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1 ABSTRACT

2 **Background:** Although inhaled corticosteroid (ICS) medication is considered the cornerstone
3 treatment for patients with persistent asthma, few ICS pharmacogenomic studies have involved
4 non-white populations.

5 **Objective:** To identify genetic predictors of ICS response in multiple population groups with
6 asthma.

7 **Methods:** The discovery group comprised African American participants from the Study of
8 Asthma Phenotypes and Pharmacogenomic Interactions by Race-ethnicity (SAPPHIRE) who
9 underwent 6 weeks of monitored ICS therapy (n=244). A genome-wide scan was performed to
10 identify single nucleotide polymorphism (SNP) variants jointly associated (i.e., the combined
11 effect of the SNP and SNP x ICS treatment interaction) with changes in asthma control. Top
12 associations were validated by assessing the joint association with asthma exacerbations in three
13 additional groups – African Americans (n=803 and n=563) and Latinos (n=1,461). RNA-seq
14 data from 408 asthma cases and 405 controls were used to examine whether genotype was
15 associated with gene expression.

16 **Results:** One variant, rs3827907, was significantly associated with ICS-mediated changes in
17 asthma control in the discovery set ($P=7.79 \times 10^{-8}$) and was jointly associated with asthma
18 exacerbations in three validation cohorts ($P=0.023$, $P=0.029$, and $P=0.041$). RNA-seq analysis
19 found the rs3827907 C-allele to be associated with lower *RNASE2* expression ($P=6.10 \times 10^{-4}$).
20 *RNASE2* encodes eosinophil-derived neurotoxin (EDN), and the rs3827907 C-allele appeared to
21 particularly influence ICS treatment response in the presence of eosinophilic inflammation (i.e.,
22 high pre-treatment EDN levels or blood eosinophil counts).

Conclusion: We identified a variant, rs3827907, which appears to influence response to ICS treatment in multiple population groups, and likely mediates its effect through eosinophils.

Clinical Implications: African Americans and Latinos are disproportionately affected by asthma and its complications. Here we identify a pharmacogenomic variant that may assist in identifying individuals from these groups who will respond to ICS treatment.

Capsule Summary: This is the first study to use a non-white study population for the discovery of pharmacogenomic predictors of asthma controller response. Variant rs3827907 genotype appeared to influenced ICS response especially in the setting of eosinophilic inflammation.

KEY WORDS: pharmacogenetics; *EDDM3B*; *RNASE2*; eosinophil-derived neurotoxin; transcriptome; eosinophils

ABBREVIATIONS:

ACT – Asthma Control Test

CHR – chromosome

ECP – eosinophil cationic protein

EDN – eosinophil derived neurotoxin

eQTL – expression quantitative trait locus (or loci)

FEV₁ – forced expiratory volume at one second

GALA II – Genes-environments & Admixture in Latino Americans Study

ICS – inhaled corticosteroid

LABA – long-acting beta-agonist

- 46 MDI – metered dose inhaler
- 47 SABA – short-acting beta-agonist
- 48 SAGE II – Study of African Americans, Asthma, Genes and Environments
- 49 SAPPHIRE – Study of Asthma Phenotypes and Pharmacogenomic Interactions by Race-
- 50 ethnicity
- 51 SNP – single nucleotide polymorphism

INTRODUCTION

African American individuals appear to be disproportionately affected by asthma and its complications with rates of asthma-related emergency room visits, hospitalizations, and deaths that are approximately 2-3 times higher when compared with white individuals.¹ Inhaled corticosteroid (ICS) medication is one of the most effective treatments for persistent asthma and its use has been associated with lower rates of severe exacerbations including death.^{2,3} However, to date there has been little to no African American representation in pharmacogenomic studies assessing genetic predictors of ICS response.⁴⁻⁶

Despite previous work by us suggesting that an individual's overall proportion of African ancestry may not be a strong determinant of ICS response,^{7,8} population-specific risk variants influencing asthma medication response may still exist. The possibility of the latter is supported by genome-wide association studies demonstrating both shared and group-specific risk variants for asthma^{9,10} and pharmacogenomic studies for other disease conditions showing population-specific variants associated with medication response.¹¹

We and others have also shown that ICS medication adherence varies widely among individuals and is on average quite poor among patients being treated for asthma.^{12,13} Not surprisingly, this variation in patient use is a major predictor of treatment response and asthma-related exacerbations.^{14,15} As a result, studies examining predictors of ICS response should account for patient medication adherence, along with dose and preparation, as all of these factors influence the level of anti-inflammatory exposure.

The Study of Asthma Phenotypes and Pharmacogenomic Interactions by Race-ethnicity (SAPPHIRE) is an ongoing prospective cohort study to understand the genetic predictors of asthma medication treatment response in a large, diverse patient population from southeast

Michigan. A subgroup of SAPPHIRE participants who met strict criteria received 6 weeks of ICS treatment to directly quantify corticosteroid response; our discovery set consisted of African American participants in this treatment group (n=244). The validation groups included African American individuals with prospective clinical information on ICS exposure and asthma exacerbations from the SAPPHIRE cohort; Latino individuals from the Genes-environments & Admixture in Latino Americans Study (GALA II); and African American individuals from the Study of African Americans, Asthma, Genes and Environments (SAGE II). The latter 2 cohorts had retrospective information on ICS use and asthma exacerbations. We also reassessed our top association in a set of European American individuals from the SAPPHIRE cohort who underwent the same 6-week ICS treatment course as the discovery group.

METHODS

Study Overview and Patient Population

The SAPPHIRE cohort participants were recruited within a large health system serving southeast Michigan and the Detroit metropolitan area. Individuals were eligible to participate if they were 12-56 years of age, had a previously documented physician diagnosis of asthma, and no prior diagnosis of congestive heart failure or chronic obstructive pulmonary disease. Study participants underwent a clinical evaluation which included a detailed questionnaire and lung function testing. Further details about the SAPPHIRE patient evaluation can be found in the online supplement.

The GALA II and SAGE II cohorts are described below and in the methods section of the online supplement. All studies had institutional review board approval at their respective institutions, and all studies required informed written consent prior to study participation.

Discovery Group

The discovery group consisted of a subset of SAPPHIRE participants who completed 6 weeks of monitored ICS treatment, had existing genome-wide genotype data, and were African American by self-report (n=244). These individuals completed the Asthma Control Test (ACT), a validated 5-question instrument used for assessing asthma control in individuals ≥ 12 years of age (Optum Inc., Eden Prairie, MN),¹⁶ both before and after ICS treatment (320 μ g of inhaled beclomethosone dipropionate hydrofluoroalkane [beclomethasone HFA] daily x 6 weeks). The phenotype for ICS response in the discovery set was the change in ACT score over the course of the treatment trial. We have previously demonstrated a “dose-response” relationship between ICS use and change in ACT score.⁸ Since the primary purpose of this study was to identify genetic factors that modify ICS treatment response, we reasoned that the strength and direction of an association would be contingent on the level of ICS exposure/adherence. Adherence was measured using an electronic counting device (DOSER-CT, Meditrack, Easton, MA) attached to the ICS metered dose inhaler.

African American SAPPHIRE participants in the discovery and validation groups were genotyped using the Axiom AFR array (Affymetrix Inc., Santa Clara, CA).¹⁷ After quality control, 574,370 of the genotyped single nucleotide polymorphisms (SNPs) were available for analysis.

Validation Groups

Validation was carried out in 4 groups. These groups included a separate set of African American individuals from the SAPPHIRE cohort, Latino individuals from GALA II, African American participants from SAGE II, and European American individuals from the SAPPHIRE cohort.

The primary validation analysis was performed in African American individuals from SAPPHIRE (n=803) who met the following criteria: available prospective clinical data on asthma exacerbations, recorded ICS fills through pharmacy claims records, existing genome-wide genotype data, and African American race-ethnicity by participant self-report. Our method of calculating ICS exposure using pharmacy data can be found in the online supplement and elsewhere.¹⁸ Additional validation was performed among Latino participants in the GALA II study (n=1,461) and African American participants in the SAGE II study (n=563). The latter two cohorts had retrospective information on asthma exacerbations. Genotyping in these cohorts was performed using the Affymetrix Axiom LAT1 array. Lastly, we had a group of European American individuals from the SAPPHIRE cohort (n=98) who underwent the same ICS treatment protocol as the discovery set; we used the change in asthma control (i.e., the change in the ACT composite score) as the outcome in this group.

