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The Pharmacogenomics of Inhaled Corticosteroids and Lung Function Decline in COPD

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Abstract

Inhaled corticosteroids (ICS) are widely prescribed for patients with chronic obstructive pulmonary disease (COPD), yet with variable outcomes and adverse reactions which may be genetically determined. The primary aim of the study was to identify the genetic determinants for FEV_1 changes related to ICS therapy. In the Lung Health Study 2 (LHS-2), 1,116 COPD patients were randomized to the ICS, triamcinolone acetonide (n=559), or placebo (n=557) with spirometry performed every 6 months for 3 years. We performed a pharmacogenomic genome-wide association study (GWAS) for the genotype-by-ICS treatment effect on 3 years of forced expiratory volume in 1 second (FEV_1) changes (estimated as slope) in 802 genotyped LHS-2 participants. Replication was performed in 199 COPD patients randomized to the ICS, fluticasone or placebo. A total of five loci showed genotype-by-ICS interaction at $P < 5x10^{-6}$; of these, SNP rs111720447 on chromosome 7 was replicated (discovery $P = 4.8x10^{-6}$, replication $P = 5.9\ x10^{-5}$) with the same direction of interaction effect. ENCODE data revealed that in glucocorticoid treated (dexamethasone) A549 alveolar cell line, glucocorticoid receptor binding sites were located near SNP rs111720447. In stratified analyses of LHS-2, genotype at SNP rs111720447 was significantly associated with rate of FEV_1 decline in patients taking ICS (C allele beta= 56.35 mL/year, 95% confidence interval (CI) = 29.96, 82.76 ml/yr) and also in patients who were assigned to placebo, though the relationship was weaker and in the opposite direction than that in the ICS group (C allele beta= -27.57 mL/year, 95% CI = -53.27, -1.87 ml/yr). The study uncovered genetic factors associated with FEV_1 changes related to ICS in COPD patients, which may provide new insight on the potential biology of steroid responsiveness in COPD.

Key words: COPD, genetics, FEV_1 decline, inhaled corticosteroids, pharmacogenomics.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) affects 384 million people and is the 3rd leading cause of death worldwide[1]. Inhaled corticosteroids (ICS) are the most commonly prescribed inhaled anti-inflammatory medications in the world for patients with COPD[2, 3]. Although currently, international and national guidelines recommend the use of ICS only for patients who experience frequent exacerbations (defined as having 2 or more exacerbations per year or a hospitalization within the previous year)[4], in clinical practice, a majority of patients without a significant history of exacerbations are prescribed these medications. Disconcertingly, the long-term use of ICS containing compounds has been associated with increased rates of pneumonia [5] and accelerated bone demineralization[6]. Although on average ICS do not modify the long-term "natural" history of COPD[7], there may be some who experience benefits while others who may experience only harm. There is a compelling need to understand the biology underlying steroid responsiveness in COPD in order to move beyond the "average" patient (which does not exist in clinical practice) to individualized therapy and most importantly to enable design of future interventions that may be able to surmount steroid insensitivity in COPD [8].

There is strong evidence to support the role of genetics in how patients respond to ICS. First, there is a wide variability in outcomes across ICS clinical trials in COPD[9, 10]. Early studies of ocular pressure have demonstrated both familial segregation and heritability in the way patients responded to glucocorticoid treatment [11] [12]. Furthermore, studies in asthma (where ICS are the most widely prescribed medications) have shown that the response to ICS is characterized by high intra-individual repeatability[13] and high inter-individual variability [14], with up to 40% of patients with asthma having no response to therapy[15]. Taken together, these data suggest that genetic variation plays an important role in ICS responses in COPD.

In the current study, our primary aim was to discover genetic loci that modify the effects of ICS on lung function as measured by changes in forced expiratory volume in 1 second (FEV_1) over time in patients with COPD. We first used data from Lung Health Study 2 (LHS-2) [7] to determine potential genetic variants that modified the effects of ICS on rate of FEV_1 decline over 3 years. We then externally validated these variants in a completely independent cohort, Advair, Biomarkers in COPD (ABC) trial [16].

METHODS

The Lung Health Study (LHS-1 and LHS-2)

The details of the LHS-2 have been published previously[7]. Briefly, LHS-2 was a multicenter randomized controlled trial (RCT) to determine the effects of inhaled corticosteroids (1,200 ug of triamcinolone) on FEV_1 decline over 3 years[17]. LHS-2 recruited subjects who were smoking or had recently quit (less than 2 years) and had previously participated in LHS-1. At the time of recruitment all subjects were between the ages of 40 and 69 years and demonstrated airflow obstruction defined by FEV_1 between 30% and 90% of predicted, in the presence of a FEV_1/ forced vital capacity (FVC) ratio of < 0.70 after bronchodilation. LHS-2 excluded subjects if they had regularly used bronchodilators or oral or inhaled corticosteroids in the previous year. A total of 1,116 subjects were randomized to receive triamcinolone acetonide (1,200 µg per day, n=559), or a matching placebo (n=557). Spirometry was performed at baseline and then every 6 months for 3 years. All spirometry measurements were performed according to the American Thoracic Society (ATS) guidelines. For this study, we used only post-bronchodilator values. The LHS is registered under ClinicalTrials.gov identifier NCT00000569, and the current analyses were performed under the University of British Columbia (UBC) ethics certificate H16-01201.

LHS Genotyping

Genotyping was performed using buffy coat samples of 4,251 European Americans who participated in LHS-1. The details of genotyping and quality control have been previously described [18]. Briefly, samples were genotyped using the Illumina Human660WQuad v.1_A BeadChip. Imputation was undertaken with the Michigan Imputation Server using the Haplotype Reference Consortium (HRC) panel. Variants were excluded if the imputation quality (r^2) was < 0.5 and if the minor allele frequency was <1%. For the 1,018 LHS-2 subjects who participated in LHS-1, 818 had genotype data available (n=410 triamcinolone and n=408 placebo arm). Of these participants, complete phenotypic information was available in 802 subjects (n=401 triamcinolone and n=401 placebo arm). LHS genotype data are available in the National Institute of Health (NH) database for genotype and phenotype (dbGaP) under study accession phs000335.v3.p2.

Genome-wide association testing

We performed a GWAS for the FEV_1 change rate (mL per year) using an additive genetic model with a genotype-by-ICS (triamcinolone vs. placebo) interaction term and adjustments for baseline FEV_1 in mL, age, sex, body mass index (BMI), smoking status and the first 5 genetic principal components (PCs) according to the following formula:

$$E[FEV_1\ slope] = \beta_0 + \beta_1 SNP \times Treatment + \beta_2 SNP + \beta_3 Treatment + \beta_4 Baseline\ FEV_1 + \beta_5 Age + \beta_6 Sex + \beta_7 BMI + \beta_8 Smoking + \beta_9 PC1 + \beta_{10} PC2 + \beta_{11} PC3 + \beta_{12} PC4 + \beta_{13} PC5.$$

Individual FEV_1 change rate was estimated using a linear model of FEV_1 (measurements at baseline, 6-month, 12-month, 24-month, and 36-month visits) over time. Genome-wide significance threshold was set to the traditional $P<5x10^{-8}$. Given the relatively small sample size of the discovery cohort (LHS-2), we defined a secondary set of criteria to identify loci that could harbor true signals of steroid responsiveness for replication. These included genetic loci that had an association with FEV_1 decline of $P < 5x10^{-6}$ in LHS-2 and region plots demonstrating genetic support from surrounding SNPs within 500 Kb of the sentinel SNP.

We conducted sensitivity analyses of the identified loci stratified according to smoking status (current smokers versus former smokers) and to COPD severity based on the GOLD (Global Initiative for Chronic Obstructive Lung Disease) spirometric grades. We also evaluated these loci in only those participants who were randomized to the ICS group.

Candidate gene approach: Lung eQTL SNPs for the glucocorticoid receptor gene *NR3C1*

The *NR3C1* gene encodes the glucocorticoid receptor (GR), which is the target receptor protein for ICS. In addition to the agnostic GWAS approach, we used a candidate gene approach to determine whether (or not) variations in the gene encoding GR modified ICS responses. To select candidate SNPs in or near the GR gene, we used the biologically determined gene regulation approach and identified the genetic variants associated with *NR3C1* gene expression in lung tissue using data from the lung expression quantitative trait loci (eQTL) study. The study details have been previously described [19-21]. Briefly, meta-analysis of the association between SNPs and mRNA expression adjusted for age, sex and smoking status was performed in non-tumor lung tissue samples from 1,111 patients who underwent lung resection surgery. The discovered eQTLs were either *cis* (1Mb from the transcription start site of gene) or *trans* (more than 1Mb away or on a different chromosome). For the purpose of this study, we evaluated the lung eQTL dataset for SNPs that were significantly associated with the expression

of probesets mapping to *NR3C1* at an FDR <5%, and then chose the SNP, which demonstrated the lowest genotype-by-ICS P value in the discovery GWAS, to represent the association of *NR3C1* gene with the phenotype of interest in the replication cohort.

Genotyping in the Replication cohort

We attempted replication of selected SNPs identified in LHS-2 by genotyping DNA obtained from blood samples of COPD patients who participated in the Advair, Biomarkers in COPD (ABC) study. The details of the ABC cohort have been previously published[16]. The ABC study was a multicenter clinical trial that evaluated the effects of inhaled fluticasone, an ICS, alone or in combination with salmeterol, a long acting beta-2 agonist (LABA), in reducing systemic inflammation in patients with COPD. All recruited participants were 40 years of age or older, and had at least 10 pack years of smoking history and an FEV_1 of < 80% of predicted with an FEV_1/FVC ratio of < 0.70 after bronchodilation. Study participants underwent a run-in phase (visit 1: enrollment) during which they received fluticasone propionate (500 mcg twice daily) for 4 weeks (visit 2: run-in phase). This was followed by a medication withdrawal phase wherein ICS and long acting bronchodilators were withdrawn for 4 weeks (visit 3: withdrawal phase). Participants were then randomly assigned to one of three arms: placebo, inhaled fluticasone (500 mcg twice daily) or inhaled fluticasone/salmeterol combination (500/50 mcg twice daily) for 4 weeks (visit 4: Randomized Controlled Trial Phase).The lung function of each subject was measured at each visit. There were 212 participants who completed all four visits, of these 199 were successfully genotyped and our analyses were based on these subjects[16]. The ABC trial is registered under ClinicalTrials.gov identifier NCT00120978 and the phenotypic data are available upon request from the authors.

Genotyping was performed at Genome Quebec using a multiplex PCR performed on 20 ng of template genomic DNA. Additional details on genotyping are available in the Supplement. The quality control procedure included inspection of genotyping intensity clusters (for a clear separation of genotypes) and deviations from the Hardy-Weinberg equilibrium.

