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]

ABSTRACT   Inhaled corticosteroids (ICS) are widely prescribed for patients with chronic obstructive pulmonary disease (COPD), yet have variable outcomes and adverse reactions, which may be genetically determined. The primary aim of the study was to identify the genetic determinants for forced expiratory volume in 1 s (FEV_1) changes related to ICS therapy.

In the Lung Health Study (LHS)-2, 1116 COPD patients were randomised 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 for the genotype-by-ICS treatment effect on 3 years of FEV_1 changes (estimated as slope) in 802 genotyped LHS-2 participants. Replication was performed in 199 COPD patients randomised to the ICS, fluticasone or placebo.

A total of five loci showed genotype-by-ICS interaction at $p < 5 \times 10^{-6}$; of these, single nucleotide polymorphism (SNP) rs111720447 on chromosome 7 was replicated (discovery $p = 4.8 \times 10^{-6}$, replication $p = 5.9 \times 10^{-5}$) with the same direction of interaction effect. ENCODE (Encyclopedia of DNA Elements) 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 β 56.36 mL·year^{-1}, 95% CI 29.96–82.76 mL·year^{-1}) and in patients who were assigned to placebo, although the relationship was weaker and in the opposite direction to that in the ICS group (C allele β −27.57 mL·year^{-1}, 95% CI −53.27– −1.87 mL·year^{-1}).

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.

Introduction

Chronic obstructive pulmonary disease (COPD) affects 384 million people and is the third 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 two or more exacerbations per year or a hospitalisation 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 demineralisation [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 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 individualised 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 characterised by high intraindividual repeatability [13] and high interindividual 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 s (FEV_1) over time in patients with COPD. We first used data from Lung Health Study (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 LHS-2 have been published previously [7]. Briefly, LHS-2 was a multicentre randomised controlled trial (RCT) to determine the effects of inhaled corticosteroids (1200 µg of triamcinolone) on FEV_1 decline over 3 years [7]. LHS-2 recruited subjects who were smoking or had recently quit (<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 30–90% 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 1116 subjects were randomised to receive triamcinolone acetonide (1200 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 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 ethics certificate H16-01201.

LHS genotyping

Genotyping was performed using buffy coat samples of 4251 European Americans who participated in LHS-1. The details of genotyping and quality control have been described previously [17]. Briefly, samples were genotyped using the Illumina Human660WQuad v.1_A BeadChip (San Diego, CA, USA).

Affiliations: [1]The University of British Columbia Center for Heart Lung Innovation, St Paul's Hospital Vancouver, BC, Canada. [2]University of Groningen, University Medical Center Groningen, Dept of Pulmonology, GRIAC research institute, Groningen, The Netherlands. [3]Pulmonary and Critical Care Medicine, School of Medicine, Johns Hopkins University, Baltimore, MD, USA. [4]Institut Universitaire de Cardiologie et de Pneumologie de Québec, Laval University, Québec, QC, Canada. [5]Dept of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA. [6]Division of Biomedical Informatics and Personalized Medicine, Dept of Medicine, University of Colorado School of Medicine, Aurora, CO, USA. [7]Division of Genetic Epidemiology, School of Medicine, Johns Hopkins University, Baltimore, MD, USA. [8]Dept of Biostatistics, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA. [9]Dept of Epidemiology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA.

Correspondence: Ma'en Obeidat, UBC Centre for Heart Lung Innovation, St Paul's Hospital, 1081 Burrard Street, Vancouver, BC V6Z 1Y6, Canada. E-mail: maen.obeidat@hli.ubc.ca

Imputation was undertaken with the Michigan Imputation Server using the Haplotype Reference Consortium panel. Variants were excluded if the imputation quality (r^2) was <0.5 and if the minor allele frequency was <1%. For the 1018 (out of the 1116) 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 Institutes of Health database for genotype and phenotype (dbGaP) under study accession phs000335.v3.p2.

Genome-wide association testing

We performed a genome-wide association study (GWAS) for the FEV1 change rate (mL·year^{-1}) using an additive genetic model with a genotype-by-ICS (triamcinolone *versus* placebo) interaction term and adjustments for baseline FEV1 in mL, age, sex, body mass index, smoking status and the first five genetic principal components (PCs) according to the following formula:

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

Individual FEV1 change rate was estimated using a linear model of FEV1 (measurements at baseline, 6-month, 12-month, 24-month and 36-month visits) over time. Genome-wide significance threshold was set to the traditional $p < 5 \times 10^{-8}$. Given the relatively small sample size of the discovery cohort (LHS-2), we defined a secondary set of criteria to identify loci that could harbour true signals of steroid responsiveness for replication. These included genetic loci that had an association with FEV1 decline of $p < 5 \times 10^{-6}$ in LHS-2 and region plots demonstrating genetic support from surrounding single nucleotide polymorphisms (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 Global Initiative for Chronic Obstructive Lung Disease (GOLD) spirometric grades. In addition, we evaluated these loci in only those participants who were randomised to the ICS group, and separately in the placebo group.