RNA-seq Data

At the time of enrollment, SAPPHIRE participants had whole blood RNA collected, preserved, and stored in PAXgene Blood RNA tubes (BD Biosciences, San Jose, CA) until the time of analysis. Sequencing libraries were constructed using TruSeq Stranded Total RNA Library Prep Kit with Ribo-Zero Globin (Illumina, San Diego, CA) on 408 African American

individuals with asthma and 405 healthy African American controls. Illumina HiSeq RNA-seq reads were mapped to human genome (build GRCh38.p5) using the software STAR and quantified at the gene level using the program RSEM.^{19, 20} We normalized raw read counts for human protein-coding genes using the variance stabilizing transformation implemented in the program DESeq2.²¹ As done by others,²² we only analyzed genes with ≥ 1 read count in $\geq 50\%$ of individuals.

Blood Eosinophil Counts and Eosinophil-Derived Neurotoxin (EDN) Protein

Measurements

Blood and serum samples were collected from SAPPHERE participants at the time of study enrollment. A complete blood count with 5-part white blood cell differential was obtained immediately for individuals with available samples. Serum specimens were placed into long-term storage at -80°C until the time of analysis. We used a commercial enzyme-linked immunosorbent assay (ELISA) kit (MBL International Corp., Woburn, MA) and followed the manufacturer's instructions to measure EDN levels in the thawed serum samples.

Statistical Analysis

A full description of the models used for each analysis can be found in the online supplement. Both the discovery and validation analyses assessed the joint association of SNP and SNP x ICS exposure interactions on asthma outcomes related to disease control. Our primary outcome for the discovery analysis was the change in asthma control (i.e., ACT score) from before to immediately following 6 weeks of ICS treatment. The conservative Bonferroni correction for this analysis resulted in significance threshold of $P\text{-value} < 8.71 \times 10^{-8}$

(0.05/574,370). In the validation analyses, we used time-to-severe exacerbation as a prospective measure of asthma control in African American SAPPHIRE participants. For validating in GALA II and SAGE II, we used a dichotomous outcome of exacerbation in the 12-month period preceding study enrollment. We defined a severe asthma exacerbation as one requiring burst oral corticosteroids, an emergency department visit, or hospitalization. For European American SAPPHIRE participants who completed 6 weeks of observed ICS treatment, we used the same model as the discovery analysis with change in ACT score as the outcome.

To assess for expression quantitative trait loci (eQTL), we used linear regression to model the relationship between rs3827907 genotype and gene expression. A Bonferroni corrected P-value $<1.56 \times 10^{-3}$ (0.05/32 genes) was considered statistically significant in the eQTL analyses.

To assess the potential effect of pre-treatment EDN levels and eosinophil counts on ICS treatment response, we assessed the relationship between rs3827907 genotype (i.e., C-allele carrier status) on the 6-week change in ACT score after stratifying by median levels of the aforementioned variables in the discovery group. To assess the impact of ICS usage, we also stratified by ICS adherence (i.e., $\leq 75\%$ and $>75\%$) based on our earlier work demonstrating that this was a meaningful threshold for asthma outcomes.¹⁵ A P-value <0.05 was considered statistically significant.

As a post hoc analysis, we imputed missing genotypes among SAPPHIRE African Americans using the program Minimac3;²³ the 1000 Genomes Project (1KGP; version 5) cosmopolitan panel was used as the reference for imputation. The models employed in the discovery and eQTL analyses were used to assess imputed variants surrounding our lead candidate, rs3827907.

RESULTS

Study Populations

Our discovery set comprised 244 African American individuals whose characteristics are shown in Table 1. Individuals in the discovery set received 6 weeks of ICS treatment, during which time we assessed for changes in asthma control. The validation samples used in this analysis are also shown. The validation groups included African American SAPHIRE participants (n=803), Latino patients from GALA II patients (n=1,461), African American patients from SAGE II (n=563), and European American SAPHIRE participants (n=98). As can be seen in Table 1, SAPHIRE participants were on average considerably older when compared with GALA II and SAGE II participants. In addition, there were no active smokers in GALA II and SAGE II as this was an exclusion criterion for participation in both of these studies.

Evaluation for ICS Pharmacogenomic Interactions in the Discovery Set

We assessed for the genetic predictors of ICS response (defined in the discovery analysis as a change in ACT score over 6 weeks of treatment). To account for differences in ICS use among patients in the discovery set, we incorporated measures of ICS adherence which had been gathered using electronic monitoring devices. Average ICS adherence was 0.76 ($\pm$ 0.22 standard deviation [SD]), representing the proportion of the prescribed amount taken. Since all patients in the discovery set were prescribed the same type and amount of ICS medication, the adherence estimate was used to account for relative differences in ICS exposure between individuals. In

our tests of association with drug response, we assessed the joint effect of genotype and a genotype x ICS exposure multiplicative interaction, while simultaneously adjusting for potential confounders. The quantile-quantile plot for this association analysis is shown in Figure E1 (online supplement). As the plot showed mild genomic inflation ($\lambda=1.09$), we adjusted our P-values accordingly using genomic control. Three SNPs – rs3827907 (chromosome [chr] 14), rs2629529 (chr 10), and rs7390625 (chr 20) – were found to have joint association P-values $<5.0 \times 10^{-7}$ prior to genomic control (Table 2). However, only SNP rs3827907, a variant located in the 3'UTR of the transcribed sequence for *EDDM3B*, had a joint test P-value, which still met the conservative Bonferroni threshold after applying genomic control – $P=7.79 \times 10^{-8}$ (Table 2, Figure 1, and Figure E2 [locus zoom plot]). For this variant, both the main effect for the SNP and the interaction term were statistically significant, suggesting that the effect of rs3827907 on asthma control was simultaneously dependent upon the degree of ICS exposure. Specifically, a seemingly detrimental effect of the rs3827907 C-allele (i.e. worsened asthma control) was reversed at prescribed levels of ICS. This relationship was also observed (Figure 2) when plotting the actual values for change in ACT score versus rs3827907 genotype stratified by ICS adherence (i.e., $\leq 75\%$ adherence [low use] and $>75\%$ [high use]). A similar pattern was observed when restricting the analytic set to individuals whose asthma was not controlled (ACT composite score ≤ 19) prior to initiating ICS treatment (Figure E3). Lastly, adjusting for local ancestry at rs3827907 (in addition to adjusting for global ancestry) did not diminish the overall statistical significance of this variant ($P=8.57 \times 10^{-8}$ after genomic control).

Based on the regression models (Table 2), a similar pattern of association was seen for SNP rs73906251 on chromosome 20 ($P=3.85 \times 10^{-7}$, joint test), where an observed detrimental association of the T-allele at this locus also appeared to be mitigated by ICS treatment. In

contrast, variant rs2629529 on chromosome 10 ($P=5.25 \times 10^{-8}$, joint test) showed a salutary association between the C-allele and asthma control, but this effect appeared to diminish with increasing ICS exposure. These two variants were no longer statistically significant after applying genomic control ($P=1.16 \times 10^{-6}$ for rs73906251 and $P=1.86 \times 10^{-7}$ for rs26295290).

To exclude the possibility that minor allele homozygotes unduly influenced our results, we repeated our analysis using a dominant genetic model (i.e., collapsing the homozygous and heterozygous carriers into a single group). The joint test under a dominant genetic model identified the same top three SNPs as the discovery analysis (data not shown). The main effect and interaction term P-values for rs3827907 were 1.12×10^{-7} and 1.45×10^{-7} , respectively ($P=6.51 \times 10^{-7}$, joint test). This relationship was also observed when plotting the actual values for change in ACT score versus rs3827907 genotype categorized dichotomously as TT homozygotes and C-allele carriers (Figure E4).

Validation Analysis

Due to the absence of African American replication populations with prospectively measured ICS treatment response on asthma control, we elected to support and validate our discovery findings using diverse cohorts with information on both ICS exposure and severe asthma exacerbation outcomes. For this analysis we used a separate group of 803 SAPPHERE participants who had both prospectively-collected clinical information and genome-wide genotype data. We used nested models to assess the joint effect of the SNP and the SNP x ICS exposure interaction on time-to-severe asthma exacerbation. The joint test P-values for rs3827907, rs2629529, and rs7390625 were 0.023, 5.65×10^{-6} , and 0.043, respectively (Table 3). Overall, rs3827907 was the only one of the three SNPs evaluated to show a direction of effect

that was consistent with the discovery analysis. The regression model suggested that the rs3827907 C-allele was associated with an increased risk of severe exacerbation and was mitigated by increasing ICS exposure (although the interaction term alone was not statistically significant). Therefore, rs3827907 appeared to affect response to ICS treatment in terms of both asthma control and exacerbations.

