Study of Asthma Phenotypes and Pharmacogenomic Interactions by Race-Ethnicity (SAPPHIRE)

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PROTOCOL

Study population

All SAPPHIRE individuals were recruited from Henry Ford Health System, whose Institutional Review Board approved this study. Informed written consent was obtained prior to the collection of any data. If the participant was a minor (age <18 years), informed written consent was obtained from a legal guardian and written assent was obtained from the participant. Individuals were eligible to participate as asthma cases if they were 12-56 years of age, had a documented physician diagnosis of asthma, and no prior diagnosis of congestive heart failure or chronic obstructive pulmonary disease.

At the time of enrollment, participants underwent a research evaluation. Lung function testing was performed using a pneumotachometer in accordance with 2005 ATS/ERS guideline recommendations. Reversible airway obstruction was assessed by administering 360μg (4 puffs) of albuterol sulfate from a standard metered dose inhaler (MDI). Spirometry was obtained before and 15 minutes after bronchodilator administration. For individuals with ≤12% improvement in their forced expiratory volume at 1 second (FEV_1), a second dose of albuterol (360μg for individuals ≥18 years and 180μg for individuals <18 years) was administered followed by lung function reassessment 15 minutes after the second dose. Individuals were considered to have reversible airway obstruction if their FEV_1 improved by >12% after bronchodilator administration.

Discovery Group

Study participants who met the following criteria were invited to undergo a 6-week course of inhaled beclomethasone dipropionate hydrofluoroalkane (beclomethasone HFA): no use of inhaled or systemic corticosteroids in the 4 weeks preceding the evaluation, a pre-bronchodilator FEV_1 that was 40-90% of predicted, reversible airway obstruction (i.e., >12% change in FEV_1 with albuterol administration), no smoking in the year prior to the evaluation and <10 pack-year smoking history in total, and not pregnant at the time of evaluation. Individuals who met these criteria and consented to undergo treatment were instructed to use 320μg (i.e., 2 puffs twice a day) of beclomethasone HFA daily via MDI (QVAR, Teva Respiratory LLC, Horsham, PA) with the use of a spacer device (AeroChamber Plus Flow-Vu spacer, Monaghan Medical Corp., Plattsburgh, NY). Treatment adherence was monitored using a DOSER-CT device (Meditrack, Easton, MA). This adherence monitoring device was fitted to each ICS MDI to count the total number of actuations. The group of individuals who completed the 6-week course of ICS treatment and had genome wide genotype data (described below) comprised the biomarker discovery group. Genome-wide genotyping was performed in African American individuals (self-reported race-ethnicity), who collectively comprised the largest population group in the SAPPHIRE cohort. As a result, the discovery sample consisted of 244 African American individuals who had both completed 6 weeks of study administered ICS treatment and genome-wide genotype data.

Genome-wide genotyping was performed using DNA isolated from whole blood and the Axiom AFR array (Affymetrix Inc., Santa Clara, CA). Quality control of the array results included a dish quality control measure ≥0.82, an overall call rate ≥97%, and no discordance between patient-reported sex and the measured X-heterozygosity. Genotyped single nucleotide polymorphisms with an overall SNP call rate <95%, a minor allele frequency (MAF) <5%, or

exact Hardy-Weinberg equilibrium p-value $<10^{-4}$ were removed from the analysis. Only autosomal SNPs were analyzed.

Validation Groups

Validation was carried out in 2 additional groups from the SAPPHIRE cohort. The primary validation set consisted of African American SAPPHIRE participants (n=803) who met the following criteria: available prospective clinical data on asthma exacerbations from available electronic health records, measures of ICS exposure through pharmacy claims records, existing genome-wide genotype data meeting QC standards, and African American race-ethnicity by participant self-report. Health records were used to identify severe asthma exacerbations and generate measures of ICS exposure over time. Severe asthma exacerbation were defined as those requiring burst oral corticosteroids, an emergency department visit, or a hospitalization.

