

The Pharmacogenomics of Inhaled Corticosteroids and Lung Function Decline in COPD

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Supplementary materials

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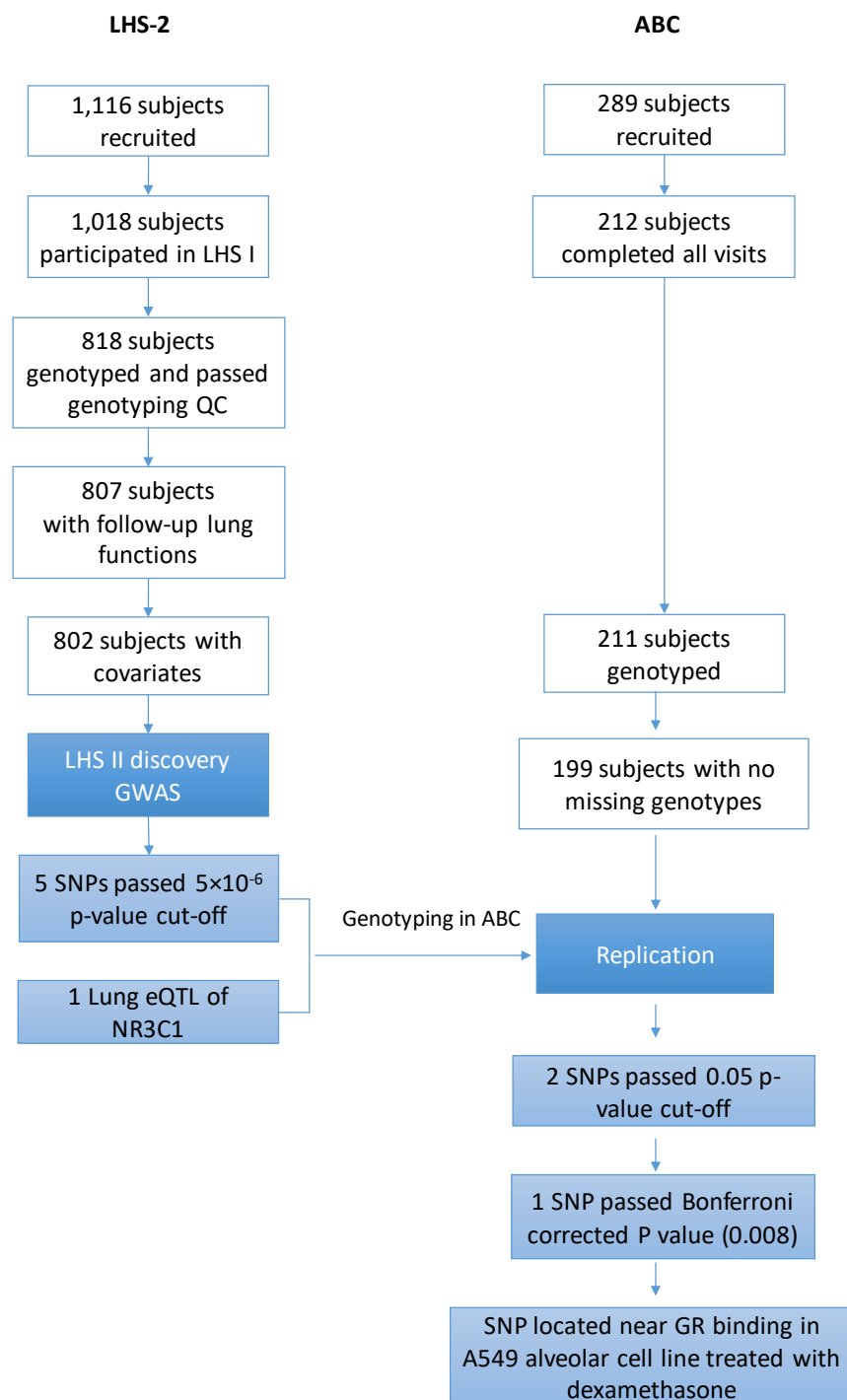
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Additional methods on genotyping in the ABC cohort