We assessed the promoted SNPs in the GALA II and SAGE II cohorts. For these cross-sectional studies, the dichotomous outcome was an asthma exacerbation in the year prior to enrollment. The respective joint test P-values for rs3827907, rs2629529, and rs7390625 were 0.029, 0.281, and 0.413 for GALA II and 0.041, 0.876, and 0.004 for SAGE II (Table 4). While the joint test was statistically significant for rs3827907 in both GALA II and SAGE II, the direction of the SNP main effect for C-allele was most consistent between African Americans in SAPPHERE (parameter estimate 0.35, Table 3) and Latinos from GALA II (parameter estimate 0.21, Table 4), but differed for African Americans in SAGE II (parameter estimate -1.04, Table 4).

The relationship between rs3827907 genotype and change in ACT score stratified by ICS adherence among 98 European American individuals in SAPPHERE who received 6 weeks of ICS treatment is shown in Figure E5 (online supplement). The rs3827907 C-allele frequency in this group was 40.3%. While not statistically significant, the direction of the allelic effect was similar to that observed in the discovery set.

Assessment of Previously Described Pharmacogenetic Variants

We also attempted to validate 32 such variants that have previously been identified in the literature.^{4-6, 24-31} The results for change in ACT score are presented in Supplementary Table E1,

and those for time-to-severe asthma exacerbation are presented in Supplementary Table E2. While none of the variants were significantly associated with a change in ACT score (Table E1), 12 (38%) of the 32 variants had a joint test $P < 0.05$ for the association with time-to-severe exacerbation (Table E2).

Expression Quantitative Trait Locus (eQTL) Analysis for rs3827907

To investigate possible functionality, we evaluated whether rs3827907 was an eQTL for genes located within 1Mb upstream and downstream of the variant. RNA-seq data derived from whole blood (i.e., whole blood transcriptome) were available for 813 African American SAPPHERE participants (408 with asthma and 405 without asthma). Expression of *EDDM3B* in whole blood was low and did not meet our criterion for evaluation ($\geq 50\%$ of individuals with ≥ 1 read count). Therefore, if rs3827907 had a substantial regulatory effect on expression in whole blood, it was likely for genes other than *EDDM3B*. The correlation in expression between the 32 genes analyzed is shown in Figure E6 of the online supplement. In the combined analysis of all 813 individuals (Table 5), rs3827907 was significantly associated with both *RNASE2* expression ($P = 6.10 \times 10^{-4}$) and *RNASE1* expression ($P = 7.92 \times 10^{-4}$) using a conservative Bonferroni threshold ($P < 0.05/32 = 1.56 \times 10^{-3}$). The association with *RNASE3* was of borderline significance ($P = 0.008$). In addition to being the most significant association in the combined sample, the parameter estimates (PE) and ranks for the eQTL association between rs3827907 and *RNASE2* expression were consistent among individuals with and without asthma (PE = -0.243, rank 3 and PE = -0.238, rank 2, respectively). The consistently negative estimates across all groups suggested that the main effect of the rs3827907 C-allele was to decrease expression of *RNASE2*.

Pre-treatment Serum Eosinophil-Derived Neurotoxin (EDN) Levels and Blood Eosinophil Counts on ICS Treatment Response

Two of the 3 genes whose expression was most strongly associated with rs3827907 genotype (i.e., *RNASE2* and *RNASE3*) in the eQTL analysis encode for proteins found in eosinophil cytoplasmic granules – namely, EDN and Eosinophil Cationic Protein, respectively. This suggested that the effect of rs3827907 on ICS treatment response may be most apparent in the setting of asthma with ongoing eosinophilic inflammation. Using banked serum from SAPPHIRE participants in the discovery set, we measured EDN levels in serum collected prior to treatment; these data were available for 243 African American individuals. We also evaluated blood eosinophil counts derived from blood cell differentials obtained immediately prior to initiating treatment. As can be seen in Figures 3 and 4, the difference in response to increasing ICS use was statistically significant and prominent in rs3827907 C-allele carriers with high (i.e., greater than the median) baseline serum levels of EDN (>2.34 ng/mL) and blood eosinophil counts (>222 cells/ μ L). The regression models which support these analyses are shown in Table E3. Replication of these findings in the 98 European American SAPPHIRE participants who also underwent ICS treatment is shown in Figure E7 (EDN stratification) and Figure E8 (blood eosinophil count stratification). While the not statistically significant, the same patterns were observed among the European American participants when compared with the African American participants, particularly for the analyses stratifying by blood eosinophil counts (Figure E8).

Assessment of Imputed Genotypes

To address the possibility that rs3827907 was not the causal variant, we imputed missing genotypes among African Americans in the SAPPHIRE cohort using the cosmopolitan panel

from the 1KGP as reference. The locus zoom plot for the joint association with ICS response including imputed genotypes is shown in Figure E9. Only one other SNP in the region, rs12891518, had a joint association P-value $<10^{-5}$ (joint test P-value without adjustment for genomic control = 3.22×10^{-7}). Evaluation of this variant in 597 African American individuals from SAPHIRE who had both RNA-seq data and genotype information found that it was less significantly associated with *RNASE2* expression when compared with rs3827907 (Table E4). Lastly, rs12891518 was not identified as a *cis*-eQTL for *any* gene in publicly available expression data sets using peripheral blood obtained from 5,311 European individuals or in any tissue included in the Genotype-Tissue Expression (GTEx) project (data not shown).^{32, 33} Taken together, these results support rs3827907 as the causal variant for ICS response in this region.

DISCUSSION

Clinical trials and epidemiologic studies have consistently found ICS therapy to be the single most effective treatment for improving asthma control and mitigating severe complications, such as life-threatening exacerbations.^{2, 34} Here we identify a novel pharmacogenomic variant, rs3827907, which appears to mediate the relationship between ICS treatment and improved asthma control among African American individuals. This variant located in the 3' UTR of *EDDM3B* on chromosome 14 also appeared to influence the relationship between ICS use and severe exacerbations among both African American and Latino individuals. It is unlikely that rs3827907 influenced *EDDM3B* expression in blood, as we found very few transcripts in our whole blood RNA-seq. We also found almost no expression of *EDDM3B* in lung (i.e., the other relevant tissue for asthma) from the GTEx project – data not shown.³³

In contrast, the eQTL analysis of rs3827907 in 813 African American SAPHIRE participants with and without asthma suggested that the C-allele was associated with lower expression of *RNASE2* and possibly *RNASE3*. The respective protein products of these genes, EDN and ECP, are major constituents of eosinophil cytoplasmic granules and are released systemically upon cell activation.³⁵ Hence, these proteins have been considered potential biomarkers of eosinophilic inflammation.³⁶⁻³⁸ Of clinical relevance, we demonstrated that in the setting of eosinophilic inflammation (as evinced by a pre-treatment serum EDN level >2.34 ng/mL or a blood eosinophil count >222 cells/ μ L), the rs3827907 C-allele distinguished African American individuals with the greatest ICS-related improvement in asthma control. Conversely, African American individuals who were homozygous for the rs3827907 T-allele did not demonstrate a clear dose-response relationship between ICS therapy and asthma control in either the presence or absence of pre-treatment eosinophilic inflammation. In short, our findings suggest that relatively easily measured biomarkers in blood (i.e., rs3827907 genotype along with blood eosinophil counts or serum EDN levels) may help identify the degree to which African American individuals will respond to ICS therapy.

Because gene expression was assessed at one time-point, we cannot comment on how rs3827907 genotype and ICS use interact to influence *RNASE2* expression over time. Similarly, without additional mechanistic studies, we cannot conclude that *RNASE2* or *RNASE3* (or their protein products EDN and ECP, respectively) were the mechanism through which the rs3827907 variant influenced ICS-associated improvements in asthma control. However, these limitations do not diminish the potential import of these biomarkers for predicting ICS response.