We standardized each available ICS preparation on the U.S. market by multiplying the number of actuations per container by the milligram dose released per actuation and dividing by the milligram strength of the lowest recommended dose for that preparation as specified in National Asthma Education and Prevention Program. Expert Panel Report 3 (ERP-3) guidelines. Applying our standardization method to participant health data, we assigned each prescription fill the value assigned for each preparation and divided by the total number of days between fills (i.e., days until the next fill) to approximate the amount of ICS used each day. We summed each daily value for 6 months and and divided by 180 to create a measure that could be interpreted as a 6-month average of the daily number of ICS doses (in units of what ERP-3 guidelines defined as a "low" dose for that preparation). This process was repeated for *each* day of each individual's follow-up to create a moving window of ICS exposure for participant in the primary validation set. We also created a dichotomous indicator variable for *each* day of follow-up to denote when a patient had used a long-acting beta-agonist (LABA) in the preceding 180 days. As time-updated proxy measures of asthma severity, we created separate exposure variables for short-acting beta-agonist (SABA) MDI and nebulizer use. The SABA measures were calculated by summing the doses filled over a 90 day moving window. Therefore, the SABA measures represented the average number of SABA doses used per day in the preceding 3-months. We also created a baseline asthma severity measure based on asthma rescue medication use in the preceding year as described by Allen-Ramey *et al*. (J Manag Care Pharm 2006; 12:310-21).

The second validation group comprised European American individuals from the SAPPHIRE cohort (n=98) who met the same selection criteria and underwent the same 6-week ICS treatment protocol as the discovery set. Because this group of European American SAPPHIRE participants had the same treatment regimen and follow-up duration as the discovery set, change in ACT composite score was used as the outcome variable. Participants in this second validation group were genotyped for the rs3827907 SNP using a TaqMan SNP Genotyping Assay (Applied Biosystems).

Statistical Analysis

In the discovery analysis, we used an additive genetic model and linear regression to assess the relationship between genotype and change in ACT score over 6 weeks of ICS treatment. To capture both an independent genetic effect and a modifying effect on ICS treatment response, we included both the main effect of each SNP and an interaction term between the SNP and ICS exposure. The full model also included individual variables for patient age, sex, ACT score at

baseline (i.e., the time of enrollment into the study), smoking status (coded as never, past, and present), the first three principal components (derived using the program EIGENSOFT and representing underlying population substructure), and ICS exposure (continuous measure based on adherence). To jointly test the effect of the SNP and the SNP x ICS interaction, we compared the full model containing all of the aforementioned variables with a reduced model (i.e., all of the variables in the full model except for the SNP and interaction term) using an F-test.

SNP associations with $P<5.0x10^{-7}$ (strict Bonferroni correction $P<0.05/574,370=8.71x10^{-8}$) from the discovery were reassessed in the validation groups. The primary validation analysis was performed in African American SAPPHIRE participants who were not involved in the 6 weeks of study-administered ICS treatment but did have longitudinal clinical information on asthma exacerbations (n=803). Proportional hazards regression was used to evaluate the association between both the SNP term and the SNP x ICS interaction term with time-to-severe asthma exacerbation. These models also accounted for patient age, sex, BMI, smoking status, ACT score at baseline (i.e., enrollment), baseline asthma severity score, LABA use (an indicator variable denoting use of an ICS-LABA combination inhaler), time-updated measures of SABA MDI and nebulizer use, the first 3 principal components, and the time-updated ICS exposure measure. A 2-degree of freedom likelihood ratio test was used to evaluate the significance of the difference in model fit for all variables as compared with a reduced model without the SNP and the SNP x ICS interaction terms.

We also assessed for replication in the 98 European American SAPPHIRE participants who completed 6-weeks of ICS treatment. The linear regression analysis was the same as used in the discovery set with the exception that only the top variant, rs3827907, was assessed, and the regression model did not include principal components to account for underlying population structure.