Candidate gene approach: lung expression quantitative trait loci 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 described previously [18–20]. Briefly, meta-analysis of the association between SNPs and mRNA expression adjusted for age, sex and smoking status was performed in nontumour lung tissue samples from 1111 patients who underwent lung resection surgery. The discovered eQTLs were either *cis* (1 Mb from the transcription start site of gene) or *trans* (>1 Mb 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 a false discovery rate (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 ABC study. The details of the ABC cohort have been published previously [16]. The ABC study was a multicentre clinical trial that evaluated the effects of inhaled fluticasone, an ICS, alone or in combination with salmeterol, a long acting β2-agonist (LABA), in reducing systemic inflammation in patients with COPD. All recruited participants were aged ⩾40 years, and had ⩾10 pack-years of smoking history and an FEV1 <80% pred with an FEV1/FVC ratio of <0.70 after bronchodilation. Study participants underwent a run-in phase (visit 1: enrolment), during which they received fluticasone propionate (500 μg 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 μg twice daily) or inhaled fluticasone/salmeterol combination (500/50 μg twice daily) for 4 weeks (visit 4: RCT 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 supplementary material. 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 visits 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 six SNPs.

$$(E[FEV_1 \text{ change}] = \beta_0 + \beta_1 SNP \times \text{Treatment} + \beta_2 SNP + \beta_3 \text{Treatment} + \beta_4 \text{Visit 3 } FEV_1 + \beta_5 \text{Age}$$
$$+ \beta_6 \text{Sex} + \beta_7 \text{BMI} + \beta_8 \text{Smoking})$$

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

Phenome-wide association study

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 assess phenome-wide association (PheWAS) for the replicated SNPs [21], which was complemented by a look-up using the GWAS catalogue [22].

Encyclopedia of DNA Elements (ENCODE) data

To determine whether the GR binding site was in close proximity to the identified SNPs, we utilised the publicly available Encyclopedia of DNA Elements (ENCODE) chromatin immunoprecipitation (ChIP) assays with sequencing (ChIP-Seq) dataset [23]. Specifically, we investigated the GR binding scores with or without dexamethasone in varying concentrations (0–100 nM) 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 S1.

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 have been published previously 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 >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 plot is presented in supplementary figure S2. The genomic inflation factor

TABLE 1 Characteristics of the discovery and replication cohorts

	LHS-2 (discovery)		ABC (replication)	
	Placebo	**ICS**	**Placebo**	**Steroid**
Subjects	401	401	36	163
Age years	55.42±6.65	55.53±6.60	66.39±10.52	69.61±9.23
BMI kg·m^{-2}	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$_1$ % pred	68.94±11.97	70.12±12.45	49.67±15.07	47.90±16.11
FEV$_1$ change[#]	−49.15±85.79	−52.56±77.54	55.00±317.66	78.90±240.34
Male	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)

Data are presented as n, mean±SD or n (%). LHS: Lung Health Study; ABC: Advair, Biomarkers in COPD; BMI: body mass index; FEV$_1$: forced expiratory volume in 1 s. [#]: FEV$_1$ slope in LHS-2 was estimated using FEV$_1$ change rate over 3 years per subject; slope in mL·year^{-1} for LHS-2, absolute change in mL for ABC.
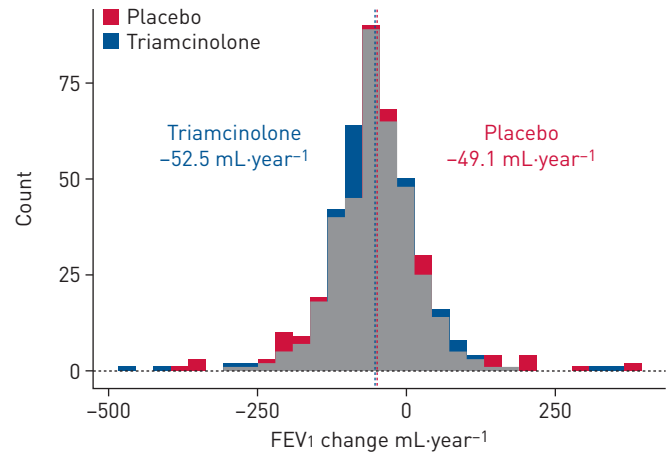
FIGURE 1 Forced expiratory volume in 1 s (FEV1) change in the Lung Health Study-2 in the triamcinolone and the placebo groups.