The association between eosinophilia and asthma severity is well described,³⁹ and it may also identify an asthma phenotype (i.e., eosinophilic asthma vs. non-eosinophilic asthma) that is

more responsive to inhaled corticosteroids.^{40, 41} Similarly, proteins released as a result of eosinophil degranulation may also be useful indicators of ongoing eosinophilic inflammation. For example, Kim *et al.* showed that EDN blood levels were associated with asthma severity in children,⁴² and Gon *et al.* found that EDN levels fell as lung function improved among adults treated with 8 weeks of omalizumab.³⁶ However, it is not clear that these protein biomarkers outperform simple counts of eosinophils in blood or sputum. Meijer *et al.* showed that ECP levels did not improve upon the use of eosinophil counts to predict corticosteroid-associated improvements in lung function and patient-reported quality of life.⁴³ One of the unique and important findings of our analysis is that we found that markers of pre-treatment eosinophilic inflammation (i.e., either EDN levels or blood eosinophil counts) were only useful in categorizing ICS responsive groups when combined with an additional genetic susceptibility marker. While our findings should be considered preliminary, they do provide a model for refining existing clinical tests by overlaying additional genetic (or “-omic”) data.

Another important feature of our study is its primary focus on U.S. minority populations, particularly African American individuals, who in general disproportionately suffer from asthma complications yet are underrepresented in genetic studies.^{44, 45} Our ability to replicate the rs3827907 association among a cohort of Latinos with asthma suggests that this variant may be influential in other populations. However, we were unable to replicate our findings in European American individuals, which may have been due in part to the much smaller sample size of this group. Additional testing in these population groups will be necessary to confirm our findings. Westra *et al.* also showed that the rs3827907 variant was an eQTL for *RNASE2*; however, in contrast to our findings, this study of the blood transcriptome in Europeans found that the rs3827907 C-allele was associated with increased *RNASE2* expression.³² It is not uncommon to

find that risk alleles, as well as their magnitude and direction of association, vary between population groups.^{9, 46}

As an ancillary analysis, we analyzed variants previously associated with ICS response in predominantly European American individuals and found that approximately 40% were associated with treatment response in African Americans with asthma (Table E2). Because the studies identifying these variants had different designs and outcomes, it is not possible to confidently comment on whether the direction of effect was consistent. Nevertheless, we believe that there are still important take away points from this analysis. First, our ability to verify previously identify ICS response variants suggests that our method was suitable for validating our discovery findings. Second, at minimum it appears that a substantial proportion of variants identified in individuals of European descent are relevant in African Americans.

There are additional study limitations to consider beyond those already mentioned. First, we did not have a replication set of African American individuals who underwent 6 weeks of observed ICS treatment analogous to the discovery set. This is due in part to the general lack of treatment trial data for minority populations. Fortunately, we were able to reassess potential pharmacogenomic interactions with respect to severe asthma exacerbations in two separate sets of African American individuals with asthma (SAPPHIRE and SAGE II) and one cohort of Latinos with asthma (GALA II). Despite finding a consistent and significant signal in our validation cohorts, characteristic differences between the discovery and validation groups (e.g., baseline differences in lung function) may have also affected the magnitude and direction of the effect estimates observed. Our primary outcome for the discovery set was a change in asthma control, as measured using the ACT, not a change in lung function, as has been used elsewhere.^{4,}

⁵ However, both the ACT and FEV₁ have been shown to be predictive of asthma

exacerbations,^{47, 48} and at least one study has suggested that the ACT is a better predictor of exacerbations.⁴⁹ We have also recently demonstrated a “dose-response” relationship between ICS use and change in ACT score,⁸ supporting its use as measure of controller response. For these reasons, we feel that change in ACT score and asthma exacerbations were reasonable phenotypes to use in the discovery and validation of pharmacogenomic variants. While we had a similar replication phenotype for European American SAPHIRE participants when compared to the discovery group, the sample size of the former was small. As a result, we could not discern whether the lack of statistical significance for rs3827907 in European Americans (despite similar patterns of association) was due to lack of power or population-specific allelic effects. This sample was also too small to be used for discovery. Lastly, we only had gene expression information from whole blood in SAPHIRE participants. It is possible that variant rs3827907 may have different associations with gene expression in other tissues and cell types. However, the eQTL association with *RNASE2*, a nearby gene which is highly expressed in eosinophils, suggests that our use of whole blood RNA-seq was both appropriate and fortuitous.

In summary, we identified a novel genetic variant, rs3827907, associated with ICS treatment response. The variant appeared to augment knowledge of pre-treatment eosinophilic inflammation in distinguishing individuals with the greatest ICS response. Future studies are needed to reassess our findings in multiple population groups, to establish the utility of this variant for predicting treatment response in additional independent samples, and to further dissect the mechanism of this pharmacogenetic interaction.

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FIGURE LEGENDS

Figure 1. Manhattan plot of the p-values from the genome wide association analysis of inhaled corticosteroid (ICS) response in the discovery set (n=244). Each p-value represents the statistical significance of jointly testing the contribution of both the SNP and SNP x ICS interaction terms. The Bonferroni-adjusted significance level ($p=8.72 \times 10^{-8}$) is shown as a solid gray line, and a p-value threshold of 10^{-5} is shown as a dashed gray line.

Figure 2. 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). Results are stratified by levels of patient ICS use ($\leq 75\%$ adherence was considered low use and $>75\%$ adherence was considered high use). Black lines connect the means for each group.

Figure 3. Effect of baseline measures of eosinophil-derived neurotoxin (EDN) on relationship between rs3827907 C-allele carrier status 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=243). Results are stratified by levels of patient ICS use ($\leq 75\%$ adherence was considered low use and $>75\%$ adherence was considered high use) and baseline serum levels of EDN. Low and high EDN levels were defined as those ≤ 2.34 ng/mL and >2.34 ng/mL (the median level for the group), respectively. Black lines connect the means for each group.

Figure 4. 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 African American participants in the SAPPHIRE cohort following 6 weeks of observed inhaled corticosteroid (ICS) treatment (n=113). Results are stratified by levels of patient ICS use ($\leq 75\%$ adherence was considered low use and $>75\%$ adherence was considered high use) and blood eosinophil counts measured before initiating ICS treatment. Low and high eosinophil counts were defined as those ≤ 222 cells/ μL and >222 cells/ μL (the median level for the group), respectively. Black lines connect the means for each group.

Table 1. Characteristics of African American individuals with asthma in both the discovery and validation sets

	Discovery Set	Validation Sets			
	SAPPHIRE (n=244)	SAPPHIRE - African Americans (n=803)	GALA II (n=1,461)	SAGE II (n=563)	SAPPHIRE – European Americans (n=98)
Age in years – mean ± SD	32.2 ± 13.0	31.9 ± 15.1	12.2 ± 3.0	13.5 ± 3.4	36 ± 14.9
Female – no. (%)	146 (59.8)	509 (63.4)	623 (42.6)	262 (46.5)	53 (54.1)
Race-ethnicity – no. (%)					
African American	244 (100.0)	803 (100.0)	--	563 (100.0)	--
European American	--	--	--	--	98 (100.0)
Latino	--	--	1,461 (100.0)	--	--
Genetic ancestry percentage – mean ± SD*					
African	79.8 ± 10.4	79.4 ± 10.4	15.6 ± 13.8	79.0 ± 12.7	NA
European	20.2 ± 10.4	20.6 ± 10.4	53.7 ± 19.4	21.0 ± 12.7	NA
Native American	--	--	30.7 ± 25.5	--	NA
Body mass index in kg/m ² – mean ± SD	32.4 ± 9.5	30.8 ± 8.8	--	--	29.6 ± 9.1
Body mass index percentile – mean ± SD†	--	--	73.3 ± 29.8	77.1 ± 24.3	--
Smoking status – no. (%)‡					
Never smoker	217 (88.9)	658 (81.9)	1,432 (98.0)	535 (95.0)	91 (92.9)
Past smoker	24 (9.9)	68 (8.5)	29 (2.0)	28 (5.0)	7 (7.1)
Current smoker	3 (1.2)	77 (9.6)	--	--	0 (0.0)
Secondhand smoke exposure – no. (%)§	76 (31.1)	219 (27.3)	306 (20.9)	163 (29.0)	18 (18.4)
Initial ACT score – mean ± SD	18.0 ± 5.2	20.6 ± 3.9	NA	NA	20.2 ± 4.5
Percent of predicted FEV ₁ in liters – mean ± SD	73.3 ± 12.9	92.5 ± 17.7	90.8 ± 16.3	99.2 ± 15.0	72.0 ± 13.6

SAPPHIRE denotes the Study of Asthma Phenotypes and Pharmacogenomic Interactions by Race-ethnicity; GALA II, the Genes-environments and Admixture in Latino Americans study; SAGE II, the Study of African Americans, Asthma, Genes, and Environments; ICS, inhaled corticosteroid; SD, standard deviation; NA, not available; kg/m², weight in kilograms per height in meters squared; ACT, asthma control test; and FEV₁, forced expiratory volume in one second.