The phenotype for the replication cohort was the FEV_1 change (in mL) over 4 weeks using data from visit 3 and 4 (i.e. immediately before and after the 4 week RCT phase). A similar linear model to the discovery GWAS was fitted for the 6 SNPs.

$$(E[FEV_1\ change] = \beta_0 + \beta_1 SNP \times Treatment + \beta_2 SNP + \beta_3 Treatment + \beta_4 Baseline\ FEV_1 + \beta_5 Age + \beta_6 Sex + \beta_7 BMI + \beta_8 Smoking)$$

A Bonferroni adjusted P value of 0.008 (multiple testing of 6 SNPs) was used as a cut-off for statistically significant replication.

Phenome-Wide association study (PheWAS)

For significantly replicated SNPs, we determined whether or not they were associated with other diseases or phenotypes. The Open Genetics Target platform was used to asses PheWAS for the replicated SNPs [22], which was complemented by a look-up using the GWAS Catalog[23].

Encyclopedia of DNA Elements (ENCODE) data

To determine whether the GR binding site was in close proximity to the identified SNPs, we utilized the publicly available ENCODE chromatin immunoprecipitation (ChIP) assays with sequencing (ChIP-Seq) dataset[24]. Specifically, we investigated the GR binding scores with or without dexamethasone in varying concentrations (0-50nM) and treatment times (GSM803358 and GSM803395) in the alveolar cell line A549.

A summary flowchart showing the study design and the number of individuals included and the main results is shown in **Supplementary Figure 1**.

RESULTS

Descriptive characteristics of LHS-2 Participants

The clinical and demographic characteristics of the LHS-2 participants (n = 802) are shown in **Table 1** along with characteristics of the ABC replication cohort. The results from LHS-2 were previously published and showed that ICS therapy did not have a significant effect on FEV_1 decline over 3 years [7] as shown in **Figure 1**.

Genome-wide association results

In the GWAS, we included 6,559,489 variants with a minor allele frequency (MAF) > 2% and imputation quality > 0.7. A total of 802 subjects who had no missing covariates were included. A Manhattan plot is shown in **Figure 2**. The quantile–quantile (QQ) plot is presented in

Supplementary Figure 2. The genomic inflation factor was 0.991, suggesting no systematic deviation in the association statistics. No loci met genome-wide significance ($P < 5x10^{-8}$); however, we identified 5 loci using the secondary criteria at a p-value cut-off of 5×10^{-6}; their GWAS summary statistics are shown in **Table 2**. Using an additional candidate gene approach, we performed a look-up for eQTLs that were significantly associated with the expression of probesets mapping to *NR3C1*. Of the 195 significant eQTLs at FDR <5%, SNP rs10057473 had an eQTL P value of P=4.16x10-7 for the probeset 100122984_TGI_at which mapped to *NR3C1* gene in the lung tissue, and was the SNP with the lowest genotype-by-ICS P value. Hence SNP rs10057473 was used to represent the association of NR3C1 gene with phenotype in discovery and replication cohorts. In the discovery pharmacogenomic GWAS of LHS-2, the GR eQTL rs10057473 was nominally associated with FEV_1 decline (P=0.02).

In addition to the interaction results, we also report in **Table 2** the SNPs and the treatment main effects. Given that the overall slope of FEV_1 change was negative, for the SNPs' main effect, a positive estimate indicated a slower decline and a negative estimate indicated a faster (accelerated) decline.

The association results for the 6 SNPs with FEV_1 changes at each follow up visit (as opposed to the overall slope used in the GWAS) are shown in **Supplementary Table 1**.

Sensitivity and stratified analyses

Additional testing of the 6 SNPs was performed in stratified analyses that included ICS users only, and placebo only groups, and in strata of current smokers vs. former smokers as well as by disease severity. The results of the stratified analyses are shown in **Table 3.** The results demonstrate that the significant SNP rs111720447 on chr7 was strongly associated with FEV_1 decline in ICS users (C allele beta= 56.35 mL/year; 95% CI=29.96, 82.76; P=3.35E-05) and to a lesser extent in the placebo group but in an opposite direction (C allele beta= -27.57 mL/year; 95% CI=-53.27, -1.87; P= 0.036) as that observed in the ICS group. The C allele at rs111720447 showed a stronger genotype-by-ICS interaction effect with FEV_1 changes in current smokers (beta = 72.74 mL/year; 95% CI=32.47, 113.00; P= 0.0004) vs. former smokers (beta=145.04 mL/year; 95% CI = 20.61, 269.46; P=0.023) and in COPD GOLD II patients (beta= 85.97 mL/year; 95% CI=38.32, 133.63; P= 0.0004), than in GOLD III and IV patients (beta = 116.33 mL/year; 95% CI=8.24, 224.42; P= 0.036). The SNP was did not show an interaction effect on FEV_1 decline in GOLD I patients (P= 0.38).

Replication in the ABC cohort

The 5 significant loci from the GWAS in LHS-2 and the eQTL SNP for *NR3C1* were directly genotyped in the ABC cohort. The ABC study design is shown in **Supplementary Figure 3**. For analytic purposes, we grouped the two treatment groups (fluticasone and fluticasone/salmeterol) into one category (treatment). A total of 199 participants were successfully genotyped for all 6 SNPs. The demographics for the 199 subjects included in the replication study are shown in **Table 1**.

To replicate the GWAS results, we calculated the FEV_1 changes (in mL) over 4 weeks using data from visit 3 and 4 (i.e. immediately before and after the 4 weeks RCT phase). Using this FEV_1 change as the response variable, a similar linear model to the discovery GWAS was then fitted for the 6 SNPs.

In the replication cohort, two SNPs showed significant ($P < 0.05$) association between FEV_1 change and genotype-by-ICS interaction with the same direction of effect as those observed in the discovery cohort. SNP rs111720447 on chromosome 7 showed strong replication with P=5.98E-05. SNP rs10057473 is the eQTL SNP for gene *NR3C1* and was also replicated with a nominal $P = 0.042$. Applying a Bonferroni corrected P value for replication of 6 SNPs ($P= 0.008$), only SNP rs111720447 on chromosome 7 showed statistically significant replication. The results for all 6 SNPs are shown in **Table 4** and the region plot for the replicated SNP at chromosome 7 is shown in **Figure 3**. SNPs rs111720447 and rs10057473 did not show deviation from the Hardy-Weinberg equilibrium ($P = 0.92$ and $P = 0.36$ for SNP rs111720447 and rs10057473, respectively), and the intensity clustering plots (**Supplementary Figure 4**) for the replicated SNPs showed clear separation of genotype clusters, indicating high quality genotyping.

Differences in FEV_1 changes related to ICS therapy stratified by genotypes of rs111720447 and rs10057473 in LHS-2 and ABC trials

Supplementary Figure 5 illustrates the changes in FEV_1 between ICS and placebo groups according to genotype for rs111720447 and rs10057473, and in LHS-2 and ABC trials. In both cohorts, individuals with the A allele in SNP rs111720447 on chr7 demonstrated on average lower FEV_1 at the end of the study if they were assigned to ICS-based therapy compared with placebo. For the glucocorticoid receptor SNP rs10057473, the G allele, which was related to increased expression of the GR encoding gene *NR3C1* in lung tissue, was associated with a higher FEV_1 in those who were assigned to ICS therapy compared with placebo.

ENCODE results

We evaluated the ChIP-Seq data from ENCODE for possible linkage disequilibrium blocks surrounding SNP rs111720447 (using r2>0.5) and identified two GR binding sites (**Figure 4**) in A549 alveolar cell lines treated with 100 nM dexamethasone for 1 hour. These GR binding sites did not map to any known genes but were in close proximity to the activator of transcription and developmental regulator (AUTS2) gene.

PheWAS results

The PheWAS plots for SNPs rs111720447 and rs10057473 are shown in **Supplementary Figure 6.** There were no significant associations with any diseases or common phenotypes for this SNP that met a Bonferroni corrected P value for the number of phenotypes tested for SNP rs111720447. In the GWAS Catalog, there were no associations that were reported for rs111720447. For the GR eQTL SNP rs10057473, the PheWAS analysis showed significant associations for the C allele with increased risk for atrial fibrillation as well decreased "comparative body size at age 10"

DISCUSSION

The 'one-size-fits all' historic approach has been widely used in drug development and clinical trials of approved drugs. In realty though, a spectrum of responses occur, and the "average" efficacy and side effects observed in any given clinical trial averages both the beneficial and the detrimental responses of a mean response. Despite their controversial effects and the large heterogeneity in patients' response, ICS are widely used in COPD. The identification of the pharmacogenomic factors that govern patients' response to ICS is crucial to their proper use in clinical practice [25]. Most importantly, these factors may also provide crucial insights on the biology of steroid responses (and non-responses) in COPD that could lead to new therapies and/or biomarkers to surmount steroid insensitivity in COPD.

In the current study, we performed a GWAS for genotype-by-ICS interaction effect on FEV_1 decline in 802 subjects from the LHS-2. No SNPs met genome-wide significance (P < 5E-8). However, by using a secondary defined criteria for discovery of P <5x10E-6, we identified an intergenic SNP on chromosome 7 (rs111720447, P=4.81E-06) that was replicated in the same direction (P=5.98E-05) in an independent cohort of 199 COPD subjects, who were treated with either fluticasone alone or in combination with salmeterol vs. placebo. Using a candidate gene

approach, we found that a regulatory variant (rs10057473) associated with lung tissue expression levels of the GR receptor-encoding gene *NR3C1* also modified the rate of FEV_1 decline related to ICS therapy. However, although this variant showed nominal P value significance (P=0.02 and 0.04 for discovery and replication, respectively and with the same direction of effect), the association did not survive correction for multiple testing (at P<0.008). The ENCODE data revealed that in glucocorticoid treated (dexamethasone) A549 alveolar cell line, GR binding sites were located near the novel ICS variant; rs111720447, which was identified in the current study. In stratified analyses, we found that the significant SNP rs111720447 on chr7 was strongly associated with FEV_1 decline in the ICS group (C allele beta= 56.36 mL/year; 95% CI=29.96, 82.76; P= 3.35E-05) and to a lesser extent in the placebo group (C allele beta= -27.57 mL/year; 95% CI=-53.27, -1.87; P= 0.036). This SNP showed a stronger genotype-by-ICS interaction effect with FEV_1 decline in current smokers (beta = 72.74 mL/year; 95% CI=32.47, 113.00; P=0.0004) vs. former smokers (beta=145.04 mL/year; 95% CI = 20.61, 269.46; P=0.023) and in COPD GOLD II patients (beta= 85.97 mL/year; 95% CI=38.32, 133.63; P= 0.0004), than in GOLD III and IV patients (beta = 116.33 mL/year; 95% CI=8.24, 224.42; P = 0.036). The effect was not significant in GOLD I patients (P=0.38). These latter data are consistent with the strong association observed between this SNP and FEV_1 changes in the ABC replication cohort, which did not have any GOLD I patients and were predominantly GOLD II patients. The precise mechanisms underlying these observations are not known and were beyond the purview of the current study.