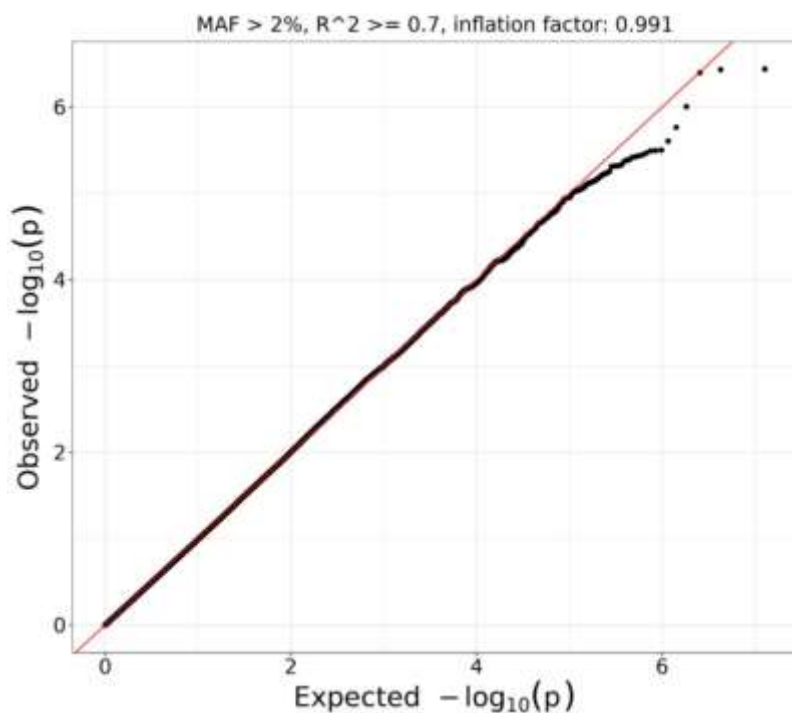
The DNA was extracted from buffy coat samples that were stored in -80 freezer storage. DNA extraction was performed with Qiagen Qiaamp DNA Mini Kit (250) (catalogue # 51306) and samples were eluted with DNase free distilled water. Each sample contained 600 ng of DNA in a total volume of 30 ul which were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