*The total proportion of continental group ancestry (i.e., global ancestry) was estimated using genome-wide genotype data and the methods described in the online supplement. Genome-wide genotype data was not available for European American individuals in the SAPPHIRE cohort.

†Body mass index percentiles were based on growth charts

(<http://www.cdc.gov/nccdphp/dnpao/growthcharts/resources/sas.htm>) and represented where each individual ranked in comparison to the age- and sex-matched population distribution.

‡Current smoking was an exclusion criterion for enrollment into GALA II and SAGE II.

§Based on participant-reported exposure to secondhand tobacco smoke at home.

||Asthma Control Test scores range from 5-25 with higher values representing better control. An ACT score ≤19 represents poor control, whereas scores >19 are considered good asthma control.

Table 2. Interactions between genetic variants and inhaled corticosteroid use on changes in asthma control among SAPPHIRE individuals in the discovery set (n=244)*

SNP	Chr	Position	Allele†	MAF†	HWE P-value‡	Gene	Parameter estimate for ICS§	P-value for ICS§	Parameter estimate for SNP§	P-value for SNP term§	Parameter estimate for SNP x ICS interaction§	P-value for interactio n term§	P-value for joint test	Adjusted P-value for joint test¶
rs3827907	14	21238798	C/T	0.13	0.59	<i>EDDM3B</i>	-0.51	0.641	-9.67	3.06x10 ⁻⁹	12.35	5.98x10 ⁻⁹	2.04x10 ⁻⁸	7.79x10 ⁻⁸
rs2629529	10	126534415	C/A	0.06	1.00	--	3.43	0.001	2.07	0.357	-7.74	7.40x10 ⁻³	5.25x10 ⁻⁸	1.86x10 ⁻⁷
rs73906251	20	29991274	T/C	0.05	0.48	--	1.89	0.065	-14.51	1.13x10 ⁻⁵	14.60	2.77x10 ⁻⁴	3.85x10 ⁻⁷	1.16x10 ⁻⁶

SAPPHIRE, denotes the Study of Asthma Phenotypes and Pharmacogenomic Interactions by Race-ethnicity; SNP, single nucleotide polymorphism; Chr, chromosome; MAF, minor allele frequency; HWE, Hardy-Weinberg equilibrium; and ICS, inhaled corticosteroid. *The phenotype was the change in Asthma Control Test (ACT) score from before to immediately following 6 weeks of inhaled corticosteroid (ICS) treatment.

†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.

‡P-values are from the exact test assessing deviation in the discovery group from the expected the Hardy-Weinberg distribution. Low P-values signify more significant deviation.

§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 ICS represents the 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]). 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 parameter estimates and P-values for the ICS, 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 (i.e., time of study enrollment), 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.

¶Adjusted using genomic control for an inflation factor of $\lambda=1.09$.

Table 3. Prospective relationship between genetic variants and inhaled corticosteroid use on asthma exacerbations within the SAPPHIRE validation population (n=803)*

SNP	Chr	Position	Allele†	MAF†	Gene	Parameter estimate for ICS‡	P-value for ICS‡	Parameter estimate for SNP‡	P-value for SNP‡	Parameter estimate for SNP x ICS interaction‡	P-value for interaction‡	P-value for joint test§
rs3827907	14	21238798	C/T	0.13	<i>EDDM3B</i>	-1.75	7.13×10^{-5}	0.35	0.007	-0.07	0.679	0.023
rs2629529	10	126534415	C/A	0.06	--	-1.75	2.06×10^{-5}	0.39	0.042	-1.27	1.58×10^{-4}	5.65×10^{-6}
rs73906251	20	29991274	T/C	0.05	--	-1.92	5.06×10^{-6}	0.22	0.173	0.28	0.182	0.043

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

*The validation analyses used Cox proportional hazards models to assess the time-to-severe asthma exacerbation (i.e., burst oral corticosteroid use, asthma-related emergency department visits, and hospitalizations for asthma).

†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 baseline assessment (i.e., the assessment at study enrollment). A *negative* parameter estimate indicates that the variable was associated with a lower risk of experiencing a severe asthma exacerbation. The parameter estimate for ICS can be interpreted as the effect of increasing ICS use (i.e., average number of ICS doses taken per day over the preceding 6 months).^{15, 18} 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 and the number of “effect” alleles on the risk of a severe asthma exacerbation. The parameter estimates and P-values for the ICS, SNP, and SNP x ICS interaction variables are shown for the model simultaneously including 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, and the first 3 principal components.

§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 4. Validation of the relationship between genetic variants and inhaled corticosteroid use on asthma exacerbations within Latinos (n=1,461) from GALA II and African Americans (n=563) from SAGE II*

Study	SNP	Chr	Position	Allele†	MAF†	Gene	Parameter estimate for ICS‡	P-value for ICS‡	Parameter estimate for SNP‡	P-value for SNP‡	Parameter estimate for SNP x ICS interaction‡	P-value for interaction‡	P-value for joint test§
GALA II	rs3827907	14	21238798	C/T	0.21	<i>EDDM3B</i>	-0.16	0.521	0.21	0.194	0.15	0.503	0.029
	rs2629529	10	126534415	C/A	0.12	--	-0.12	0.634	0.17	0.405	0.08	0.766	0.281
	rs73906251	20	29991274	T/C	0.02	--	-0.12	0.611	-0.45	0.312	1.08	0.224	0.413
SAGE II	rs3827907	14	21238798	C/T	0.16	<i>EDDM3B</i>	-1.01	0.183	-1.04	0.021	0.96	0.053	0.041
	rs2629529	10	126534415	C/A	0.06	--	-0.93	0.204	0.15	0.794	-0.01	0.995	0.876
	rs73906251	20	29991274	T/C	0.08	--	-0.89	0.204	-0.69	0.219	-0.12	0.851	0.004

GALA II denotes the Genes-environments and Admixture in Latino Americans study; SAGE II, the Study of African Americans, Asthma, Genes, and Environments; SNP, single nucleotide polymorphism; Chr, chromosome; MAF, minor allele frequency; and ICS, inhaled corticosteroid.

*The validation analyses used logistic regression to model the *retrospective* relationship between severe asthma exacerbation in the year prior to study enrollment (i.e., burst oral corticosteroid use, asthma-related emergency department or unscheduled physician visits, and hospitalizations for asthma) and both SNPs and SNP x ICS interactions.

†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 among individuals with asthma in each study.

‡The parameter estimates represent the risk of having a severe asthma exacerbation in the year prior to baseline assessment (i.e., the assessment at study enrollment). A *negative* parameter estimate indicates that the variable was associated with a lower risk of experiencing a severe asthma exacerbation. The parameter estimate for ICS represents the effect of ICS, a dichotomous indicator variable for participant-reported ICS use at the time of study enrollment. 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 ICS use at enrollment and the number of “effect” alleles on the risk of a severe asthma exacerbation. In both GALA II and SAGE II, ICS use was represented as a single dichotomous variable based on patient reported use at the time of enrollment. The parameter estimates and P-values for the ICS, 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 percentile, secondhand smoke exposure, medication step at enrollment (a proxy for asthma severity based on Expert Panel Report-3 guidelines),³⁰ and the first 3 principal components.

§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.