To date, a number of GWAS for ICS responses have been reported but all in asthma patients. In one study the authors integrated the pharmacodynamic properties of drug responses with GWAS data, and then modelled drug effect-dose relationships through mathematical equations based on repeated measures of drug response at multiple dosages[26]. The authors found that there were multiple ICS response-associated SNPs that mapped to several genes and intergenic regions [26]. Another study reported a pharmacogenetic effect of ICS response for variant rs37973 in the glucocorticoid-induced transcript 1 gene (GLCCI1)[15]. Interestingly, studies in COPD patients, which attempted to replicate the pharmacogenetic effect of rs37973, have yielded conflicting results. Although one study of 462 Caucasians failed to identify an association[27], another study of 204 Chinese COPD patients demonstrated a significant association with ICS response[28]. The reasons behind these conflicting data for rs37973 in COPD are not clear, but it may be due to differences in the underlying disease severity, sample sizes (low power), medications that were evaluated including different potencies of ICS and use

of combination drugs rather than ICS monotherapy and differential follow up times across studies. In the current study, SNP rs37973 was not associated with FEV_1 decline related to ICS use (P>0.05). This could indicate that this SNP has no effect on ICS response in COPD patients, or that it is not associated with long term FEV_1 response.

Uncovering the mechanism of GWAS associated variants is a challenging task. The novel variant we report on chromosome 7 has no effect on gene expression in lung tissue but is located near a GR binding site in the airway alveolar cell line A549 after treatment with dexamethasone. This provides a potential mechanism underlying the genetic association. The variant could alter the structure or charge of the GR complex and thus modify its downstream effects. The nearest gene to the associated SNP and the GR binding sites is the AUTS2 gene, which is reported to be associated with multiple neurological disorders including autism and neuron development as well as non-neurological disorders such as acute lymphoblastic leukemia (ALL) [29].

Without any additional information, it is challenging to distinguish noise from true biological signals in GWAS that do not meet the genome-wide cut-offs. At the molecular level, one of the most important factors that drive therapeutic responsiveness to inhaler medications is the variation and/or expression of the receptor in lung tissue or on infiltrating immune cells that penetrate airways and orchestrate the local inflammatory responses. For corticosteroids, the target is the glucocorticoid receptor (GR) that is encoded by the nuclear receptor 3C1 (*NR3C1*) gene[30]. Thus, it is biologically plausible that genetic variants in *NR3C1* gene may modulate the effects of ICS on FEV_1 decline. In the current study the variant that regulates *NR3C1* expression was associated with differential FEV_1 decline between triamcinolone and placebo groups (P=0.02) which was nominally replicated in the ABC cohort (P=0.04) but did not meet Bonferroni corrected P value for multiple testing. The direction of effect was that the genetic variant associated with decreased *NRC31* expression was associated with accelerated FEV_1 decline in the triamcinolone group.

The current study has a number of limitations. First, owing to the study's relatively small sample size, there is a possibility of Type 2 error in missing potentially significant genotype–by-treatment interaction effect on FEV_1 decline. Nevertheless, with direct genotyping of candidate loci emerging from the discovery GWAS we have uncovered novel locus related to how COPD patients responded to ICS. Second, the mechanism underlying the novel locus on chromosome

7 is not yet understood, although ENCODE data suggest that the variant is located near a GR binding site in relevant alveolar cell lines treated with dexamethasone (corticosteroid). Third, while the clinical trial setting has a number of important methodological advantages including mitigation against indication bias and confounding in treatment response from non-compliance, finding an "ideal" external cohort for replication is extremely challenging. In this study, we used the ABC cohort for replication, which had some notable differences compared with LHS-2 including age, smoking status and severity of airflow limitation. The ABC trial used a more potent and lipid-soluble ICS (fluticasone) and also contained an ICS-LABA arm in contrast to LHS-2 which used triamcinolone without any LABA. Furthermore, the RCT component of the ABC trial was over 4 weeks (and largely evaluated the effects of ICS withdrawal on FEV_1), while LHS-2 had at least 3 years of follow-up (and largely evaluated the effects of ICS addition to standard therapy on FEV_1). In analysis stratified by follow up year in LHS-2 (**Supplementary Table 1**), the genetic effect became stronger for the reported SNPs at 24 and 36 months follow up, arguing against a concentrated "early" effect that can explain the replication of chromosome 7 locus in the ABC trial, which had only a 4 week RCT duration. A more likely explanation is the inclusion of GOLD II and III patients in whom the association of chromosome 7 SNP was much stronger compared to GOLD I patients, who made up a significant proportion of LHS-2. The findings encourage the use of genetics for recent RCTs with various follow-up durations and treatment regimens (e.g. triple inhaler therapies). Finally, although the variants uncovered may offer potential new insights into the biology and biomarkers of ICS responses in COPD, its clinical translation is uncertain and beyond the purview of the present study. Clinical translation will require fine-tuning of the biomarker parameters and phenotypes as well as additional replication in real-life cohorts.

In conclusion, the current study has uncovered a novel genetic variant associated with long term effect of ICS on FEV_1 decline in COPD patients that should be evaluated in future studies for biological and clinical translation. This observation may provide new insights on the biology of ICS responses in COPD, as well as for the design and validation of novel therapeutics to surmount the challenges of steroid insensitivity in COPD.

REFERENCES

1. Adeloye D, Chua S, Lee C, Basquill C, Papana A, Theodoratou E, Nair H, Gasevic D, Sridhar D, Campbell H, Chan KY, Sheikh A, Rudan I, Global Health Epidemiology Reference G. Global and regional estimates of COPD prevalence: Systematic review and meta-analysis. *Journal of global health* 2015: 5(2): 020415.
2. Price D, West D, Brusselle G, Gruffydd-Jones K, Jones R, Miravitlles M, Rossi A, Hutton C, Ashton VL, Stewart R, Bichel K. Management of COPD in the UK primary-care setting: an analysis of real-life prescribing patterns. *Int J Chron Obstruct Pulmon Dis* 2014: 9: 889-904.
3. Barnes PJ, Bonini S, Seeger W, Belvisi MG, Ward B, Holmes A. Barriers to new drug development in respiratory disease. *European Respiratory Journal* 2015: 45(5): 1197-1207.
4. Vogelmeier CF, Criner GJ, Martinez FJ, Anzueto A, Barnes PJ, Bourbeau J, Celli BR, Chen R, Decramer M, Fabbri LM, Frith P, Halpin David MG, López Varela MV, Nishimura M, Roche N, Rodriguez-Roisin R, Sin DD, Singh D, Stockley R, Vestbo J, Wedzicha JA, Agusti A. Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Lung Disease 2017 Report: GOLD Executive Summary. *European Respiratory Journal* 2017: 49(3): 1700214.
5. Suissa S. Number needed to treat in COPD: exacerbations versus pneumonias. *Thorax* 2013: 68(6): 540-543.
6. Scanlon PD, Connett JE, Wise RA, Tashkin DP, Madhok T, Skeans M, Carpenter PC, Bailey WC, Buist AS, Eichenhorn M, Kanner RE, Weinmann G. Loss of bone density with inhaled triamcinolone in Lung Health Study II. *Am J Respir Crit Care Med* 2004: 170(12): 1302-1309.
7. Group" LHSR. Effect of Inhaled Triamcinolone on the Decline in Pulmonary Function in Chronic Obstructive Pulmonary Disease. *New England Journal of Medicine* 2000: 343(26): 1902-1909.
8. Schork NJ. Personalized medicine: Time for one-person trials. *Nature* 2015: 520(7549): 609-611.
9. Calverley PM, Anderson JA, Celli B, Ferguson GT, Jenkins C, Jones PW, Yates JC, Vestbo J, investigators T. Salmeterol and fluticasone propionate and survival in chronic obstructive pulmonary disease. *The New England journal of medicine* 2007: 356(8): 775-789.
10. Lapperre TS, Snoeck-Stroband JB, Gosman MM, Jansen DF, van Schadewijk A, Thiadens HA, Vonk JM, Boezen HM, Ten Hacken NH, Sont JK, Rabe KF, Kerstjens HA, Hiemstra PS, Timens W, Postma DS, Sterk PJ. Effect of fluticasone with and without salmeterol on pulmonary outcomes in chronic obstructive pulmonary disease: a randomized trial. *Annals of internal medicine* 2009: 151(8): 517-527.
11. Armaly MF. The heritable nature of dexamethasone-induced ocular hypertension. *Archives of ophthalmology (Chicago, Ill : 1960)* 1966: 75(1): 32-35.
12. Schwartz JT, Reuling FH, Feinleib M, Garrison RJ, Collie DJ. Twin heritability study of the effect of corticosteroids on intraocular pressure. *Journal of Medical Genetics* 1972: 9(2): 137-143.
13. Drazen JM, Silverman EK, Lee TH. Heterogeneity of therapeutic responses in asthma. *British medical bulletin* 2000: 56(4): 1054-1070.
14. Szefler SJ, Martin RJ, King TS, Boushey HA, Cherniack RM, Chinchilli VM, Craig TJ, Dolovich M, Drazen JM, Fagan JK, Fahy JV, Fish JE, Ford JG, Israel E, Kiley J, Kraft M, Lazarus SC, Lemanske RF, Jr., Mauger E, Peters SP, Sorkness CA. Significant variability in response to inhaled corticosteroids for persistent asthma. *The Journal of allergy and clinical immunology* 2002: 109(3): 410-418.
15. Tantisira KG, Lasky-Su J, Harada M, Murphy A, Litonjua AA, Himes BE, Lange C, Lazarus R, Sylvia J, Klanderman B, Duan QL, Qiu W, Hirota T, Martinez FD, Mauger D, Sorkness C, Szefler S, Lazarus SC, Lemanske RF, Jr., Peters SP, Lima JJ, Nakamura Y, Tamari M, Weiss ST. Genomewide Association between GLCCI1 and Response to Glucocorticoid Therapy in Asthma. *New England Journal of Medicine* 2011: 365(13): 1173-1183.
16. Don DS, Man SFP, Darcy DM, Gordon F, Mark F, Eric W, Ernest Y, Rajesh RM, Warren R, Lyle SM, Eric W, Robert LC, Dave W, Wen QG, Roxanne R. The Effects of Fluticasone with or without Salmeterol