Analyses were performed using R statistical software.

FIGURE LEGENDS

Figure E1. Quantile-quantile (Q-Q) plot of expected vs. observed genome-wide joint association test results before (A) and after (B) applying genomic control. Each joint test estimated the combined effect of a single nucleotide polymorphism (SNP) and its interaction with inhaled corticosteroid (ICS) use on changes in asthma control occurring over 6 weeks of ICS treatment. Asthma control was measured using the composite Asthma Control Test (ACT) score. The models also adjusted for patient age, sex, ACT score at baseline (i.e., the time of study enrollment), smoking status, the first three principal components, and ICS adherence. The Q-Q plot before applying genomic control (plot A) showed mild inflation (λ=1.09); this was corrected via genomic control (plot B).

Figure E2. LocusZoom plot of rs3827907 on chromosome 14. Single nucleotide polymorphisms located within a 200 kilobase window upstream and downstream of rs3827907 are shown. P-values were generated using the joint (SNP and SNP*ICS combined) test of association with changes in asthma control occurring over 6 weeks of ICS treatment. The SNP color represents the level of correlation between each SNP and rs3827907. Genes located in the region are shown beneath the plotted SNPs.

Figure E3. Relationship between rs3827907 genotype and absolute change in Asthma Control Test (ACT) composite score among African American participants in the Study of Asthma Phenotypes and Pharmacogenomic Interactions by Race-ethnicity (SAPPHIRE) following 6 weeks of observed inhaled corticosteroid (ICS) treatment. Results are restricted to the 129 individuals whose asthma was not controlled at baseline (ACT score ≤19) and are stratified by levels of patient ICS use (≤75% adherence was considered low use and >75% adherence was considered high use). Black lines connect the means for each group.

Figure E4. Relationship between rs3827907 genotype and absolute change in Asthma Control Test (ACT) composite score among African American participants in the SAPPHIRE cohort following 6 weeks of observed inhaled corticosteroid (ICS) treatment (n=244). The rs3827907 genotype is categorized in TT homozygotes and C-allele carriers (denoted C- and representing individuals with either the CT or CC genotypes). Results are stratified by levels of patient ICS use (≤75% adherence was considered low use and >75% adherence was considered high use). Black lines connect the means for each group.

Figure E5. Relationship between rs3827907 genotype and absolute change in Asthma Control Test (ACT) composite score among European American participants in the Study of Asthma Phenotypes and Pharmacogenomic Interactions by Race-ethnicity (SAPPHIRE) cohort following 6 weeks of observed inhaled corticosteroid (ICS) treatment (n=98). Results are stratified by levels of patient ICS use (≤75% adherence was considered low use and >75% adherence was considered high use). Black lines connect the means for each group.

Figure E6. Correlation of expression between protein-coding genes located within 1 megabase upstream and downstream of rs3827907 on chromosome 14. Gene expression is based on read counts from RNA-seq data obtained from 158 African American participants from the Study of

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Asthma Phenotypes and Pharmacogenomic Interactions by Race-ethnicity (SAPPHIRE). Read counts were normalized using the program DESeq2 (https://bioconductor.org/packages/release/bioc/html/DESeq2.html). Gene expression of *EDDM3B* in whole blood was low and did not meet our criterion for evaluation (≥50% of individuals with read counts >0); therefore, it is not listed among the 32 genes evaluated for this region.

Figure E7. Effect of baseline measures of eosinophil-derived neurotoxin (EDN) on the relationship between rs3827907 C-allele carrier status and absolute change in Asthma Control Test (ACT) composite score among European American participants in the SAPPHIRE cohort following 6 weeks of observed inhaled corticosteroid (ICS) treatment (n=96). Results are stratified by levels of patient ICS use (≤75% adherence was considered low use and >75% adherence was considered high use) and pre-treatment serum levels of EDN. Low and high EDN levels were defined as those ≤2.34 ng/mL and >2.34 ng/mL, respectively. Black lines connect the means for each group.