713 **Table 5. Expression quantitative trait locus analysis for rs3827909 and its association with nearby gene expression on**
 714 **chromosome 14 among African American SAPHIRE participants with (n=408) and without asthma (n=405)***

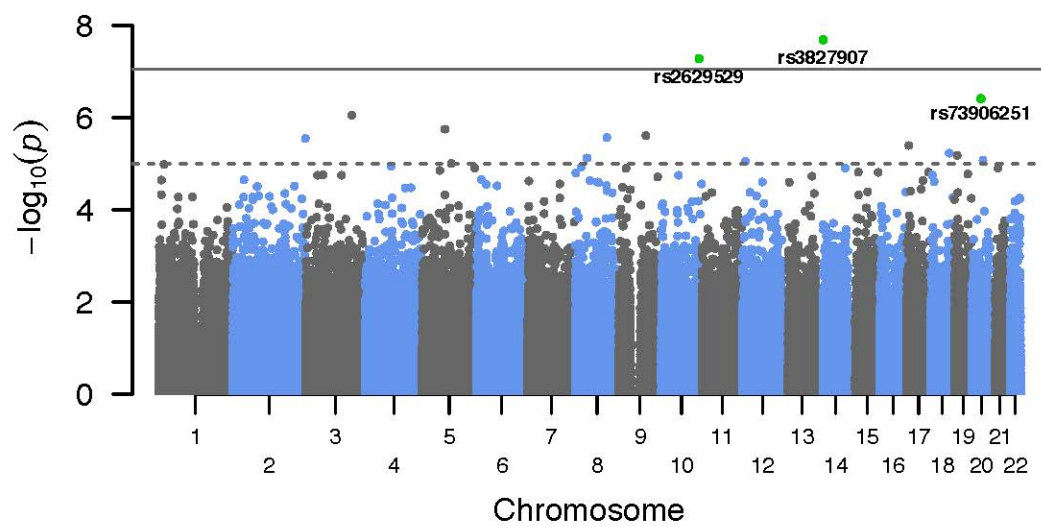
Gene	Start	End	Distance to rs3827907 (bp)†	Rank	Cases (n=408)		Rank	Controls (n=405)		Rank	Total (n=813)	
					Parameter estimate±	P-value		Parameter estimate±	P-value		Parameter estimate±	P-value
OR11H4	20242739	20243820	+526,819	27	-0.046	0.666	8	-0.088	0.339	18	-0.066	0.346
TTC5	20256558	20305994	+464,645	6	-0.195	0.058	24	-0.018	0.851	8	-0.109	0.121
CCNB1IP1	20311368	20333312	+437,327	25	-0.055	0.575	31	0.003	0.975	23	-0.027	0.703
PARP2	20343582	20357905	+412,734	4	0.211	0.025	4	0.134	0.202	5	0.173	0.014
TEP1	20365667	20413429	+357,210	10	-0.117	0.230	21	-0.028	0.779	17	-0.074	0.292
KLHL33	20428811	20435642	+334,997	24	0.054	0.571	12	-0.080	0.437	27	-0.011	0.874
OSGEP	20446411	20455105	+315,534	2	-0.246	0.015	5	-0.107	0.273	4	-0.179	0.110
APEX1	20455191	20457772	+312,867	28	-0.036	0.715	20	0.038	0.701	32	0.000	0.998
TMEM55B	20457719	20461612	+309,027	22	0.062	0.526	16	0.070	0.488	19	0.066	0.348
PNP	20468954	20477094	+293,545	20	-0.067	0.501	7	0.098	0.323	26	0.013	0.850
RNASE10	20505537	20511169	+259,470	9	0.142	0.178	15	0.065	0.485	9	0.104	0.138
RNASE4	20684100	20701215	+69,424	14	0.101	0.321	3	0.183	0.060	6	0.141	0.045
ANG	20684177	20698971	+71,668	21	-0.069	0.503	11	-0.087	0.360	14	-0.078	0.267
RP11-903H12.5	20684587	20700576	+70,063	7	-0.187	0.062	17	-0.062	0.531	7	-0.126	0.072
RNASE6	20781051	20782467	-10,412	13	-0.102	0.319	9	-0.091	0.346	10	-0.097	0.170
RNASE1	20801228	20803278	-30,589	5	-0.210	0.027	1	-0.262	0.012	2	-0.235	7.92x10 ⁻⁴
RNASE3	20891399	20892348	-120,760	1	-0.261	0.007	6	-0.109	0.288	3	-0.187	0.008
RNASE2	20955452	20956436	-184,813	3	-0.243	0.018	2	-0.238	0.013	1	-0.240	6.10x10 ⁻⁴
METTL17	20989770	20997035	-219,131	23	-0.059	0.533	10	-0.095	0.360	15	-0.077	0.275
NDRG2	21016763	21070872	-246,124	15	-0.090	0.355	18	-0.061	0.549	16	-0.076	0.280
ARHGEF40	21070270	21090240	-299,631	11	-0.114	0.250	14	-0.072	0.472	12	-0.094	0.184
ZNF219	21090046	21104722	-319,407	12	0.109	0.251	13	0.078	0.458	11	0.094	0.183
TMEM253	21098937	21103724	-328,298	29	-0.036	-0.725	23	0.021	0.830	28	-0.008	0.910
HNRNPC	21209136	21269494	-438,497	17	0.081	0.420	19	-0.044	0.655	24	0.020	0.771
RPGRIP1	21287939	21351301	-517,300	19	0.076	0.449	27	0.006	0.954	22	0.042	0.552
SUPT16H	21351472	21384266	-580,833	30	0.002	0.982	28	0.004	0.963	30	0.003	0.962
CHD8	21385194	21456126	-614,555	32	0.001	0.990	26	0.007	0.942	29	0.004	0.954
RAB2B	21459020	21476973	-688,381	16	0.085	0.368	30	0.005	0.965	21	0.046	0.514
TOX4	21476597	21499175	-705,958	18	0.076	0.441	22	0.028	0.783	20	0.052	0.457
METTL3	21498133	21511375	-727,494	8	0.154	0.116	29	0.005	0.963	13	0.081	0.248
SALL2	21521081	21537216	-750,442	31	0.002	0.986	32	-0.001	0.994	31	0.001	0.993
OR10G2	21633836	21634940	-863,197	26	-0.046	0.641	25	0.011	0.916	25	-0.019	0.792

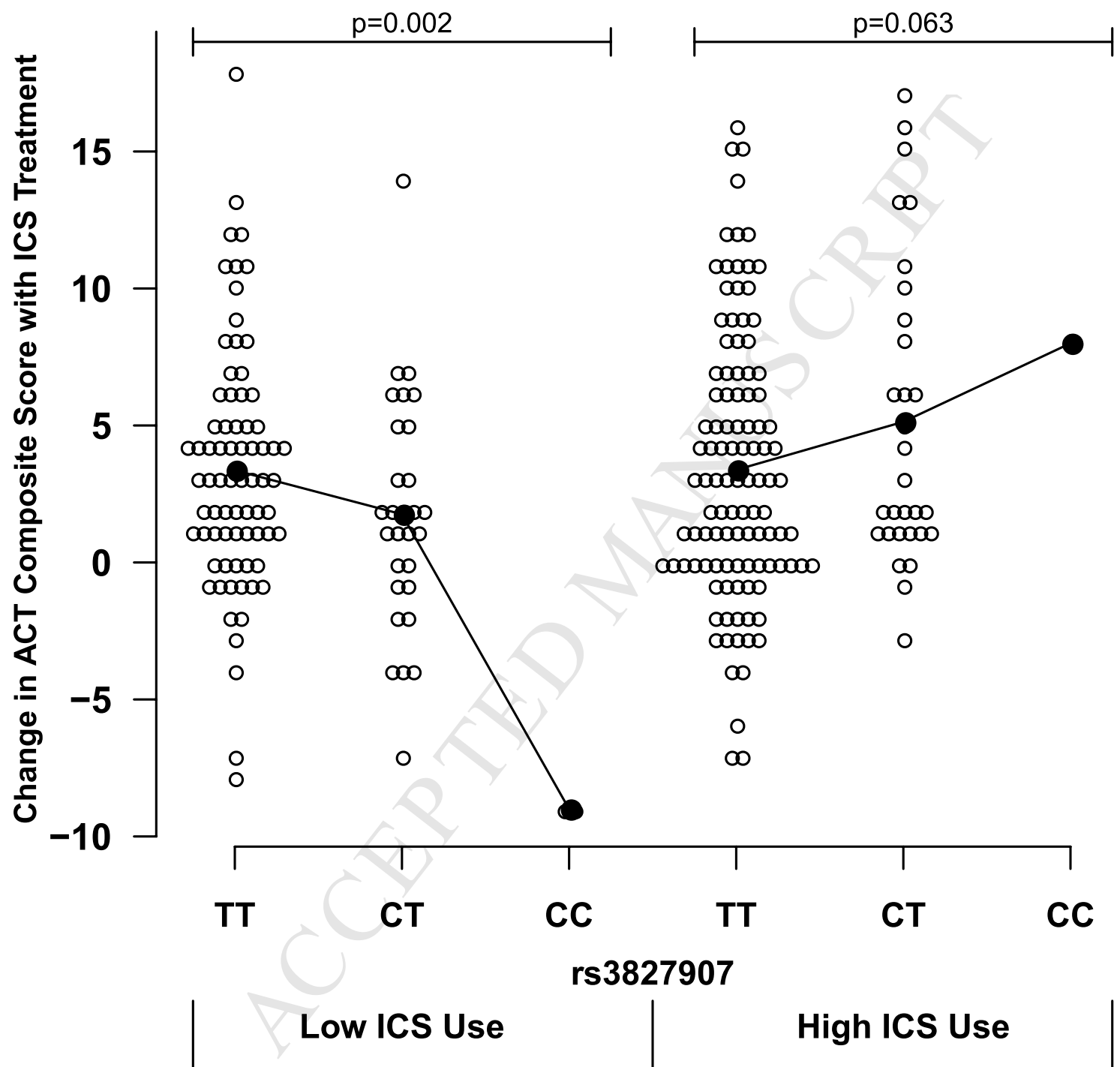
715 SAPHIRE denotes the Study of Asthma Phenotypes and Pharmacogenomic Interactions by Race-ethnicity, and bp, nucleotide base
 716 pair.

