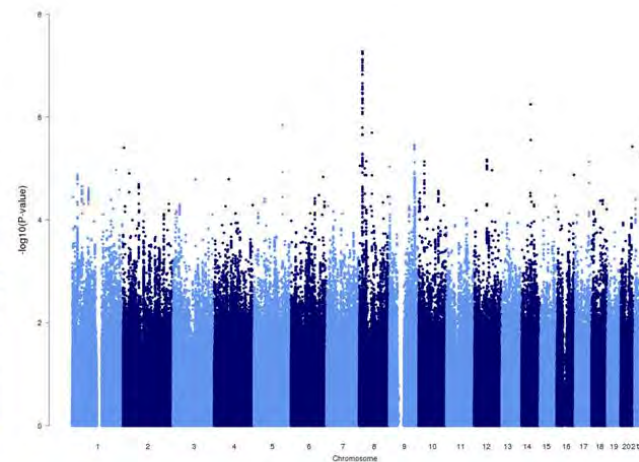
**A** - FEV1 decline in non-asthmatics



**B** - FEV1 decline in asthmatics



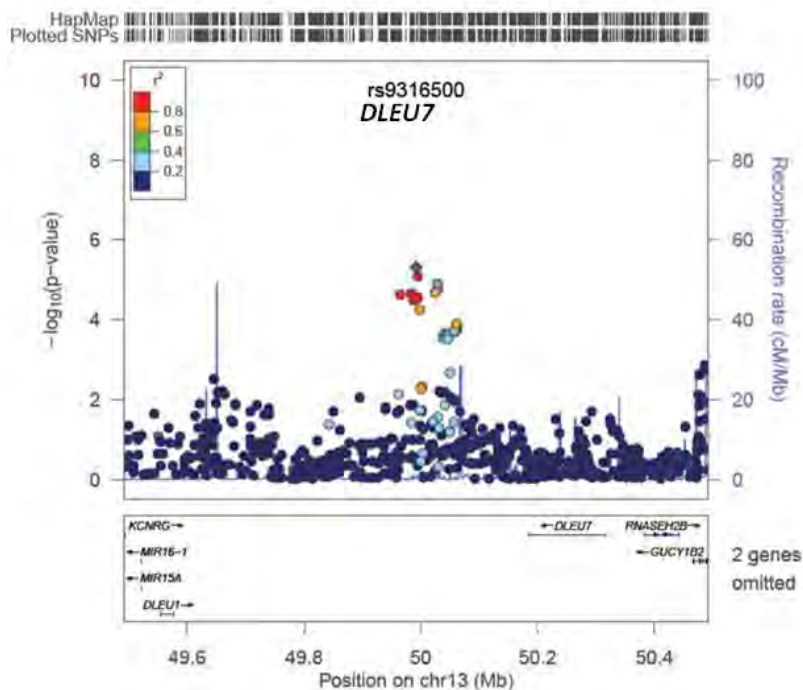
**C** - FEV1/FVC decline in non-asthmatics



**D** - FEV1/FVC decline in asthmatics

**Figure 2**

**A**



**B**

rs9316500, *DLEU7*

	allele	beta	standard	P	P Het
study	frequency	estimate	error	study	between studies
<b>DISCOVERY PHASE</b>					
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	
<b>pooled FE (n=2860)</b>		<b>0.135</b>	<b>0.030</b>	<b>4.81E-06</b>	<b>0.613</b>
<b>REPLICATION PHASE</b>					
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	
<b>pooled FE (n=10858)</b>		<b>0.033</b>	<b>0.015</b>	<b>0.026</b>	<b>0.124</b>
<b>DISCOVERY &amp; REPLICATION pooled FE overall (n=13518)</b>		<b>0.053</b>	<b>0.013</b>	<b>5.70E-05</b>	<b>0.011</b>

FE = fixed effect meta-analysis estimate