Figure E8. Effect of baseline blood eosinophil counts on relationship between rs3827907 C-allele carrier status and absolute change in Asthma Control Test (ACT) composite score among European American participants in the SAPPHIRE cohort following 6 weeks of observed inhaled corticosteroid (ICS) treatment (n=94). Results are stratified by levels of patient ICS use (≤75% adherence was considered low use and >75% adherence was considered high use) and blood eosinophil counts measured before ICS treatment. Low and high eosinophil counts were defined as those ≤222 cells/μL and >222 cells/ μL, respectively. Black lines connect the means for each group.

Figure E9. LocusZoom plot of rs3827907 on chromosome 14 including imputed variants. Single nucleotide polymorphisms located within a 200 kilobase window upstream and downstream of rs3827907 are shown. P-values were generated using the joint (SNP and SNP*ICS combined) test of association with changes in asthma control occurring over 6 weeks of ICS treatment. The SNP color represents the level of correlation between each SNP and rs3827907. After res3827907, the next most significant variant, imputed SNP rs12891518, is also labeled. Genes located in the region are shown beneath the plotted SNPs.

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Table E1. Interactions between previously reported pharmacogenetic variants and inhaled corticosteroid use on changes in asthma control among individuals in the discovery set (n=244)*

SNP	Chr	Position	Imputation Quality†	Allele‡	MAF‡	Gene	Parameter estimate for SNP§	P-value for SNP term ‖	Parameter estimate for SNP x ICS interaction§	P-value for interaction term ‖	P-value for joint test¶
rs10044254	5	15,783,596	0.96	G/A	0.35	FBXL7	2.49	0.060	-3.08	0.063	0.167
rs2395672	6	37,428,577	0.75	A/G	0.03	CMTR1	2.38	0.583	-1.72	0.750	0.566
rs6924808	6	98,358,575	0.95	G/A	0.39		0.41	0.733	-0.70	0.651	0.861
rs6456042	6	166,534,742	0.96	A/C	0.35	T	1.18	0.369	-1.33	0.413	0.657
rs3127412	6	166,535,561	0.96	C/T	0.35	T	1.18	0.369	-1.33	0.413	0.657
rs37972	7	8,007,509	0.92	T/C	0.18	GLCCI1	-0.20	0.910	0.44	0.840	0.918
rs2691529	7	77,803,275	0.89	C/T	0.24	MAGI2	-0.86	0.564	0.77	0.685	0.713
rs6467778	7	138,178,222	0.95	A/G	0.17	TRIM24	-1.43	0.354	1.36	0.481	0.521
rs10481450	8	9,798,246	0.80	A/T	0.13		2.88	0.092	-2.63	0.248	0.061
rs3793371	8	144,664,299	0.97	A/G	0.04	EEF1D (NAPRT1)	2.03	0.761	-2.32	0.760	0.954
rs4271056	9	38,232,043	1.00	C/T	0.23		-2.10	0.106	2.86	0.087	0.230
rs7850103	9	118,137,835	0.99	A/C	0.15	DEC1 (TNC)	2.14	0.263	-3.57	0.132	0.126
rs1353649	11	20,253,599	0.99	A/G	0.40		-1.45	0.203	2.31	0.113	0.194
rs4980524	11	63,959,259	0.99	C/A	0.29	STIP1	0.38	0.736	-0.82	0.563	0.655
rs6591838	11	63,959,356	0.99	G/A	0.21	STIP1	0.43	0.736	-0.53	0.744	0.944
rs2236647	11	63,964,605	0.99	C/T	0.28	STIP1	-1.03	0.452	1.15	0.494	0.740
rs1144764	11	77,870,486	0.94	T/C	0.39	KCTD21-AS1 (ALG8)	-1.75	0.191	2.38	0.161	0.367
rs12891009	14	74,083,846	0.93	T/C	0.26	ACOT6/HEATR4 (ACOT4)	-0.18	0.884	-0.24	0.877	0.580
rs2230155	15	59,510,189	0.90	A/G	0.41	MYO1E	0.84	0.448	-1.38	0.341	0.550
rs12438740	15	59,515,767	1.00	A/G	0.41	MYO1E	0.58	0.601	-0.97	0.507	0.744
rs242941	17	43,892,520	1.00	C/A	0.37	CRHR1	0.16	0.883	0.00	0.999	0.880
rs242939	17	43,895,579	1.00	C/T	0.32	CRHR1	2.19	0.081	-2.82	0.085	0.215
rs1876828	17	43,911,525	0.99	T/C	0.03	CRHR1	5.29	0.459	-5.67	0.497	0.701
rs2240017	17	45,810,919	0.38	G/C	0.01	TBX21	4.87	0.916	-3.57	0.963	0.560
rs1380657	17	48,598,785	1.00	G/A	0.12	MYCBPAP (SPATA20)	2.95	0.113	-4.70	0.052	0.081
rs6504666	17	48,601,328	1.00	G/A	0.16	MYCBPAP (SPATA20)	0.99	0.544	-1.21	0.565	0.831
rs9303988	18	6,667,583	0.67	T/C	0.44		1.10	0.348	-1.29	0.397	0.631
rs279728	20	45,080,421	1.00	T/C	0.23		0.75	0.551	-0.98	0.538	0.826
rs2037925	21	40,699,931	0.95	C/T	0.33	(BRWD1)	-2.27	0.098	2.38	0.155	0.192
rs2836987	21	40,706,594	0.94	C/T	0.31	(BRWD1)	-1.53	0.272	1.65	0.334	0.503
rs138335	22	41,227,086	0.83	C/G	0.07	ST13	1.30	0.652	-2.64	0.456	0.350
rs138337	22	41,231,053	0.94	G/A	0.42	ST13	0.79	0.504	-1.09	0.465	0.759