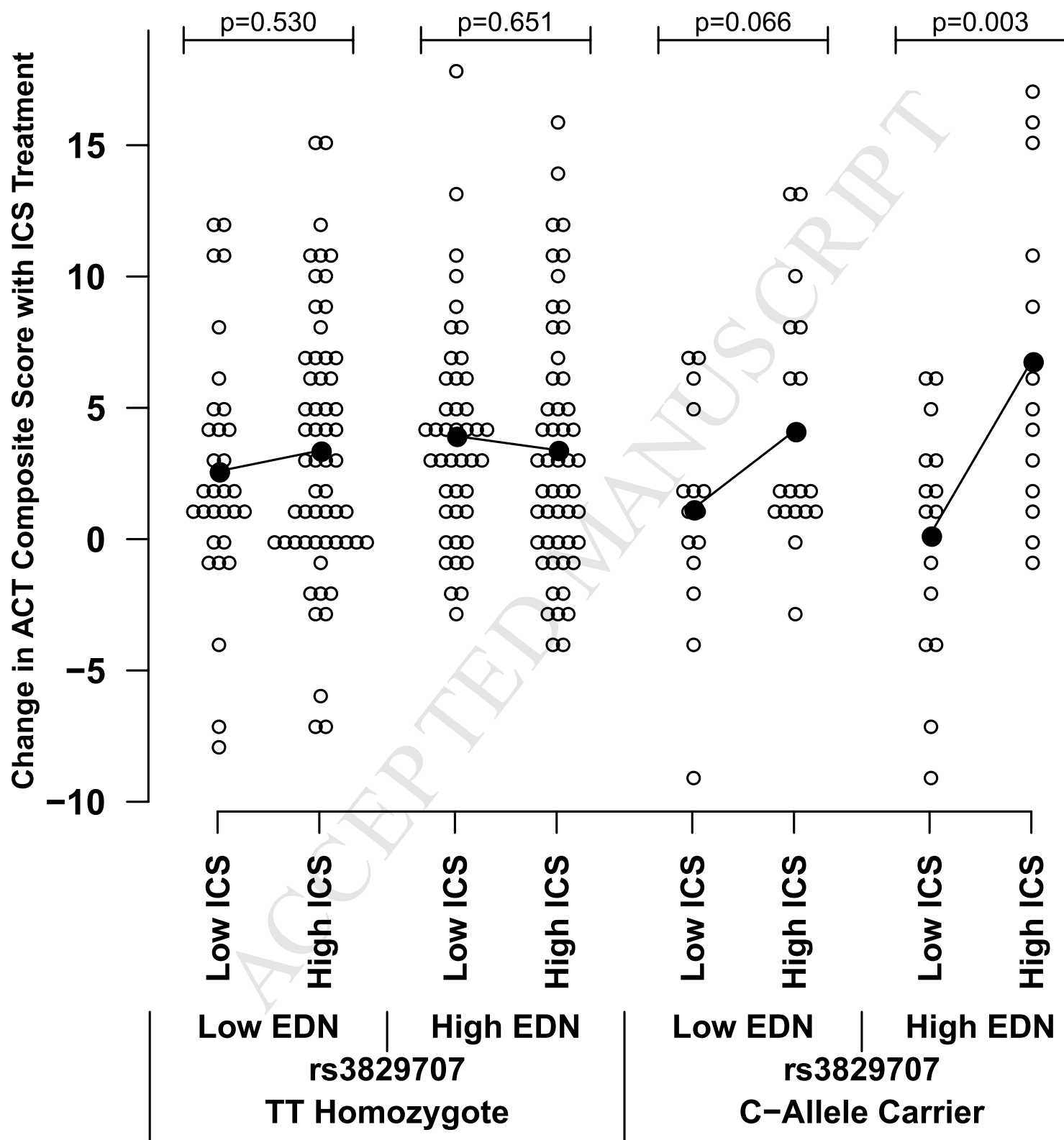
717 *Gene expression was measured using RNA-seq data derived from whole blood RNA. To assess whether rs3827907 was a cis-
 718 expression quantitative trait locus for nearby genes, we restricted the analysis to genes located within 1 mega-base upstream and
 719 downstream of the variant.

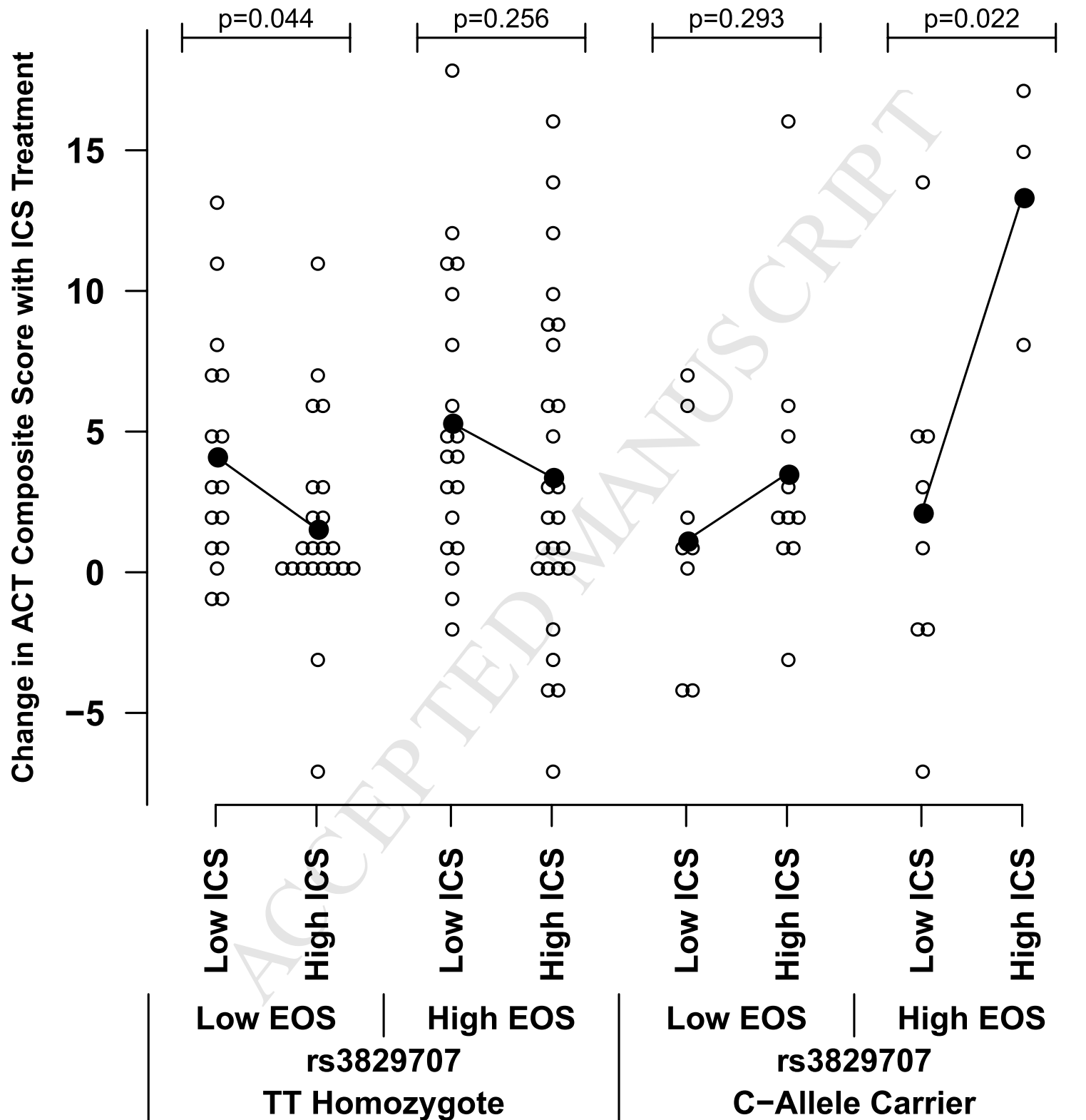
†Positive (+) distance indicates that the start codon for the gene is located 5' to rs3827907 on sense strand, whereas negative (-) distance indicates that the start codon for the gene is located 3' to the variant on the sense strand.