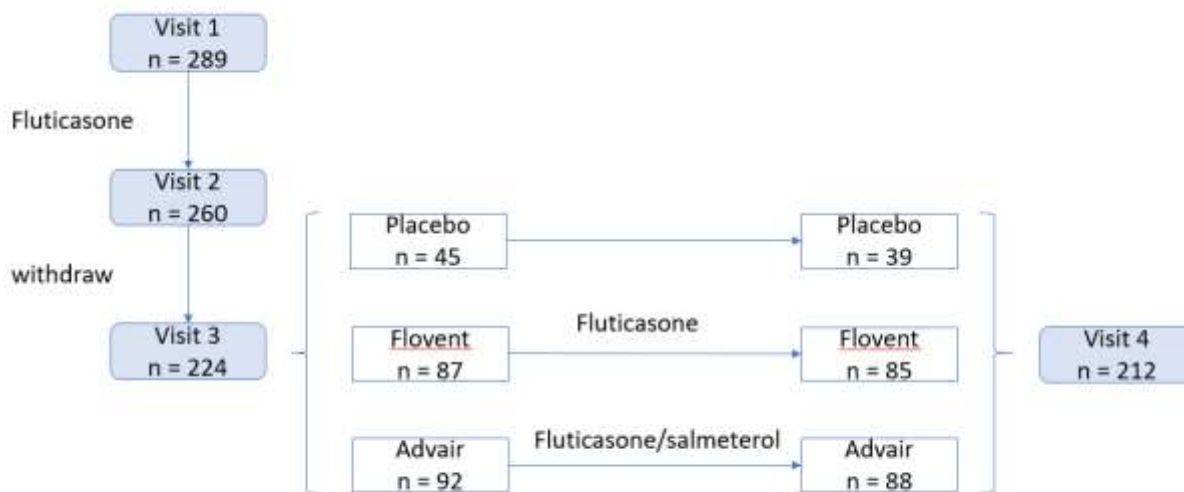
Supplementary Figures



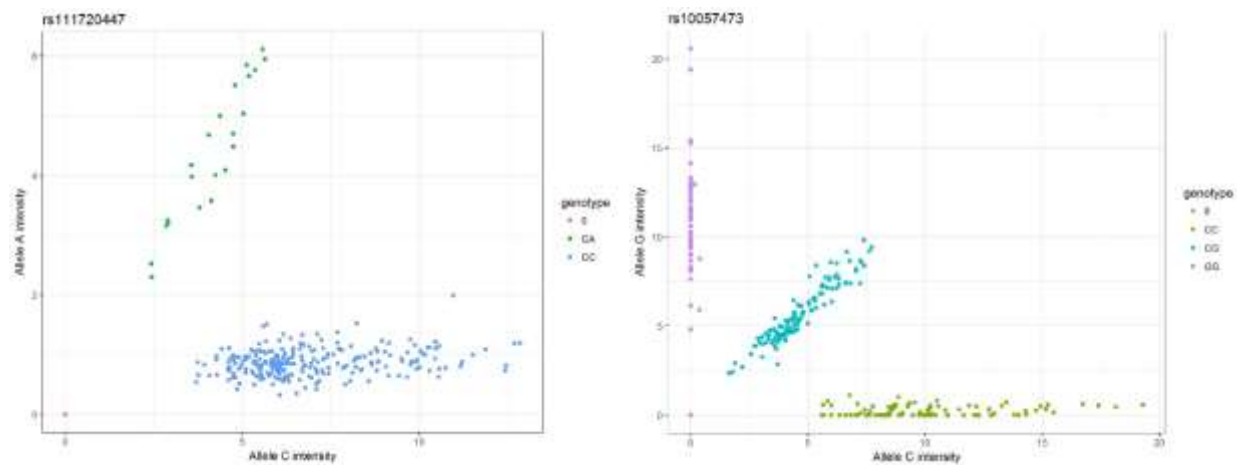
Supplementary Figure S1. A summary Flowchart of the study design with the number of individuals included and results.



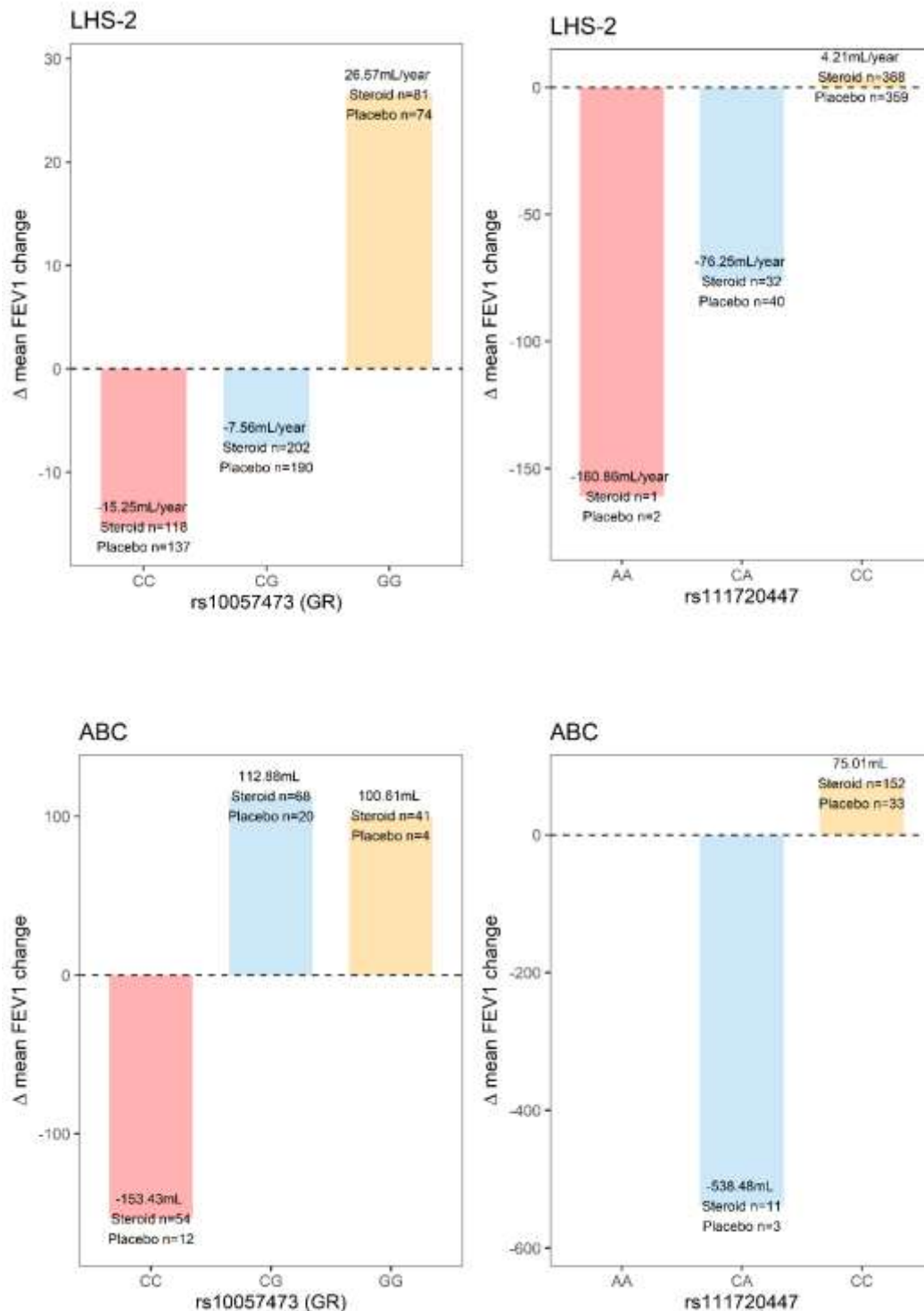
Supplementary Figure S2. Quantile-quantile (QQ) plot of ICS response GWAS in LHS-2. The plot shows the observed P values ($-\log_{10}$ scale) on the Y axes, and the expected P values ($-\log_{10}$ scale) on the X axes. The red line represents where the observed P values equal to the expected P values.