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SNP denotes single nucleotide polymorphism; Chr, chromosome; MAF, minor allele frequency; and ICS, inhaled corticosteroid.
*SNPs were selected from variants previously reported to be associated with ICS response. The outcome phenotype was the change in asthma control test (ACT) score from before to immediately following 6 weeks of inhaled corticosteroid (ICS) treatment.
†The imputation quality score is the MACH-based imputation r^2 value, which ranges from zero to one. Values closer to one indicate higher imputation quality.
‡The "effect" allele is shown first, and the referent allele follows. The allele frequency of the "effect" allele is provided, and in the current table, this estimate is based on the observed frequency in the discovery set.
§The parameter estimates demonstrate the direction and magnitude for a change in the ACT score over the course of 6 weeks of ICS treatment. A *positive* parameter estimate indicates that the variable was associated with an improvement in asthma control over the course of treatment. The parameter estimate for the SNP can be interpreted as the effect on asthma control for each additional "effect" allele (i.e., none [=0], one [=1], or two alleles [=2]). The parameter estimate for the interaction term can be interpreted as the combined effect of increasing ICS use (i.e., going from zero to complete adherence to the prescribed dose [a continuous variable ranging from 0 to 1]) and the number of "effect" alleles on the change in asthma control over 6 weeks of ICS treatment.
‖The P-values for the SNP and SNP x ICS interaction variables are shown for the model simultaneously including these variables, as well as adjusting for patient age, sex, ACT score at baseline enrollment into the study, smoking status (coded as never, past, and present), the first three principal components, and ICS adherence.
¶The F-test assessed the significance of the difference in model fit between the full model (i.e., all of the aforementioned variables included) and the reduced model (i.e., the full model minus both the SNP and SNP x ICS interaction terms). In other words, a *joint test* was used to simultaneously assess the combined significance of the SNP and SNP x ICS interaction terms.