on Systemic Biomarkers of Inflammation in Chronic Obstructive Pulmonary Disease. *American Journal of Respiratory and Critical Care Medicine* 2008: 177(11): 1207-1214.
17. Lung Health Study Research G, Wise R, Connett J, Weinmann G, Scanlon P, Skeans M. Effect of inhaled triamcinolone on the decline in pulmonary function in chronic obstructive pulmonary disease. *The New England journal of medicine* 2000: 343(26): 1902-1909.
18. Hansel NN, Ruczinski I, Rafaels N, Sin DD, Daley D, Malinina A, Huang L, Sandford A, Murray T, Kim Y, Vergara C, Heckbert SR, Psaty BM, Li G, Elliott WM, Aminuddin F, Dupuis J, O'Connor G, Doheny K, Scott AF, Boezen HM, Postma DS, Smolonska J, Zanen P, Mohamed Hoesein FA, de Koning HJ, Crystal R, Tanaka T, Ferrucci L, Silverman E, Wan E, Vestbo J, Lomas DA, Connett J, Wise RA, Neptune ER, Mathias RA, Paré PD, Beaty TH, Barnes KC. Genome-wide study identifies two loci associated with lung function decline in mild to moderate COPD. *Human genetics* 2013: 132(1): 79-90.
19. Obeidat M, Nie Y, Fishbane N, Li X, Bosse Y, Joubert P, Nickle DC, Hao K, Postma DS, Timens W, Sze MA, Shannon CP, Hollander Z, Ng RT, McManus B, Miller BE, Rennard S, Spira A, Hackett TL, Lam W, Lam S, Faner R, Agusti A, Hogg JC, Sin DD, Pare PD. Integrative Genomics of Emphysema Associated Genes Reveals Potential Disease Biomarkers. *American journal of respiratory cell and molecular biology* 2017.
20. Obeidat M, Hao K, Bosse Y, Nickle DC, Nie Y, Postma DS, Laviolette M, Sandford AJ, Daley DD, Hogg JC, Elliott WM, Fishbane N, Timens W, Hysi PG, Kaprio J, Wilson JF, Hui J, Rawal R, Schulz H, Stubbe B, Hayward C, Polasek O, Jarvelin MR, Zhao JH, Jarvis D, Kahonen M, Franceschini N, North KE, Loth DW, Brusselle GG, Smith AV, Gudnason V, Bartz TM, Wilk JB, O'Connor GT, Cassano PA, Tang W, Wain LV, Soler Artigas M, Gharib SA, Strachan DP, Sin DD, Tobin MD, London SJ, Hall IP, Pare PD. Molecular mechanisms underlying variations in lung function: a systems genetics analysis. *The Lancet Respiratory medicine* 2015: 3(10): 782-795.
21. Hao K, Bossé Y, Nickle DC, Paré PD, Postma DS, Laviolette M, Sandford A, Hackett TL, Daley D, Hogg JC, Elliott WM, Couture C, Lamontagne M, Brandsma C-A, van den Berge M, Koppelman G, Reicin AS, Nicholson DW, Malkov V, Derry JM, Suver C, Tsou JA, Kulkarni A, Zhang C, Vessey R, Opiteck GJ, Curtis SP, Timens W, Sin DD. Lung eQTLs to Help Reveal the Molecular Underpinnings of Asthma. *PLoS Genetics* 2012: 8(11): e1003029.
22. Carvalho-Silva D, Pierleoni A, Pignatelli M, Ong C, Fumis L, Karamanis N, Carmona M, Faulconbridge A, Hercules A, McAuley E, Miranda A, Peat G, Spitzer M, Barrett J, Hulcoop DG, Papa E, Koscielny G, Dunham I. Open Targets Platform: new developments and updates two years on. *Nucleic Acids Research* 2018: 47(D1): D1056-D1065.
23. Buniello A, MacArthur JAL, Cerezo M, Harris LW, Hayhurst J, Malangone C, McMahon A, Morales J, Mountjoy E, Sollis E, Suveges D, Vrousgou O, Whetzel PL, Amode R, Guillen JA, Riat HS, Trevanion SJ, Hall P, Junkins H, Flicek P, Burdett T, Hindorff LA, Cunningham F, Parkinson H. The NHGRI-EBI GWAS Catalog of published genome-wide association studies, targeted arrays and summary statistics 2019. *Nucleic acids research* 2019: 47(D1): D1005-d1012.
24. ENCODE. An integrated encyclopedia of DNA elements in the human genome. *Nature* 2012: 489(7414): 57-74.
25. Wu AC, Gay C, Rett MD, Stout N, Weiss ST, Fuhlbrigge AL. Pharmacogenomic test that predicts response to inhaled corticosteroids in adults with asthma likely to be cost-saving. *Pharmacogenomics* 2015: 16(6): 591-600.
26. Wang Y, Tong C, Wang Z, Wang Z, Mauger D, Tantisira KG, Israel E, Szefler SJ, Chinchilli VM, Boushey HA, Lazarus SC, Lemanske RF, Wu R. Pharmacodynamic genome-wide association study identifies new responsive loci for glucocorticoid intervention in asthma. *Pharmacogenomics J* 2015: 15(5): 422-429.

27. Mosteller M, van den Berge M, Hosking L, Timens W, Hiemstra PS, Crim C, Postma DS, Ghosh S. Genetic evaluation of the effect of GLCCI1 rs37973 on corticosteroid response in chronic obstructive pulmonary disease. *COPD Research and Practice* 2017: 3(1): 2.
28. Lei Y, Gao Y, Chen J, Li M, Wu X, Ning Q, Zhao J, Xiong W, Xu Y, Xie J. GLCCI1 rs37973: a potential genetic predictor of therapeutic response to inhaled corticosteroids in Chinese chronic obstructive pulmonary disease patients. *Scientific reports* 2017: 7: 42552.
29. Oksenberg N, Ahituv N. The role of AUTS2 in neurodevelopment and human evolution. *Trends in genetics : TIG* 2013: 29(10): 600-608.
30. Kino T. Glucocorticoid Receptor. *In:* De Groot LJ, Chrousos G, Dungan K, Feingold KR, Grossman A, Hershman JM, Koch C, Korbonits M, McLachlan R, New M, Purnell J, Rebar R, Singer F, Vinik A, eds. Endotext, South Dartmouth (MA), 2000.

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Authors' Contributions

Conceived and designed the study: MO, PDP, DDS.
Lung eQTL data collection and analysis: MO, MVDB, PJ, KH, CAB, SFPM
Lung Health Study data and genotyping: MO, XL, NNH, NR, RM, IR, THB, KCB
Study data analyses: MO, XL, AF.
Wrote the manuscript: MO, DDS.
Discussed the results and implications and commented on the manuscript at all stages: all co-authors.

TABLES

Table 1. Characteristics of the discovery and replication cohorts.

FEV1 slope in LHS-2 was estimated using FEV_1 change rate over 3 years per subject, Variables men and current smokers are expressed as total number (% of column totals); all other variables are expressed as mean (SD).

	LHS-2 (discovery)		ABC (replication)	
Variable	**Placebo**	**ICS**	**Placebo**	**Steroid**
N	401	401	36	163
Age, year	55.42 (6.65)	55.53 (6.60)	66.39 (10.52)	69.61 (9.23)
BMI, kg/m2	26.43 (4.70)	26.39 (4.47)	26.19 (5.91)	27.99 (5.76)
FEV_1, mL	2292.12 (643.53)	2351.37 (608.25)	1428.61 (522.12)	1403.68 (590.35)
FEV_1, % predicted	68.94 (11.97)	70.12 (12.45)	49.67 (15.07)	47.90 (16.11)
FEV_1 change Slope in mL/year for LHS Absolute change in mL for ABC	-49.15 (85.79)	-52.56 (77.54)	55.00 (317.66)	78.90 (240.34)
Men	252 (62.84%)	267 (66.58%)	22 (61.11%)	106 (65.03%)
Current smokers	354 (88.28%)	358 (89.28%)	14 (38.89%)	50 (30.67%)

Table 2. **Genetic loci associated with FEV_1 decline in LHS-2**.

Chr: chromosome. Alleles: coded/non-coded. SNP Effect: SNP main effect. Treatment Effect: Treatment main effect (triamcinolone vs. placebo). SNP × Treatment: SNP-treatment interaction effect. Given that the overall slope of FEV_1 change was negative, forthe SNP's main effect,a positive beta estimate indicates less (or slower) decline and a negative beta estimate indicates more (or accelerated) decline.

SNP	Chr	Position	Alleles (coded/ non-coded)	Coded allele frequency	SNP Effect		Treatment Effect		SNP × Treatment	
					Beta mL/year	P	Beta mL/year	P	Beta mL/year	P
rs10057473	5	142887378	G/C	44.3%	-12.7	2.8E-02	-19.1	3.9E-02	18.6	2.4E-02
rs111720447	7	68703305	C/A	95.2%	-29.5	1.8E-02	-168.3	4.1E-06	86.6	4.8E-06
rs10108679	8	2179372	G/C	43.9%	-17.6	5.3E-03	-38.1	5.3E-05	41.1	3.1E-06
rs1361249	10	7007283	C/T	70.1%	24.5	5.8E-05	55.8	4.1E-05	-42.6	1.7E-06
rs117989968	11	128639945	T/C	97.8%	126.1	3.0E-06	338.0	4.1E-06	-173.4	3.2E-06
rs12433619	14	71679203	A/G	48.2%	21.4	2.3E-04	34.6	4.3E-04	-38.7	2.5E-06

Table 3. Stratified analyses results for the 6 SNPs from the ICS response discovery GWAS.

The table shows the results for the 6 SNPs that were selected for replication in the ABC cohort. The table shows the association results in treatment strata (ICS only and placebo only groups), as well as current vs. former smokers and in the different GOLD stages. The estimates shown are in mL/year for the SNP effect for ICS and Placebo groups, for SNP-by-ICS interaction, and for smoking status and COPD sensitivity analyses. The models used in each analyses were the following:

Treatment group: FEV_1 slope ~ SNP + baseline FEV1 + age + sex + BMI + smoking status + PC1 + PC2 + PC3 + PC4 + PC5
Smoking status: FEV_1 slope ~ SNP*treatment group + baseline FEV1 + age + sex + BMI + PC1 + PC2 + PC3 + PC4 + PC5
COPD GOLD stage: FEV_1 slope ~ SNP* treatment group + baseline FEV1 + age + sex + BMI + smoking status +PC1 + PC2 + PC3 + PC4 + PC5