was 0.991, suggesting no systematic deviation in the association statistics. No loci met genome-wide significance ($p<5\times10^{-8}$); however, we identified five 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 $p=4.16\times10^{-7}$ 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 interaction 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 FEV1 decline (p=0.02).

In addition to the interaction results, we report in table 2 the SNPs and the treatment main effects. Given that the overall slope of FEV1 change was negative, for the SNPs' main effect, a positive estimate indicates a slower decline and a negative estimate indicates a faster (accelerated) decline.

The association results for the six SNPs with FEV1 change rate at each follow-up visit (as opposed to the overall slope used in the GWAS) are shown in supplementary table S1.
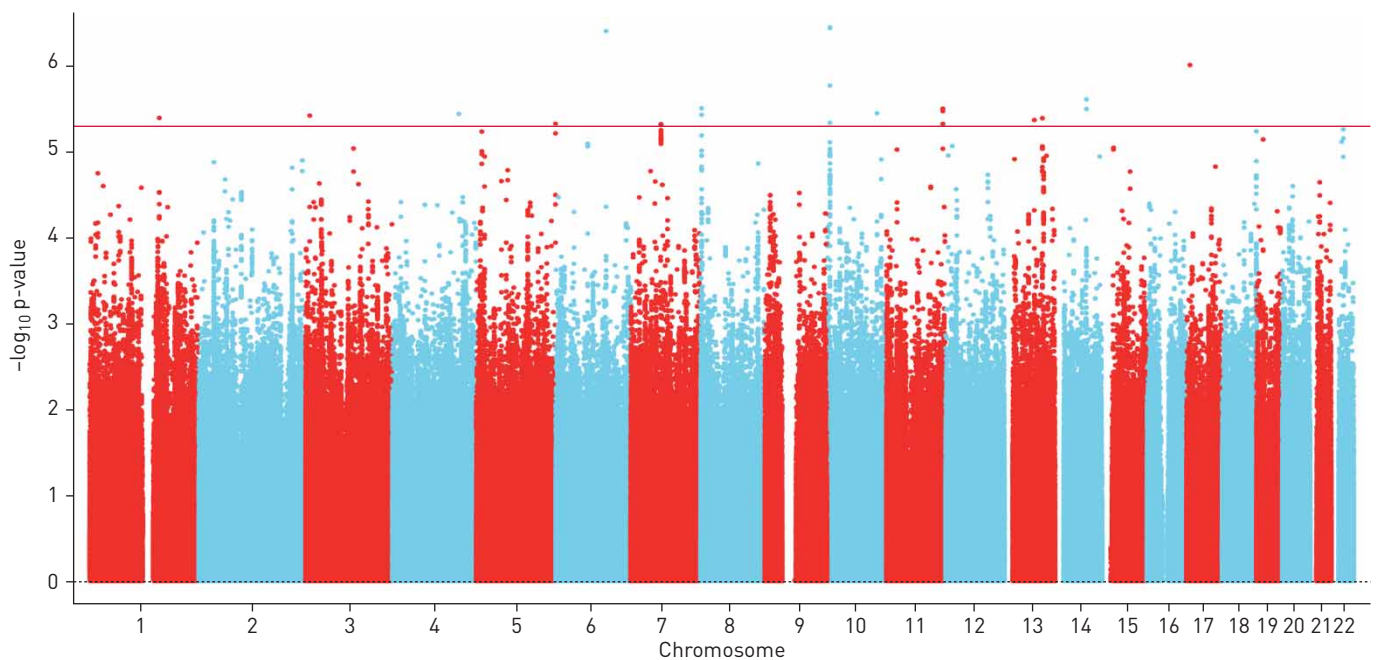

FIGURE 2 Manhattan plot of genome-wide association study for genotype-by-inhaled corticosteroid interaction in the Lung Health Study-2. The plot shows the p-values ($-\log_{10}$ scale) on the y-axis and the single nucleotide polymorphism positions across 22 autosomal chromosomes on the x-axis. The horizontal red line represents the p-value cut-off of 5×10^{-6}.