Supplementary Figure S3. ABC cohort study design.



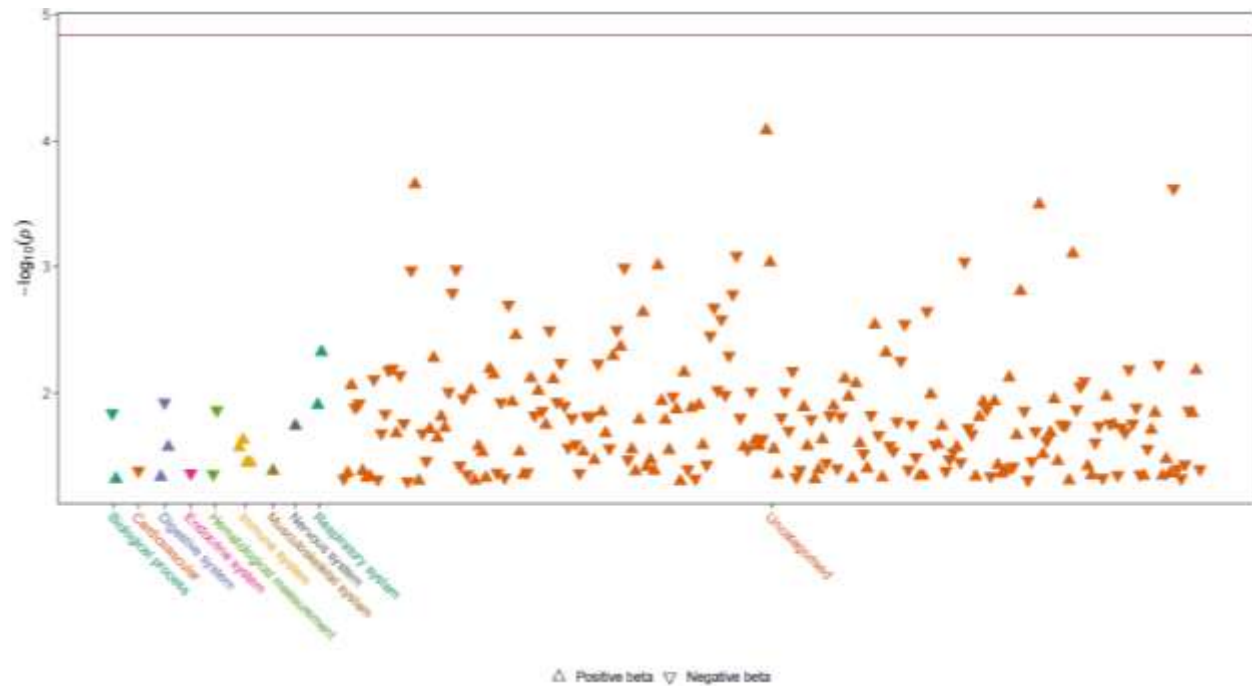
Supplementary Figure S4. Intensity clustering plots for the replicated SNPs