				Treatment group						Smoking status strata						COPD GOLD stage strata								
				ICS			Placebo			Current smokers			Former smokers			COPD GOLD I			COPD GOLD II			COPD GOLD III & IV		
				n = 401			n = 401			n = 712			n = 90			n = 162			n = 576			n = 63		
SNP	CHR	Position	alleles	estimate	95% CI	p	estimate	95% CI	p	estimate	95% CI	p	estimate	95% CI	p	estimate	95% CI	p	estimate	95% CI	p	estimate	95% CI	p
rs10057473	5	142887378	G/C	6.04	(-4.96, 17.03)	0.2809	-13.37	(-25.16, -1.58)	0.0263	22.60	(5.47, 39.74)	0.0098	-17.41	(-69.57, 34.76)	0.5084	-2.03	(-36.57, 32.51)	0.9077	22.96	(3.32, 42.61)	0.0220	10.62	(-46.52, 67.77)	0.7104
rs11720447	7	68703305	C/A	56.36	(29.96, 82.76)	3.35E-05	-27.57	(-53.27, -1.87)	0.0356	72.74	(32.47, 113.00)	0.0004	145.04	(20.61, 269.46)	0.0229	45.26	(-55.36, 145.89)	0.3755	85.97	(38.32, 133.63)	0.0004	116.33	(8.24, 224.42)	0.0355
rs10108679	8	2179372	G/C	22.01	(10.62, 33.41)	0.0002	-16.28	(-29.44, -3.11)	0.0156	39.42	(21.08, 57.76)	2.78E-05	42.96	(-10.20, 96.12)	0.1117	62.11	(24.45, 99.77)	0.0014	35.61	(14.73, 56.49)	0.0009	18.62	(-33.91, 71.16)	0.4796
rs1361249	10	7007283	C/T	-16.86	(-29.00, -4.71)	0.0066	23.85	(11.40, 36.31)	0.0002	-42.42	(-60.82, -24.03)	6.97E-06	-32.89	(-91.52, 25.74)	0.2675	-23.08	(-60.60, 14.44)	0.2260	-52.84	(-73.89, -31.79)	1.08E-06	-3.63	(-72.79, 65.53)	0.9164
rs117989968	11	128639945	T/C	-48.87	(-97.01, -0.74)	0.0466	122.82	(68.29, 177.34)	1.24E-05	-178.59	(-254.47, -102.70)	4.56E-06	-83.06	(-759.90, 593.78)	0.8076	-214.72	(-864.21, 434.77)	0.5146	-171.93	(-246.83, -97.04)	7.93E-06	-2917.73	(-8214.03, 2378.57)	0.2737
rs12433619	14	71679203	A/G	-16.76	(-27.63, -5.90)	0.0026	22.14	(10.15, 34.12)	0.0003	-38.20	(-55.20, -21.20)	1.19E-05	-64.15	(-118.18, -10.12)	0.0206	-43.16	(-80.96, -5.37)	0.0255	-35.86	(-55.08, -16.64)	0.0003	-46.59	(-96.28, 3.10)	0.0655

Table 4. Replication results for ICS pharmacogenomics loci in the ABC cohort.

Chr: chromosome. Alleles: coded/non-coded. SNP Effect: SNP main effect. Treatment Effect: Treatment main effect (triamcinolone vs. placebo). SNP × Treatment: SNP-treatment interaction effect. SNPs meeting Bonferroni corrected P values are shown in bold

SNP	Chr	Position	Alleles (coded/ non-coded)	Coded allele frequency	SNP Effect		Treatment Effect		SNP × Treatment	
					Beta mL	P	Beta mL	P	Beta mL	P
rs10057473	5	142887378	G/C	44.7%	-108.7	0.1	-105.9	0.1	145.7	0.04
rs111720447	**7**	**68703305**	**C/A**	**96.5%**	**-657.7**	**1.3E-05**	**-1278.0**	**8.2E-05**	**670.8**	**6.0E-05**
rs10108679	8	2179372	G/C	45.5%	68.2	0.2	80.6	0.3	-72.3	0.26
rs1361249	10	7007283	C/T	71.1%	-5.7	0.9	-26.7	0.9	26.9	0.77
rs117989968	11	128639945	T/C	98.2%	92.4	0.7	424.3	0.4	-209.9	0.45
rs12433619	14	71679203	A/G	55.0%	91.6	0.1	48.4	0.6	-37.9	0.56

FIGURE LEGENDS

Figure 1. The FEV_1 change in LHS-2 in the triamcinolone and the placebo groups.

Figure 2. Manhattan plot of GWAS in LHS-2. The plot shows the P values (-log10 scale) on the Y axes and the SNP positions across 22 autosomal chromosomes on the X axes. The horizontal red line represents the p-value cut-off of $5x10^{-6}$.

Figure 3. Region plot of the ICS pharmacogenomics loci associated with FEV_1 decline in LHS-2 and replicated in ABC cohort. The Y axis represent the P values (–log10 scale) and the X axis is the genomic position. Gene names and their corresponding coordinates are shown below. The sentinel SNP is shown as a purple diamond and the color coding of SNPs reflects the degree of linkage disequilibrium (LD) with the sentinel SNP using 1000G reference. The horizontal red line represents the p-value cut-off of $5x10^{-7}$. The horizontal green line represents the p-value cut-off of $5x10^{-6}$.

Figure 4. Glucocorticoid receptor (GR) binding in proximity of rs11720447 A) LD block surrounding rs11720447 (r2>0.5) based on the 1000 genomes. B) GR ChiP-Seq on the lung cell line (A549) treated with 100 nM dexamethasone for 1 hour. Abbreviations GR= Glucocorticoid receptor, and DEX= dexamethasone.

FIGURES

Figure 1

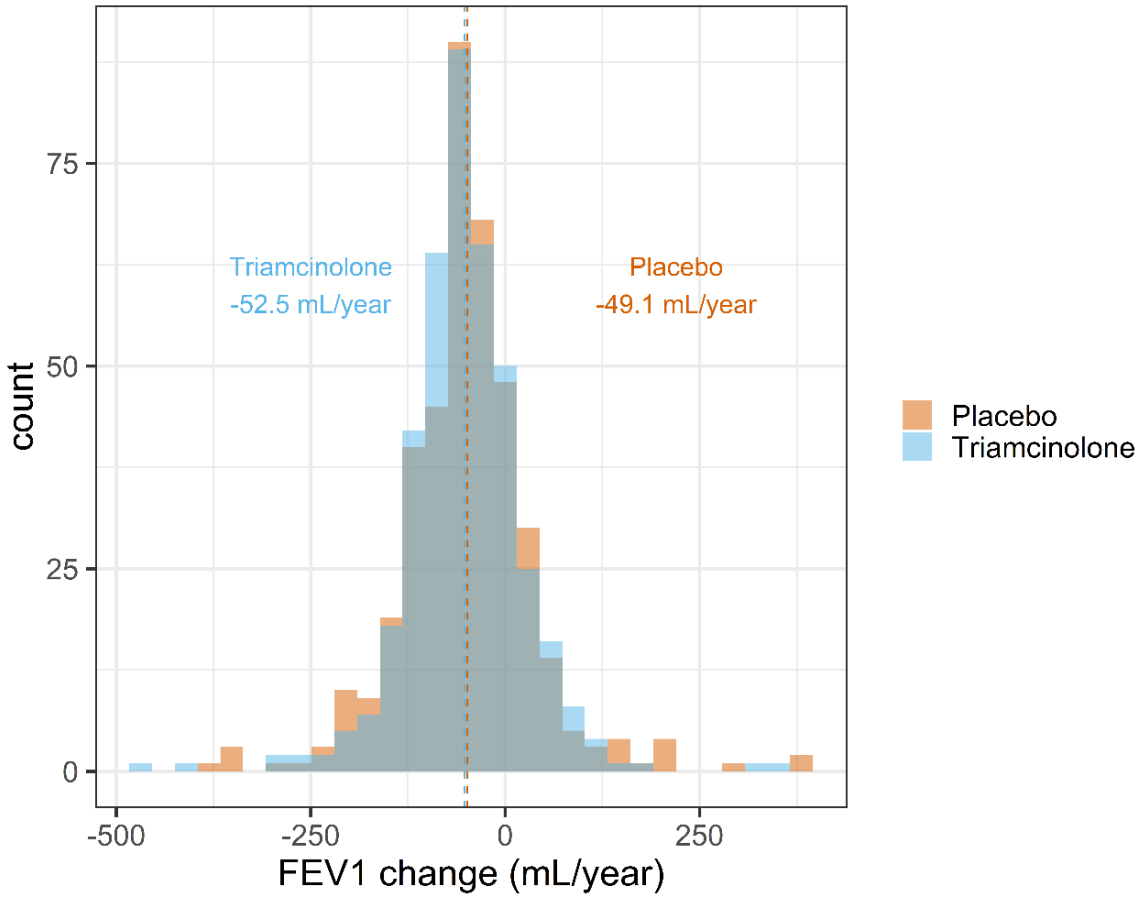
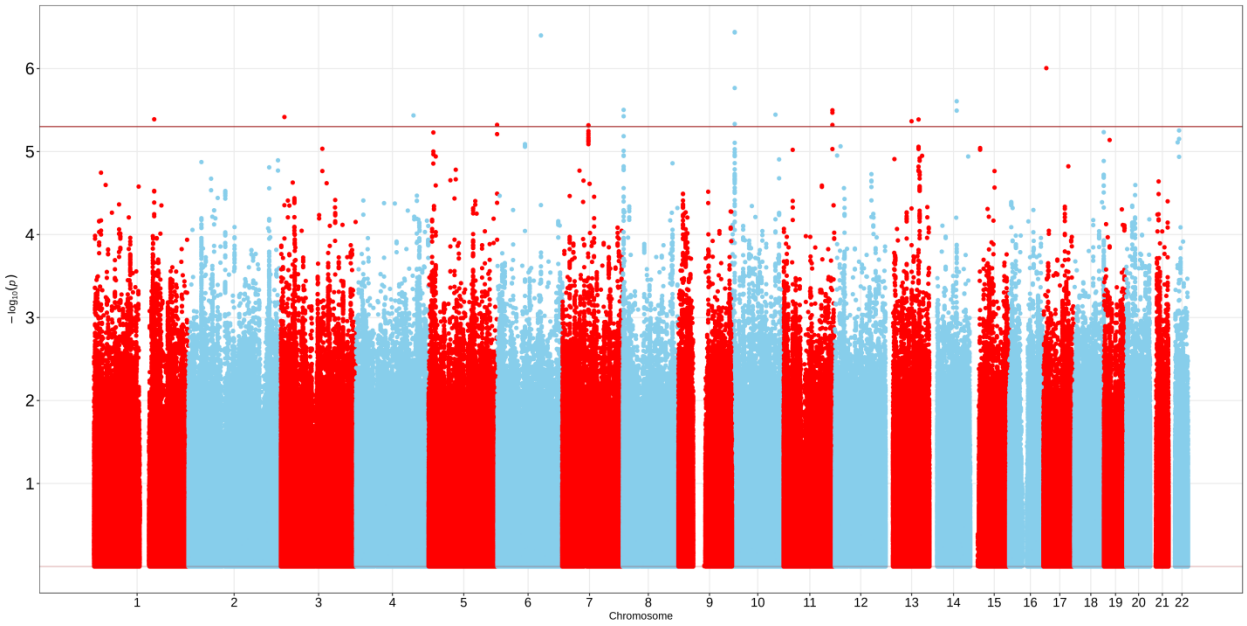