TABLE 2 Genetic loci associated with forced expiratory volume in 1 s (FEV1) decline in the Lung Health Study-2

SNP	Chromosome	Position	Alleles (coded/ noncoded)	Coded allele frequency %	SNP effect[#]		Treatment effect[¶]		SNP × treatment[+]	
					β mL·year^{-1}	p-value	β mL·year^{-1}	p-value	β mL·year^{-1}	p-value
rs10057473	5	142887378	G/C	44.3	−12.7	2.8×10^{-2}	−19.1	3.9×10^{-2}	18.6	2.4×10^{-2}
rs111720447	7	68703305	C/A	95.2	−29.5	1.8×10^{-2}	−168.3	4.1×10^{-6}	86.6	4.8×10^{-6}
rs10108679	8	2179372	G/C	43.9	−17.6	5.3×10^{-3}	−38.1	5.3×10^{-5}	41.1	3.1×10^{-6}
rs1361249	10	7007283	C/T	70.1	24.5	5.8×10^{-5}	55.8	4.1×10^{-5}	−42.6	1.7×10^{-6}
rs117989968	11	128639945	T/C	97.8	126.1	3.0×10^{-6}	338.0	4.1×10^{-6}	−173.4	3.2×10^{-6}
rs12433619	14	71679203	A/G	48.2	21.4	2.3×10^{-4}	34.6	4.3×10^{-4}	−38.7	2.5×10^{-6}

Given that the overall slope of FEV1 change was negative, for the single nucleotide polymorphism's (SNP) main effect, a positive β estimate indicates less (or a slower) decline and a negative β estimate indicates more (or an accelerated) decline. [#]: SNP main effect; [¶]: treatment main effect (triamcinolone versus placebo); [+]: SNP–treatment interaction effect.

Sensitivity and stratified analyses

Additional testing of the six SNPs was performed in stratified analyses that included ICS users only, and placebo-only groups, and in strata of current smokers versus 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 chromosome 7 was strongly associated with FEV1 decline in ICS users (C allele β 56.36 mL·year^{-1}, 95% CI 29.96–82.76; p=3.35×10^{-5}) and to a lesser extent in the placebo group, but in an opposite direction (C allele β −27.57 mL·year^{-1}, 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 FEV1 changes in current smokers (β 72.74 mL·year^{-1}, 95% CI 32.47–113.00; p=0.0004) versus former smokers (β 145.04 mL·year^{-1}, 95% CI 20.61–269.46; p=0.023) and in COPD GOLD II patients (β 85.97 mL·year^{-1}, 95% CI 38.32–133.63; p=0.0004), than in GOLD III and IV patients (β 116.33 mL·year^{-1}, 95% CI 8.24–224.42; p=0.036). The SNP did not show an interaction effect on FEV1 decline in GOLD I patients (p=0.38).

Replication in the ABC cohort

The five 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 S3. 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 six 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 FEV1 changes (in mL) over 4 weeks using data from visits 3 and 4 (*i.e.* immediately before and after the 4-week RCT phase). Using this FEV1 change as the response variable, a similar linear model to the discovery GWAS was then fitted for the six SNPs.

In the replication cohort, two SNPs showed significant (p<0.05) association between FEV1 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=5.98×10^{-5}. 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 six SNPs (p=0.008), only SNP rs111720447 on chromosome 7 showed statistically significant replication. The results for all six 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 S4) for the replicated SNPs showed clear separation of genotype clusters, indicating high-quality genotyping.

Differences in FEV1 changes related to ICS therapy stratified by genotypes of rs111720447 and rs10057473 in LHS-2 and ABC trials

Supplementary figure S5 illustrates the changes in FEV1 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 chromosome 7 demonstrated on average lower FEV1 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 FEV1 in those who were assigned to ICS therapy compared with placebo.