Table E2. Interactions between previously reported pharmacogenetic variants and inhaled corticosteroid use on severe asthma exacerbations among African American SAPPHIRE participants in the validation set (n=803)*

SNP	Chr	Position	Imputation Quality†	Allele‡	MAF‡	Gene	Parameter estimate for SNP§	P-value for SNP term‖	Parameter estimate for SNP x ICS interaction§	P-value for interaction term‖	P-value for joint test¶
rs10044254	5	15,783,596	0.96	G/A	0.34	FBXL7	-0.18	0.115	0.13	0.433	0.282
rs2395672	6	37,428,577	0.75	A/G	0.04	CMTR1	0.28	0.326	-0.88	**0.036**	**0.020**
rs6924808	6	98,358,575	0.95	G/A	0.42		0.00	0.981	-0.18	0.212	0.331
rs6456042	6	166,534,742	0.96	A/C	0.36	T	0.10	0.367	0.09	0.524	0.294
rs3127412	6	166,535,561	0.96	C/T	0.36	T	0.10	0.367	0.09	0.524	0.294
rs37972	7	8,007,509	0.92	T/C	0.21	GLCCI1	0.20	0.104	-0.22	0.188	0.233
rs2691529	7	77,803,275	0.89	C/T	0.26	MAGI2	0.16	0.154	-0.04	0.742	0.336
rs6467778	7	138,178,222	0.95	A/G	0.16	TRIM24	-0.40	**0.017**	0.21	0.304	**0.039**
rs10481450	8	9,798,246	0.80	A/T	0.13		0.13	0.437	0.07	0.717	0.508
rs3793371	8	144,664,299	0.97	A/G	0.04	EEF1D (NAPRT1)	-0.71	**0.016**	-0.29	0.361	**$3.27x10^{-4}$**
rs4271056	9	38,232,043	1.00	C/T	0.22		0.07	0.587	0.16	0.319	0.316
rs7850103	9	118,137,835	0.99	A/C	0.11	DEC1 (TNC)	0.77	**$6.38x10^{-7}$**	-0.46	**0.030**	**$1.87x10^{-5}$**
rs1353649	11	20,253,599	0.99	A/G	0.42		-0.03	0.762	0.19	0.106	0.215
rs4980524	11	63,959,259	0.99	C/A	0.32	STIP1	0.15	0.189	-0.21	0.109	0.218
rs6591838	11	63,959,356	0.99	G/A	0.21	STIP1	0.13	0.336	-0.27	0.117	0.271
rs2236647	11	63,964,,605	0.99	C/T	0.29	STIP1	-0.02	0.856	0.03	0.837	0.975
rs1144764	11	77,870,486	0.94	T/C	0.39	KCTD21-AS1 (ALG8)	0.13	0.218	-0.03	0.839	0.415
rs12891009	14	74,083,846	0.93	T/C	0.27	ACOT6/HEATR4 (ACOT4)	0.31	**0.012**	-0.36	**0.029**	**0.026**
rs2230155	15	59,510,189	0.90	A/G	0.38	MYO1E	0.29	**0.014**	-0.06	0.750	**0.028**
rs12438740	15	59,515,767	1.00	A/G	0.38	MYO1E	0.30	**0.012**	-0.07	0.699	**0.024**
rs242941	17	43,892,520	1.00	C/A	0.42	CRHR1	-0.02	0.829	0.15	0.183	0.367
rs242939	17	43,895,579	1.00	C/T	0.31	CRHR1	0.04	0.734	0.13	0.368	0.433
rs1876828	17	43,911,525	0.99	T/C	0.06	CRHR1	-0.69	**0.007**	0.45	**0.026**	**0.010**
rs2240017	17	45,810,919	0.38	G/C	0.01	TBX21	-0.60	0.334	3.55	**$2.52x10^{-6}$**	**0.010**
rs1380657	17	48,598,785	1.00	G/A	0.13	MYCBPAP (SPATA20)	-0.16	0.348	-0.13	0.504	0.262
rs6504666	17	48,601,328	1.00	G/A	0.16	MYCBPAP (SPATA20)	0.08	0.572	-0.06	0.663	0.840
rs9303988	18	6,667,583	0.67	T/C	0.39		0.01	0.948	-0.10	0.438	0.672
rs279728	20	45,080,421	1.00	T/C	0.21		0.48	**$3.36x10^{-4}$**	0.23	0.191	**$1.43x10^{-5}$**
rs2037925	21	40,699,931	0.95	C/T	0.37	(BRWD1)	-0.34	**0.003**	-0.07	0.538	**0.001**
rs2836987	21	40,706,594	0.94	C/T	0.34	(BRWD1)	-0.19	0.103	-0.08	0.457	**0.047**
rs138335	22	41,227,086	0.83	C/G	0.08	ST13	0.19	0.298	-0.22	0.470	0.575