Figure 2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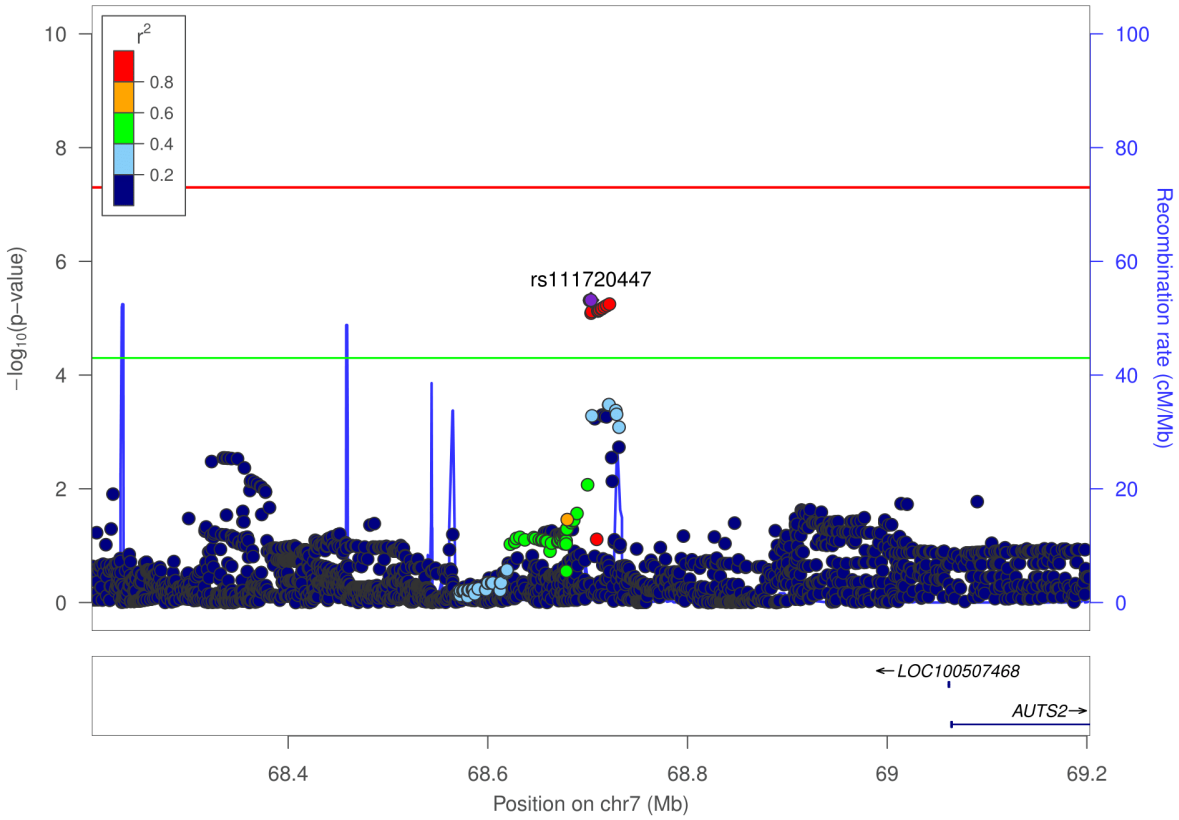
Chromosome

Figure 3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0.4
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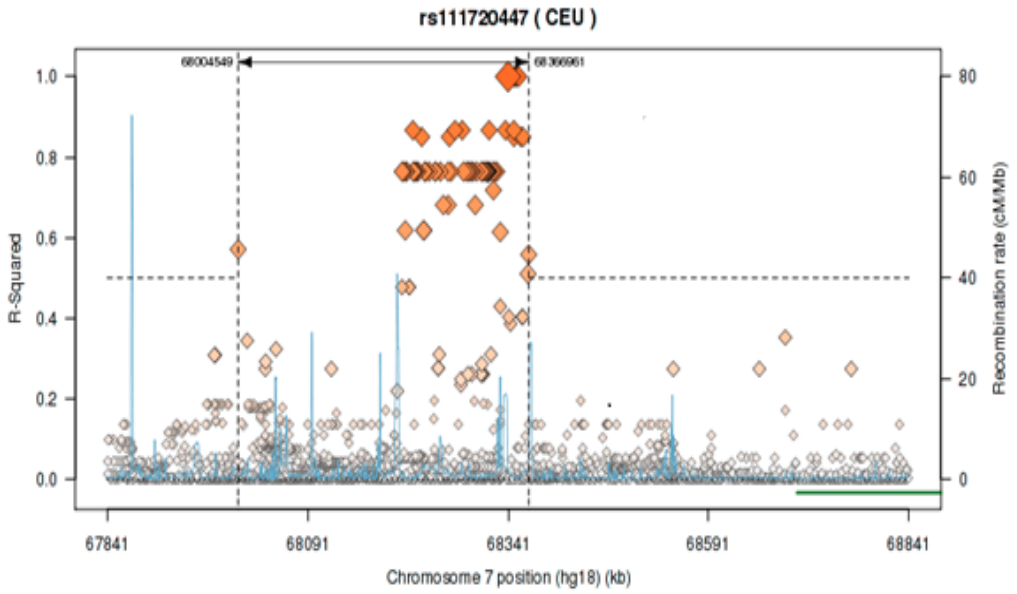
AUTS2
Position on chr7 (Mb)

Figure 4

A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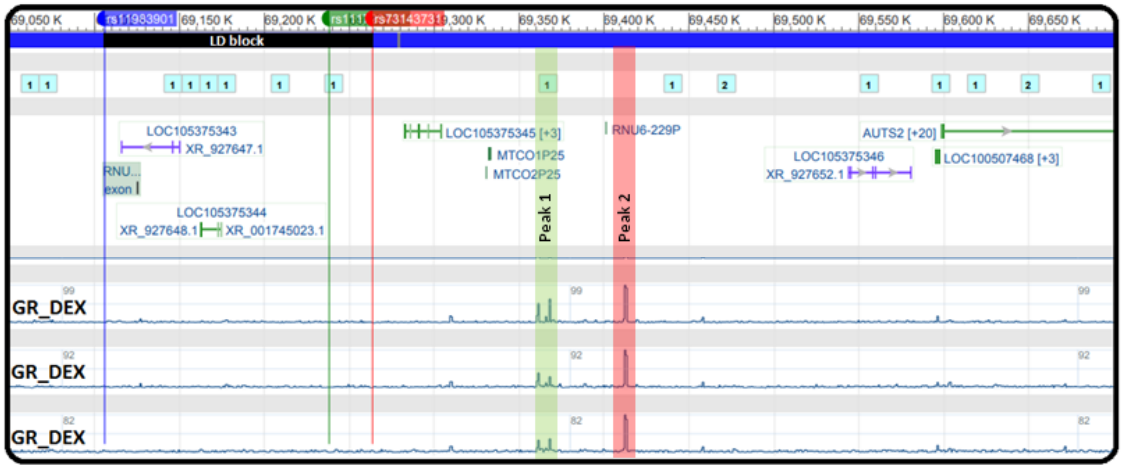
68341
68591
68841

B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LOC100507468 [+3]
LOC105375344
XR_927648.1
XR_001745023.1
Peak 1
Peak 2
GR_DEX
GR_DEX
GR_DEX

The Pharmacogenomics of Inhaled Corticosteroids and Lung Function Decline in COPD

Ma'en Obeidat[1], Alen Faiz[2], Xuan Li[1], Maarten van den Berge[2], Nadia N. Hansel[3], Philippe Joubert[4], Ke Hao[5], Corry-Anke Brandsma[2], Nicholas Rafaels[6], Rasika Mathias[7], Ingo Ruczinski[8], Terri H. Beaty[9], Kathleen C. Barnes[6], S. F. Paul Man[1], Peter D. Paré[1], and Don D. Sin[1]