TABLE 3 Stratified analyses results for the six single nucleotide polymorphisms (SNPs) from the inhaled corticosteroid (ICS) response discovery genome-wide association study

| SNP | Chromosome | Position | Alleles | Treatment group[#] | | | | | | Smoking status strata[¶] | | | | | | COPD GOLD stage strata[+] | | | | | | | | |
| | | | | ICS | | | Placebo | | | Current smokers | | | Former smokers | | | COPD GOLD I | | | COPD GOLD II | | | COPD GOLD III and IV | | |
				Estimate	95% CI	p-value	Estimate	95% CI	p-value	Estimate	95% CI	p-value	Estimate	95% CI	p-value	Estimate	95% CI	p-value	Estimate	95% CI	p-value	Estimate	95% CI	p-value
Subjects n					401			401			712			90			162			576			63	
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
rs111720447	7	68703305	C/A	56.36	(29.96–82.76)	3.35×10^{-5}	−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)	2.78×10^{-5}	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)	6.97×10^{-6}	−32.89	(−91.52–25.74)	0.2675	−23.08	(−60.60–14.44)	0.2260	−52.84	(−73.89–−31.79)	1.08×10^{-6}	−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)	1.24×10^{-5}	−178.59	(−254.47–−102.70)	4.56×10^{-6}	−83.06	(−759.90–593.78)	0.8076	−214.72	(−864.21–434.77)	0.5146	−171.93	(−246.83–−97.04)	7.93×10^{-6}	−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)	1.19×10^{-5}	−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

The table shows the results for the six SNPs that were selected for replication in the Advair, Biomarkers in COPD (chronic obstructive pulmonary disease) cohort. The table shows the association results in treatment strata (ICS-only and placebo-only groups), as well as current *versus* former smokers and in the different Global Initiative for Chronic Obstructive Lung Disease (GOLD) stages. The estimates shown are in mL·year^{-1} for the SNP effect for ICS and placebo groups, for SNP-by-ICS interaction, and for smoking status and COPD sensitivity analyses. FEV_1: forced expiratory volume in 1 s; BMI: body mass index; PC: principal component. [#]: FEV_1 slope~SNP+baseline FEV_1+age+sex+BMI+smoking status+PC1+PC2+PC3+PC4+PC5; [¶]: FEV_1 slope~SNP*treatment group+SNP+treatment group+baseline FEV_1+age+sex+BMI+PC1+PC2+PC3+PC4+PC5; [+]: FEV_1 slope~SNP* treatment group+SNP+treatment group + baseline FEV_1+age+sex+BMI+smoking status +PC1+PC2+PC3+PC4+PC5.

TABLE 4 Replication results for inhaled corticosteroid pharmacogenomics loci in the Advair, Biomarkers in COPD cohort

SNP	Chromosome	Position	Alleles (coded/noncoded)	Coded allele frequency	SNP effect[#]		Treatment effect[¶]		SNP × treatment[+]	
					β mL	p-value	β mL	p-value	β mL	p-value
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**	**1.3×10⁻⁵**	**−1278.0**	**8.2×10⁻⁵**	**670.8**	**6.0×10⁻⁵**
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

Single nucleotide polymorphisms (SNPs) meeting Bonferroni corrected p-values are shown in bold. COPD: chronic obstructive pulmonary disease. [#]: SNP main effect; [¶]: treatment main effect (triamcinolone *versus* placebo); [+]: SNP–treatment interaction effect.

ENCODE results

We evaluated the ChIP-Seq data from ENCODE for possible linkage disequilibrium blocks surrounding SNP rs111720447 (using $r^2>0.5$) and identified two GR binding sites (figure 4) in A549 alveolar cell lines treated with 100 nM dexamethasone for 1 h. 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 S6. 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 catalogue, there were no associations that were reported for rs111720447. For the GR eQTL SNP
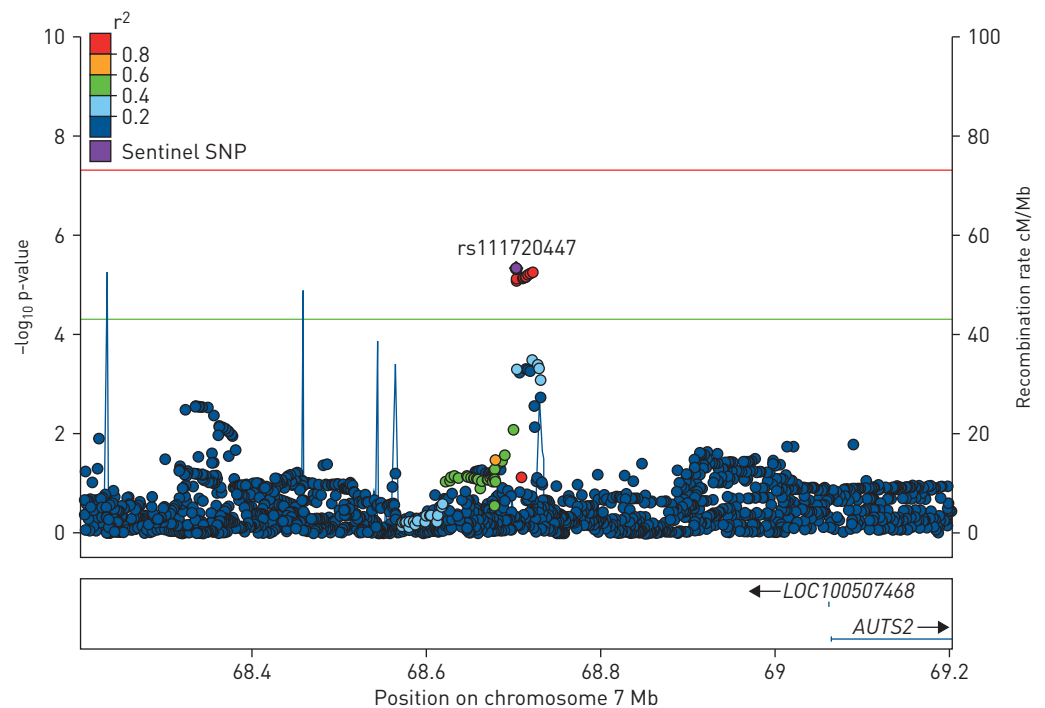