rs138337	22	41,231,053	0.94	G/A	0.41	ST13	0.11	0.305	0.00	0.985	0.471

SAPPHIRE denotes Study of Asthma Phenotypes and Pharmacogenomic Interactions by Race-ethnicity; SNP, single nucleotide polymorphism; Chr, chromosome; MAF, minor allele frequency; and ICS, inhaled corticosteroid.

*SNPs were selected from variants previously reported to be associated with ICS response. Cox proportional hazards models were used to assess associations with time-to-severe asthma exacerbation (i.e., burst oral corticosteroid use, asthma-related emergency department visits, and hospitalizations for asthma).

†The imputation quality score is the MACH-based imputation r^2 value, which ranges from zero to one. Values closer to one indicate higher imputation quality.

‡The "effect" allele is shown first, and the referent allele follows. The allele frequency of the "effect" allele is provided, and in the current table, this estimate is based on the observed frequency in the validation set.

§The parameter estimates represent the risk (i.e., hazard) of having a severe asthma exacerbation following the initial (baseline) assessment at study entry. A *negative* parameter estimate indicates that the variable was associated with a lower risk of experiencing a severe asthma exacerbation. The parameter estimate for the SNP can be interpreted as the effect on exacerbation risk for each additional "effect" allele (i.e., none [=0], one [=1], or two alleles [=2]). The parameter estimate for the interaction term can be interpreted as the combined effect of increasing ICS use (i.e., average number of ICS doses taken per day – see Methods Section and Supplemental Methods Section for more details on how longitudinal ICS exposure measures were created) and the number of "effect" alleles on the risk of a severe asthma exacerbation.

‖The P-values for the SNP and SNP x ICS interaction variables are shown for the model simultaneously including these variables, as well as adjusting for patient age, sex, BMI, smoking status, ACT score at baseline (i.e., time of study enrollment), baseline asthma severity score, LABA use (an indicator variable denoting use of an ICS-LABA combination inhaler), time-updated measures of SABA MDI and nebulizer use, the first 3 principal components, and the time-updated ICS exposure measure.

¶The likelihood ratio test assessed the significance of the difference in model fit between the full model (i.e., all of the aforementioned variables included) and the reduced model (i.e., the full model minus both the SNP and SNP x ICS interaction terms). In other words, a *joint test* was used to simultaneously assess the combined significance of the SNP and SNP x ICS interaction terms.

Table E3. Relationship between rs3827907 genotype and inhaled corticosteroid use on change in asthma control stratified by pre-treatment eosinophilia African American individuals in the SAPPHIRE cohort*