Supplementary materials

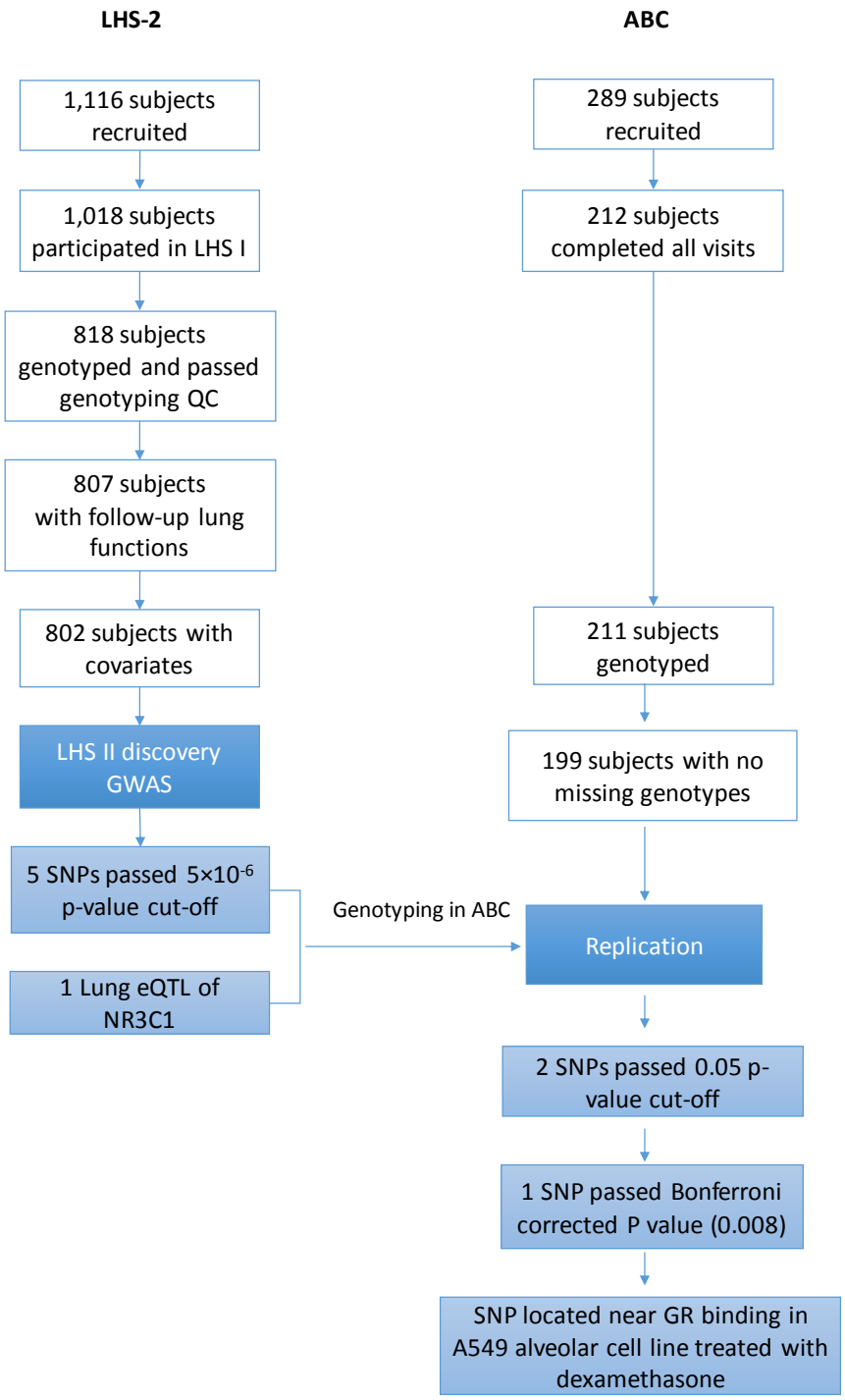
Contents

Additional methods on genotyping in the ABC cohort

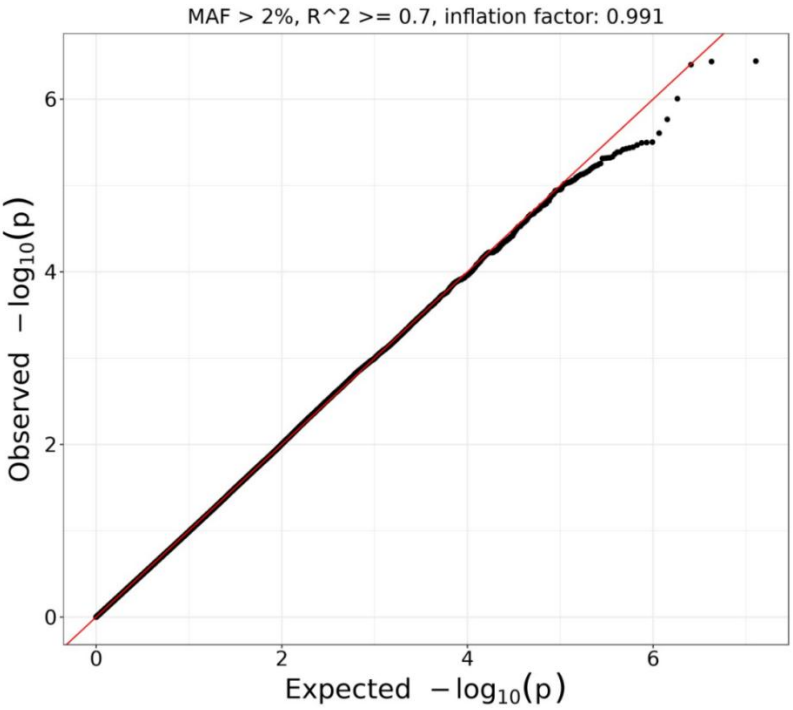
The DNA was extracted from buffy coat samples that were stored in -80 freezer storage. DNA extraction was performed with Qiagen Qiaamp DNA Mini Kit (250) (cataloge # 51306) and samples were eluted with DNase free distilled water. Each sample contained 600 ng of DNA in a total volume of 30 ul which were sent to Genome Quebec for genotyping.

At Genome Quebec, a multiplex PCR was performed on 20 ng of template genomic DNA in a 5uL reaction mixture containing: 0.1uL (0.5 U) HotStar Taq enzyme (QIAGEN), 0.625uL of 10X HotStar Buffer, 0.325uL of 25mM (total) MgCl2, 0.25uL of 10mM dNTP mix, 0.55uL of forward and reverse primer pool (1uM) and 1.15uL of water. The amplification cycling: 95c 15min, 45x (95c 20sec, 56c 30sec, 72c 60sec), 72c 3min, hold 4c. A few PCR reactions are ran on QIAxcel (QIAGEN) to assess the amplification (1uL of PCR in 9uL of DNA Dilution Buffer (QIAGEN)). This is followed by a shrimp-alkaline-phosphatase treatment to remove the unused nucleotides (0.2uL of SAP Buffer, 0.3uL of SAP and 1.5uL of water). SAP cycling: 37c 40min, 85c 10min, hold 4c. Next, a primer extension reaction (iPLEX Gold, Agena Bioscience) is performed with 0.94uL of extension primer mix, 0.2uL of iPlex Terminator, 0.2uL of iPlex Buffer, 0.041uL of iPlex Thermo Sequenase and 0.619uL of water. The products are desalted using 6mg of resin (Agena Bioscience) and spotted on a 384-point SpectroCHIP (Agena Bioscience) using a nanodispenser. The distinct masses were determined by MALDI-TOF mass-spectrometry and data were analyzed using MassARRAY Typer Analyser software.

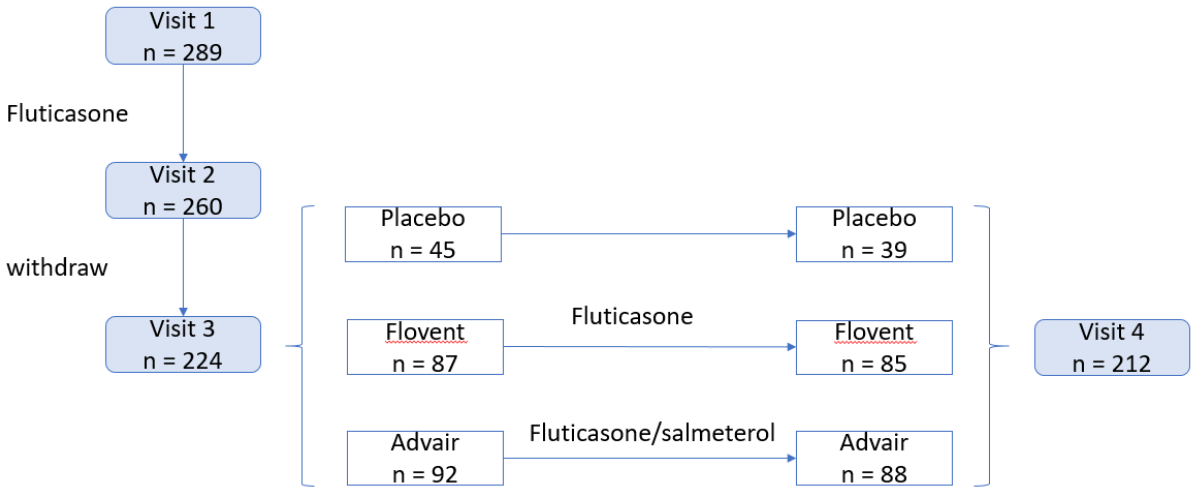
Supplementary Figures



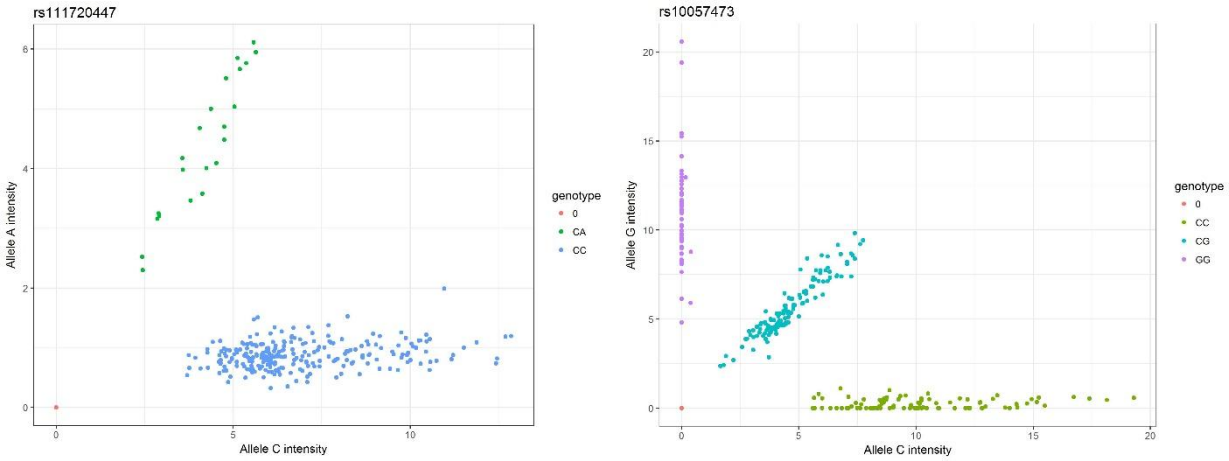
Supplementary Figure 1. A summary Flowchart of the study design with the number of individuals included and results.



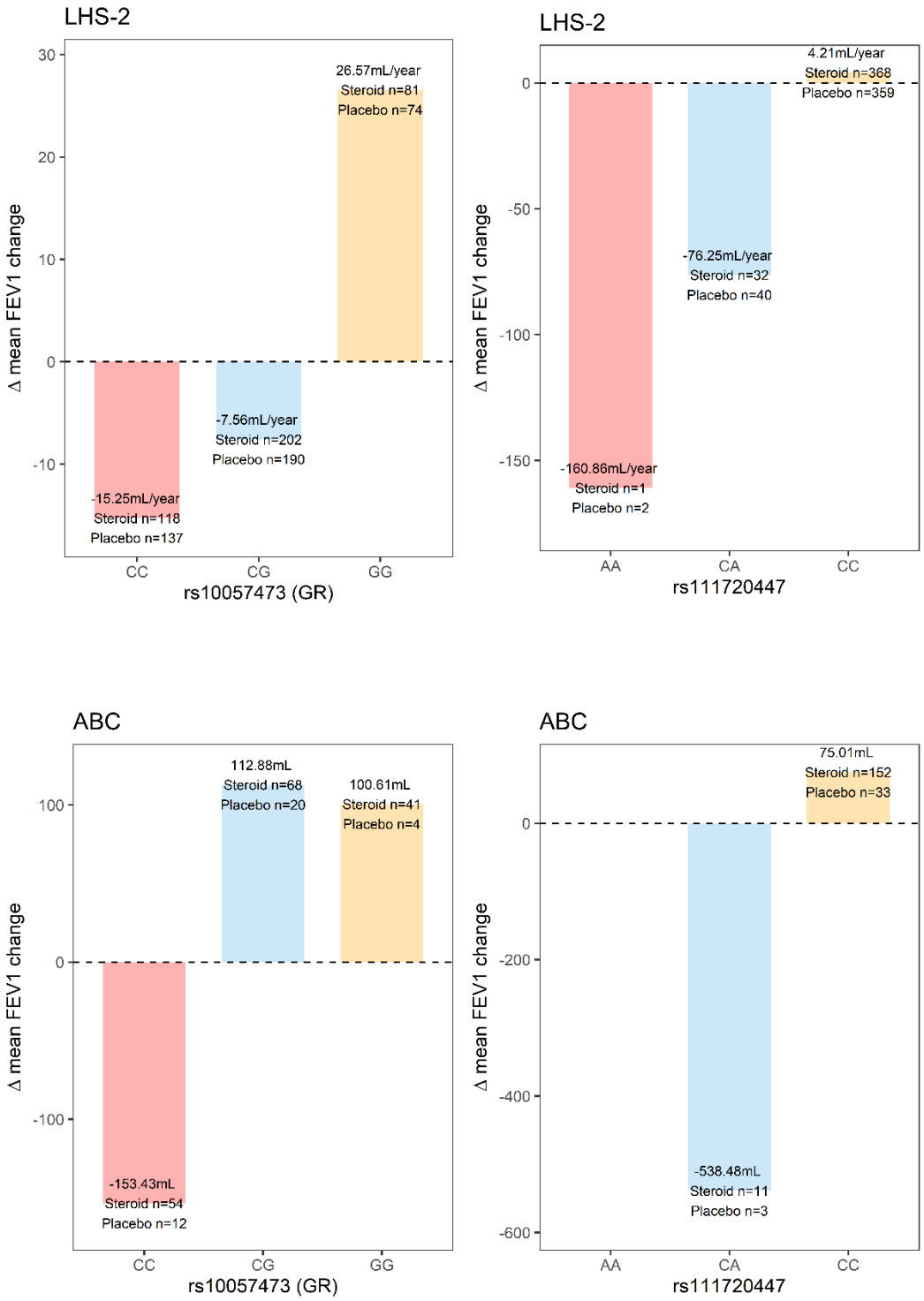
Supplementary Figure 2. Quantile–quantile (QQ) plot of ICS response GWAS in LHS-2. The plot shows the observed P values (-log10 scale) on the Y axes, and the expected P values (-log10 scale) on the X axes. The red line represents where the observed P values equal to the expected P values.