FIGURE 3 Region plot of the inhaled corticosteroid pharmacogenomics loci associated with forced expiratory volume in 1 s decline in the Lung Health Study (LHS)-2 and replicated in Advair, Biomarkers in COPD (ABC) cohort. The y-axis represents the p-values (−log₁₀ scale) and the x-axis is the genomic position. Gene names and their corresponding coordinates are shown below the plot. The colour coding of single nucleotide polymorphisms (SNPs) reflects the degree of linkage disequilibrium with the sentinel SNP using 1000G reference. The horizontal red line represents the p-value cut-off of 5×10^{-8}. The horizontal green line represents the p-value cut-off of 5×10^{-5}.
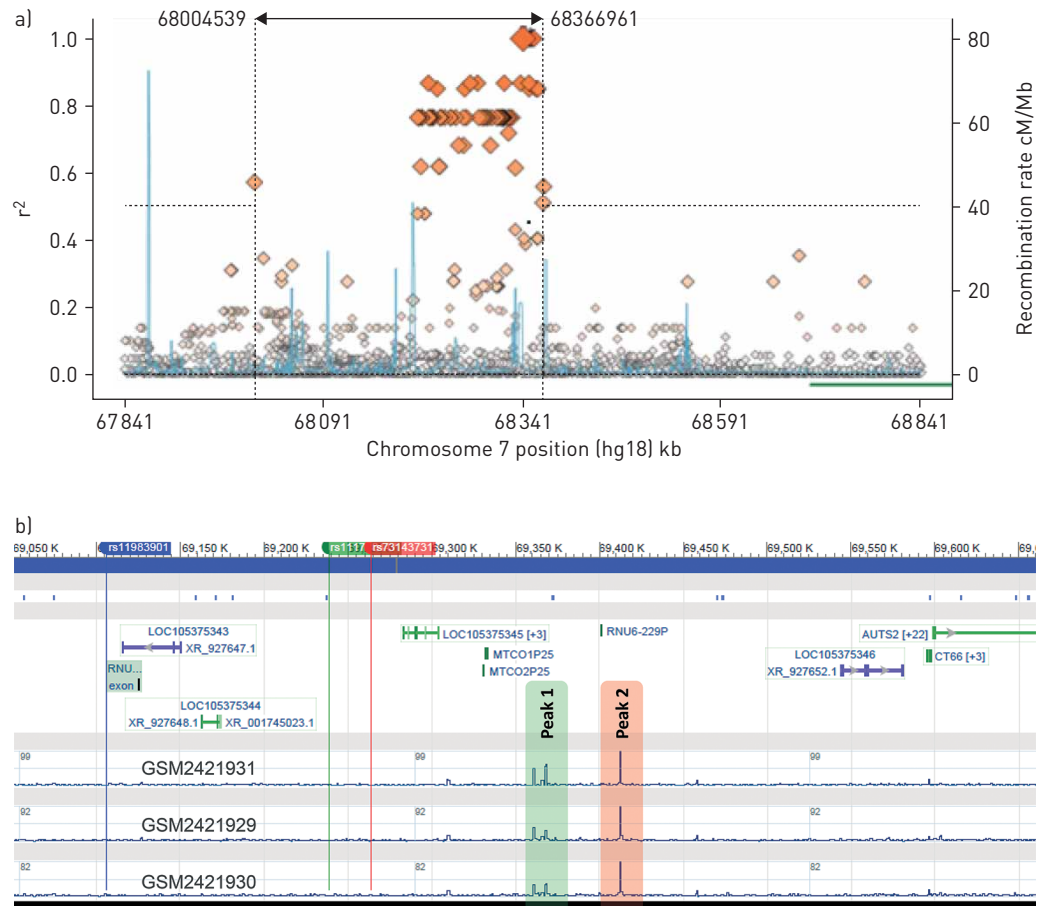
FIGURE 4 Glucocorticoid receptor (GR) binding in proximity of rs111720447. a) Linkage disequilibrium block surrounding rs111720447 (r^2>0.5) based on the 1000 genomes; b) GR chromatin immunoprecipitation assays with sequencing on the lung cell line (A549) treated with 100 nM dexamethasone for 1 h.