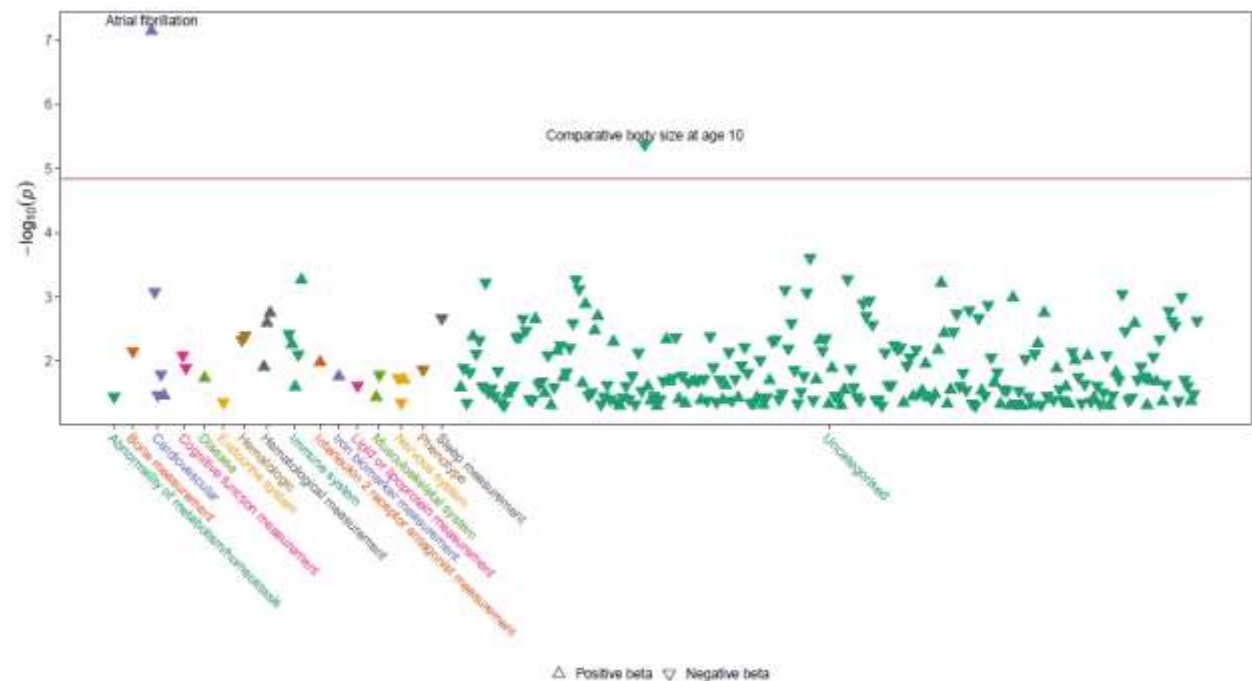
Supplementary Figure S5. Difference in FEV1 change between the ICS and Placebo group in LHS and ABC stratified by genotypes of rs10057473 and rs111720447. The Y axis represents the difference between ICS and placebo in mean FEV1 change rate. The X axis shows the genotypes for each SNP. For SNP rs10057473 (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 S6. 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 $-\log_{10}$ 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 S1. 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).