Supplementary Figure 3. ABC cohort study design.



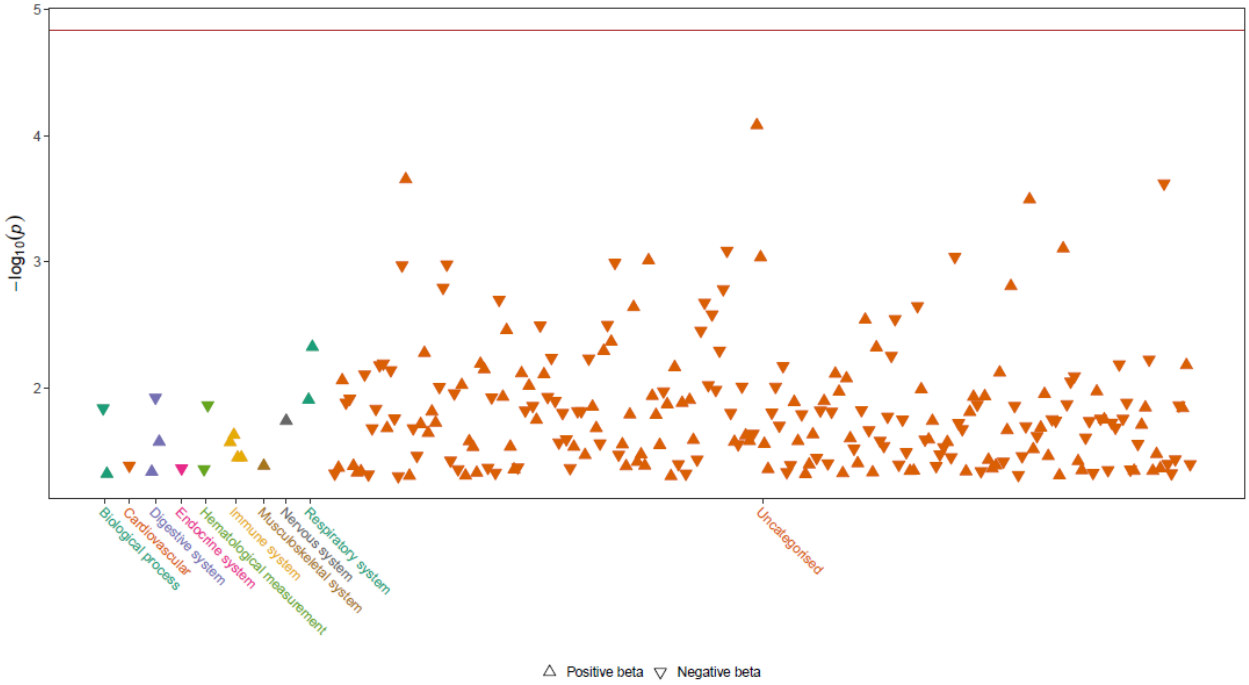
Supplementary Figure 4. Intensity clustering plots for the replicated SNPs



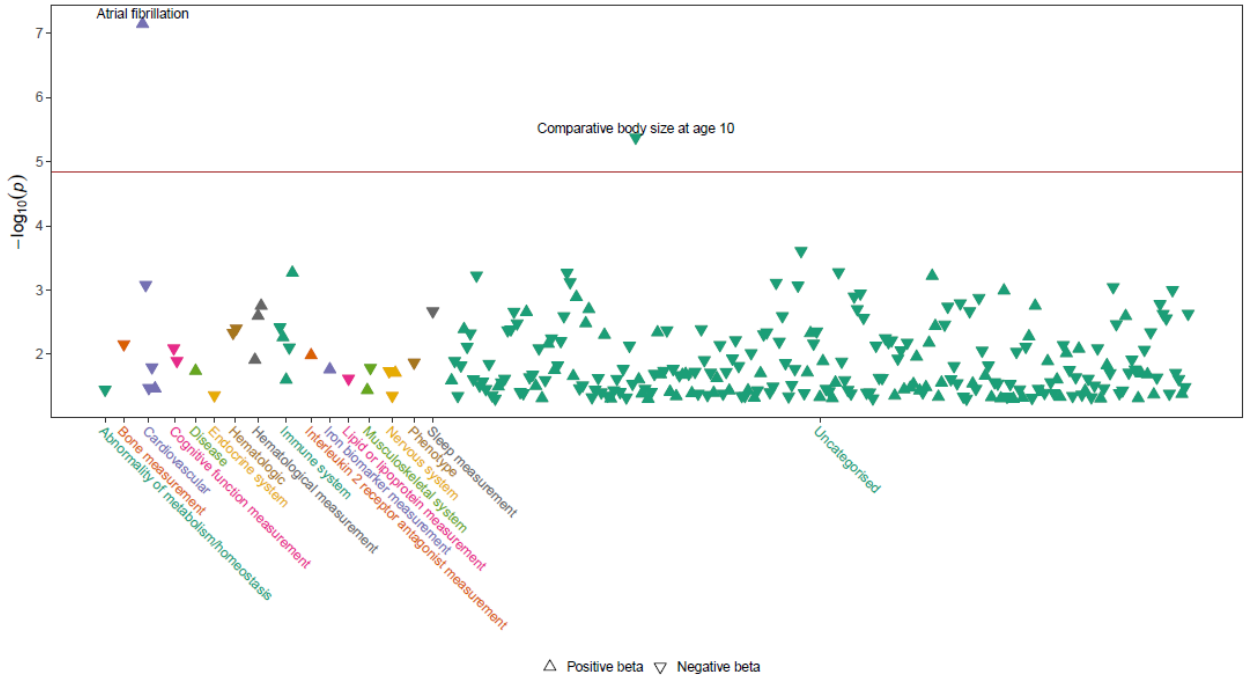
Supplementary Figure 5. Difference in FEV1 change between the ICS and Placebo group in LHS and ABC stratified by genotypes of rs10057473 and rs111720447. The Y axis represents the difference between ICS and placebo in mean FEV1 change rate. The X axis shows the genotypes for each SNP. For SNP rs10057473 (the eQTL SNP for GR gene), the genotype GG is associated with a relative increase of FEV1 change in ICS compared to placebo in both LHS-2 and ABC. The G allele was also associated with increased expression of the GR receptor in lung tissue.

For SNP rs111720447 the CC genotype is associated with slightly improved FEV1 in both LHS-2 and ABC cohorts, while the CA and AA in LHS-2 and CA genotypes in ABC were associated with accelerated loss of FEV1 between ICS and placebo. A positive value indicates that ICS therapy improved lung function compared to placebo; whereas a negative value indicates an opposite effect.

A) PheWAS plot for SNP rs111720447



B) PheWAS plot for SNP rs10057473



Supplementary Figure 6. PheWAS plots for SNPs rs111720447 (A) and rs10057473 (B).

The X axis shows the different phenotypes tested in the UK Biobank. The Y axis shows the –log10 P value for association with phenotype. The red horizontal line represent the Bonferroni corrected threshold for the PheWAS adjusted for the number of phenotypes tested. The estimates are based on the A allele for rs111720447 and on the C allele for SNP rs10057473.

Supplementary Tables

			6 month			12 month			24 month			36 month		
SNP ID	CHR	alleles	SNP effect	Treatment effect	Interaction effect	SNP effect	Treatment effect	Interaction effect	SNP effect	Treatment effect	Interaction effect	SNP effect	Treatment effect	Interaction effect
rs10057473	5	G/C	5.90 (0.79)	14.21 (0.69)	28.27 (0.38)	6.65 (0.58)	-9.14 (0.63)	13.76 (0.42)	-11.86 (0.11)	-25.88 (0.03)	25.29 (0.018)	-12.73 (0.028)	-19.11 (0.039)	18.58 (0.024)
rs111720447	7	C/A	-21.09 (0.67)	-92.64 (0.52)	69.87 (0.34)	0.56 (0.98)	-97.54 (0.2)	52.91 (0.18)	-29.26 (0.075)	-176.06 (0.00023)	90.26 (0.00027)	-29.48 (0.018)	-168.28 (4.1e-06)	86.61 (4.8e-06)
rs10108679	8	G/C	-15.74 (0.53)	44.24 (0.24)	-2.78 (0.94)	-9.23 (0.48)	-29.64 (0.13)	38.52 (0.035)	-19.61 (0.017)	-40.58 (0.00093)	43.12 (0.00016)	-17.63 (0.0053)	-38.13 (5.3e-05)	41.07 (3.1e-06)
rs1361249	10	C/T	5.06 (0.83)	135.00 (0.011)	-67.21 (0.053)	24.44 (0.054)	88.39 (0.0018)	-60.71 (0.00097)	28.85 (0.00027)	74.76 (2.2e-05)	-56.60 (9.2e-07)	24.53 (5.8e-05)	55.84 (4.1e-05)	-42.56 (1.7e-06)
rs117989968	11	T/C	135.08 (0.19)	465.39 (0.095)	-216.15 (0.13)	81.87 (0.14)	254.85 (0.092)	-127.69 (0.095)	134.92 (0.00011)	416.70 (1.2e-05)	-213.74 (9.1e-06)	126.14 (3e-06)	338.03 (4.1e-06)	-173.37 (3.2e-06)
rs12433619	14	A/G	-15.43 (0.5)	26.39 (0.49)	14.62 (0.65)	1.46 (0.9)	28.65 (0.16)	-25.86 (0.13)	21.15 (0.005)	37.41 (0.0034)	-42.24 (7.1e-05)	21.43 (0.00023)	34.63 (0.00043)	-38.69 (2.5e-06)

Supplementary Table 1. The effect of the pharmacogenomic loci from the discovery GWAS on FEV1 change in each follow-up visit. The effects are shown as estimates (P values).