rs10057473, the PheWAS analysis showed significant associations for the C allele with increased risk for atrial fibrillation as well as 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 reality, 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 patient response to ICS is crucial to their proper use in clinical practice [24]. Most importantly, these factors may also provide crucial insights on the biology of steroid responses (and nonresponses) 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 FEV1 decline in 802 subjects from the LHS-2. No SNPs met genome-wide significance ($p<5\times10^{-8}$). However, by using a secondary defined criteria for discovery of $p<5\times10^{-6}$, we identified an intergenic SNP on chromosome 7 (rs111720447, $p=4.81\times10^{-6}$) that was replicated in the same direction ($p=5.98\times10^{-5}$) in an independent cohort of 199 COPD subjects, who were treated with either fluticasone alone or in combination with salmeterol *versus* placebo. Using a candidate-gene approach, we found that a regulatory variant (rs10057473) associated with lung tissue expression levels of the GR-encoding gene *NR3C1* also modified the rate of FEV1 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 chromosome 7 was strongly associated with FEV$_1$ decline in the ICS group (C allele β 56.36 mL·year^{-1}, 95% CI 29.96–82.76; p=3.35×10^{-5}) and to a lesser extent in the placebo group (C allele β −27.57 mL·year^{-1}, 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 (β 72.74 mL·year^{-1}, 95% CI 32.47–113.00; p=0.0004) *versus* former smokers (β 145.04 mL·year^{-1}, 95% CI 20.61–269.46; p=0.023) and in COPD GOLD II patients (β 85.97 mL·year^{-1}, 95% CI 38.32–133.63; p=0.0004), than in GOLD III and IV patients (β 116.33 mL·year^{-1}, 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 [25]. The authors found that there were multiple ICS response-associated SNPs that mapped to several genes and intergenic regions [25]. 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 [26], another study of 204 Chinese COPD patients demonstrated a significant association with ICS response [27]. The reasons behind these conflicting data for rs37973 in COPD are not clear, but 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 leukaemia [28].

Without any additional information, it is challenging to distinguish noise from true biological signals in GWAS that do not meet the genome-wide cut-off. 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 GR, which is encoded by the nuclear receptor 3C1 (*NR3C1*) gene [29]. 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-ICS 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 noncompliance, 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 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 FEV1), while LHS-2 had ⩾3 years of follow-up (and largely evaluated the effects of ICS addition to standard therapy on FEV1). In analysis stratified by follow-up year in LHS-2 (supplementary table S1), 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 FEV1 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, *et al.* Global and regional estimates of COPD prevalence: systematic review and meta-analysis. *J Glob Health* 2015; 5: 020415.
2   Price D, West D, Brusselle G, *et al.* 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, *et al.* Barriers to new drug development in respiratory disease. *Eur Respir J* 2015; 45: 1197–1207.
4   Vogelmeier CF, Criner GJ, Martinez FJ, *et al.* Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Lung Disease 2017 Report: GOLD Executive Summary. *Eur Respir J* 2017; 49: 1700214.
5   Suissa S. Number needed to treat in COPD: exacerbations *versus* pneumonias. *Thorax* 2013; 68: 540–543.
6   Scanlon PD, Connett JE, Wise RA, *et al.* Loss of bone density with inhaled triamcinolone in Lung Health Study II. *Am J Respir Crit Care Med* 2004; 170: 1302–1309.
7   Lung Health Study Research Group. Effect of inhaled triamcinolone on the decline in pulmonary function in chronic obstructive pulmonary disease. *N Engl J Med* 2000; 343: 1902–1909.
8   Schork NJ. Personalized medicine: time for one-person trials. *Nature* 2015; 520: 609–611.
9   Calverley PM, Anderson JA, Celli B, *et al.* Salmeterol and fluticasone propionate and survival in chronic obstructive pulmonary disease. *N Engl J Med* 2007; 356: 775–789.
10   Lapperre TS, Snoeck-Stroband JB, Gosman MM, *et al.* Effect of fluticasone with and without salmeterol on pulmonary outcomes in chronic obstructive pulmonary disease: a randomized trial. *Ann Intern Med* 2009; 151: 517–527.
11   Armaly MF. The heritable nature of dexamethasone-induced ocular hypertension. *Arch Ophthalmol* 1966; 75: 32–35.
12   Schwartz JT, Reuling FH, Feinleib M, *et al.* Twin heritability study of the effect of corticosteroids on intraocular pressure. *J Med Genet* 1972; 9: 137–143.
13   Drazen JM, Silverman EK, Lee TH. Heterogeneity of therapeutic responses in asthma. *Br Med Bull* 2000; 56: 1054–1070.