Sample	Sample size	rs3827907 C-allele status		ICS use		SNP*ICS interaction	
		Parameter estimate†	P-value	Parameter estimate†	P-value	Parameter estimate†	P-value
All	243	-2.569	5.38×10^{-4}	0.097	0.851	4.405	1.52×10^{-5}
High EDN‡	121	-3.838	9.87×10^{-5}	-0.014	0.984	5.485	1.35×10^{-4}
Low EDN‡	122	-1.154	0.314	0.365	0.658	3.319	0.029
High Eosinophils§	56	-2.720	0.238	-0.777	0.567	7.050	0.041
Low Eosinophils§	57	-2.365	0.154	-0.753	0.533	3.722	0.075
C-allele carrier	61	--	--	4.304	4.61×10^{-5}	--	--
High EDN‡	28	--	--	5.986	0.002	--	--
Low EDN‡	33	--	--	4.247	0.003	--	--
High Eosinophils§		--	--	10.234	0.516	--	--
Low Eosinophils§		--	--	2.765	0.116	--	--
TT homozygotes	182	--	--	0.14	0.782	--	--
High EDN‡	93	--	--	0.131	0.830	--	--
Low EDN‡	89	--	--	0.327	0.713	--	--
High Eosinophils§	45	--	--	-0.627	0.621	--	--
Low Eosinophils§	39	--	--	-1.079	0.415	--	--

SAPPHIRE denotes Study of Asthma Phenotypes and Pharmacogenomic Interactions by Race-ethnicity; ICS, inhaled corticosteroid; SNP, single nucleotide polymorphism; and EDN, eosinophil-derived neurotoxin.

*The analysis was restricted to SAPPHIRE participants from the discovery set with available data and who underwent 6 weeks of observed ICS treatment. Change is asthma control was taken as the change in Asthma Control Test composite score from before to after ICS treatment.

†Parameter estimates represent the effect of a one-unit increase in the variable listed. The rs3827907 genotype was collapsed into C-allele carriers (=1) and TT homozygotes (=0). Inhaled corticosteroid use was dichotomously categorized into those whose adherence was ≤75% (low use) and >75% (high use) over course of the 6-week treatment regimen. The interaction term represents the combined effect of rs3827907 C-allele carrier status and level of ICS use. The models used to create the parameter estimates simultaneously

included all of the variables shown, as well as variables to adjust for patient age, sex, BMI, smoking status, ACT score at baseline, and first 3 principal components. Positive parameter estimates suggest that the variable was associated with an improvement in asthma control over the 6-week ICS treatment trial.

‡The study population was stratified based on the median EDN serum level for the entire group with these data (n=243) prior to initiating ICS treatment. A baseline EDN value ≤2.34 ng/mL was considered low and a value >2.34 ng/mL was considered high.

§The study population was also stratified based on the median blood eosinophil for the entire group with these data (n=133) prior to initiating ICS treatment. A baseline blood eosinophil count ≤222 cells/μL was considered low and a count >222 cells/μL was considered high.

Table E4. Relationship between both rs3827907 and rs12891519 genotype and *RNASE2* gene expression in peripheral blood among African American participants with and without asthma from the SAPPHIRE cohort.

Groups	rs3827907 variant		rs12891518 variant	
	Parameter estimate*	P-value	Parameter estimate*	P-value
All participants (n=597)	-0.237	0.004	-0.235	0.008
Asthma cases (n=408)	-0.243	0.018	-0.220	0.047
Controls (n=189)	-0.224	0.103	-0.267	0.069

SAPPHIRE denotes the Study of Asthma Phenotypes and Pharmacogenomic Interactions by Race-ethnicity.

*Linear regression was used to examine the relationship between both rs3827907 genotype (coded as TT=0, CT=1, CC=2) and rs12891518 genotype (code AA=0, AT=1, and TT=2) and gene expression (dependent variable). The model adjusted for patient age, sex, BMI, the absolute cell counts for each of the 5 white blood cell types measured (i.e., neutrophils, monocytes, lymphocytes, eosinophils, and basophils from complete blood counts obtained at the time that the RNA was collected), sequence batch, and the first 30 probabilistic estimation of expression residuals (PEERs). The parameter estimates are the adjusted genotype main effect estimates for rs3827907 and rs12891518. A negative parameter estimate suggests that the minor allele (i.e., the C-allele for rs3827907 and the T-allele for rs12891518) was associated with decreased *RNASE2* gene expression.