14 Szefler SJ, Martin RJ, King TS, *et al.* Significant variability in response to inhaled corticosteroids for persistent asthma. *J Allergy Clin Immunol* 2002; 109: 410–418.

15 Tantisira KG, Lasky-Su J, Harada M, *et al.* Genomewide association between GLCCI1 and response to glucocorticoid therapy in asthma. *N Engl J Med* 2011; 365: 1173–1183.

16 Sin DD, Man SF, Marciniuk DD, *et al.* The effects of fluticasone with or without salmeterol on systemic biomarkers of inflammation in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2008; 177: 1207–1214.

17 Hansel NN, Ruczinski I, Rafaels N, *et al.* Genome-wide study identifies two loci associated with lung function decline in mild to moderate COPD. *Hum Genet* 2013; 132: 79–90.

18 Obeidat M, Nie Y, Fishbane N, *et al.* Integrative genomics of emphysema associated genes reveals potential disease biomarkers. *Am J Respir Cell Mol Biol* 2017; 57: 411–418.

19 Obeidat M, Hao K, Bossé Y, *et al.* Molecular mechanisms underlying variations in lung function: a systems genetics analysis. *Lancet Respir Med* 2015; 3: 782–795.

20 Hao K, Bossé Y, Nickle DC, *et al.* Lung eQTLs to help reveal the molecular underpinnings of asthma. *PLoS Genet* 2012; 8: e1003029.

21 Carvalho-Silva D, Pierleoni A, Pignatelli M, *et al.* Open Targets Platform: new developments and updates two years on. *Nucleic Acids Res* 2019; 47: D1056–D1065.

22 Buniello A, MacArthur JAL, Cerezo M, *et al.* The NHGRI-EBI GWAS Catalog of published genome-wide association studies, targeted arrays and summary statistics 2019. *Nucleic Acids Res* 2019; 47: D1005–D1012.

23 ENCODE Project Consortium. An integrated encyclopedia of DNA elements in the human genome. *Nature* 2012; 489: 57–74.

24 Wu AC, Gay C, Rett MD, *et al.* Pharmacogenomic test that predicts response to inhaled corticosteroids in adults with asthma likely to be cost-saving. *Pharmacogenomics* 2015; 16: 591–600.

25 Wang Y, Tong C, Wang Z, *et al.* Pharmacodynamic genome-wide association study identifies new responsive loci for glucocorticoid intervention in asthma. *Pharmacogenomics J* 2015; 15: 422–429.

26 Mosteller M, van den Berge M, Hosking L, *et al.* Genetic evaluation of the effect of *GLCCI1* rs37973 on corticosteroid response in chronic obstructive pulmonary disease. *COPD Res Pract* 2017; 3: 2.

27 Lei Y, Gao Y, Chen J, *et al.* GLCCI1 rs37973: a potential genetic predictor of therapeutic response to inhaled corticosteroids in Chinese chronic obstructive pulmonary disease patients. *Sci Rep* 2017; 7: 42552.

28 Oksenberg N, Ahituv N. The role of AUTS2 in neurodevelopment and human evolution. *Trends Genet* 2013; 29: 600–608.

29 Kino T. Glucocorticoid receptor. *In:* LJ De Groot, G Chrousos, K Dungan, *et al.*, eds. Endotext. 2000. www.endotext.org