‡Linear regression was used to examine the relationship between rs3827907 genotype (assuming an additive genetic model where genotypes were coded as TT=0, CT=1, CC=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 from the model. A positive parameter estimate suggests that an increasing dose of the C-allele is associated with increased expression of a given gene, whereas a negative parameter estimate suggests that the C-allele is associated with decreased gene expression. Rank represents the P-value order for the association between rs3827907 genotype and gene expression; the most statistically significant P-value is ranked first.









ONLINE SUPPLEMENT

Integrative approach identifies corticosteroid response variant in diverse populations with asthma

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METHODS SUPPLEMENT

The Study of Asthma Phenotypes and Pharmacogenomic Interactions by Race-ethnicity (SAPPHIRE) cohort

All SAPPHIRE individuals were recruited from a large health system, which serves southeast Michigan and the Detroit metropolitan area. Individuals were eligible to participate as asthma cases if they were 12-56 years of age, had a previously documented physician diagnosis of asthma, and no prior diagnosis of congestive heart failure or chronic obstructive pulmonary disease. Control participants were recruited from the same patient population and met the same entry criteria as case patients, but they had no prior diagnosis of asthma.

At the time of enrollment, SAPPHIRE participants underwent a thorough evaluation which included lung function testing. Lung function testing was performed using a Fleisch-type pneumotachometer in accordance with 2005 ATS/ERS guideline recommendations.¹ Reversible airway obstruction was assessed by administering 360 μ g (4 puffs) of albuterol sulfate hydrofluoroalkane (HFA) from a standard metered dose inhaler (MDI). Lung function was measured before and 15 minutes after bronchodilator administration. A second dose of albuterol (360 μ g for individuals $\geq$ 18 years and 180 μ g for individuals $<$ 18 years) was administered to individuals who demonstrated $\leq$ 12% improvement in their forced expiratory volume at 1 second (FEV₁), and lung function was reassessed 15 minutes after the second dose. Individuals were considered to have reversible airway obstruction if their FEV₁ improved by $>$ 12% after bronchodilator administration.² By virtue of health plan enrollment, SAPPHIRE participants also had retrospective and prospective records of health care utilization (e.g., prescription medication fills and clinical visits).

A subset of the above study participants with asthma met criteria to be treated with a 6-week course of inhaled beclomethasone dipropionate hydrofluoroalkane (beclomethasone HFA) in order to assess inhaled corticosteroid (ICS) response. These additional eligibility criteria were as follows: no use of inhaled or systemic corticosteroids in the 4 weeks preceding the evaluation, a pre-bronchodilator FEV₁ that was 40-90% of predicted,³ reversible airway obstruction (i.e., $>$ 12% change in FEV₁ following bronchodilator 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 participate were instructed to use 320 μ g (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). Adherence to the 6-week ICS treatment regimen was estimated 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.^{4,5} ICS exposure was calculated as the number of actuations counted divided by the total prescribed amount (i.e., the number of prescribed puffs per day [4 puffs] multiplied by the actual number of days between evaluations). The discovery set for this analysis consisted of SAPPHIRE participants who completed the 6-week course of ICS treatment trial, had existing genome-wide genotype data, and were African American by self-report (n=244). A group of European American participants (n=98) also met the same criteria and completed 6 weeks of ICS treatment. This latter group did not undergo genome-wide genotyping.

Whole blood DNA isolated from African American SAPPHIRE participants was used for genome-wide genotyping. This genotyping was performed using the Axiom AFR array (Affymetrix Inc., Santa Clara, CA). To be included in the analysis, an individual's array results

had to have a dish quality control measure ≥ 0.82 , an overall call rate $\geq 97\%$, and no discordance between patient-reported sex and the measured X-heterozygosity. We removed SNPs from the analysis which had an overall SNP call rate $< 95\%$, minor allele frequency (MAF) $< 5\%$, and exact Hardy-Weinberg equilibrium p-value $< 10^{-4}$. Only autosomal SNPs were analyzed.

For European American individuals in SAPPHERE, we genotyped the rs3827907 SNP using a TaqMan SNP Genotyping Assay (Applied Biosystems, Foster City, CA). Genotyping was performed on a 7900HT Fast Real-Time PCR System (Applied Biosystems).

At the time of enrollment, some SAPPHERE participants also provided a blood sample for RNA analysis. Whole blood was collected into PAXgene Blood RNA tubes for RNA preservation. Samples were stored at -80°C prior to processing.

A subset of African American SAPPHERE participants with stored RNA was selected for RNA-sequencing (RNA-seq) – 408 asthma cases and 405 healthy controls. Whole blood RNA was isolated in an automated fashion using QuickGene-810 (FujiFilm Life Sciences and Autogen, Holliston, MA). RNA purity and concentration were assessed by spectrophotometer (NanoDrop, Thermo Fisher Scientific Inc.) and Bioanalyzer 2100 (Agilent Technologies Inc., Santa Clara, CA). RNA sequencing libraries were created using Illumina's TruSeq Stranded Total RNA Library Prep Kit with Ribo-Zero Globin. Fragment size distribution was assessed using the Bioanalyzer 2100. RNA-seq was performed on an Illumina HiSeq 2500 after an optimizing run on an Illumina MiSeq. The resulting FASTQ files were first evaluated using FastQC v0.11.4 (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc>), and the results of multiple samples were aggregated using MultiQC.⁶ BBDuk version 36.49 (<https://sourceforge.net/projects/bbmap/>) was used to process the raw FASTQ files; low quality regions and those containing adaptor sequences were trimmed. Ensembl Homo sapiens GRCh38.84 (build GRCh38.p5) was used as the reference genome, and RNA-seq reads were mapped to the reference genome using STAR.⁷ For post-alignment quality control, we used RSeQC v2.6.4 and deepTools.^{8,9} Reads for protein-encoding genes were quantified at the gene level using RSEM.¹⁰ Raw read counts were normalized using the variance stabilizing transformation procedure in DESeq2.¹¹ Only protein-encoding genes with one or more read counts in $\geq 50\%$ of individuals were included in the analysis. Since 216 of 813 the African American individuals with RNA-seq data did not have genome-wide genotype data, we genotyped those with missing rs3827907 SNP information using the previously described TaqMan SNP Genotyping Assay.

Validation Groups

Validation was carried out in 4 groups. These groups included a separate sets of African American and European American individuals from the SAPPHERE cohort, Latino individuals from GALA II, and African American participants from SAGE II.

The primary validation set consisted of a group of African American SAPPHERE participants (n=803) who met the following criteria: available prospective clinical data on asthma exacerbations, measures of ICS exposure through electronic claims records, existing genome-wide genotype data, and African American race-ethnicity by participant self-report. Longitudinal medical records were used to identify severe asthma exacerbations and generate measures of ICS exposure over time. We defined a severe asthma exacerbation as one requiring burst oral corticosteroids, an emergency department visit, or a hospitalization. This definition is consistent with consensus recommendations from the American Thoracic Society and the European Respiratory Society.¹²

As we have done previously,^{13, 14} we used pharmacy claims records to estimate ICS exposure over time among SAPPHERE participants in the validation set. We have shown that our procedure captures >99% of ICS prescription fills among health plan members.¹⁵ To create a standardized measure for all ICS preparations we multiplied the number of actuations per container by the milligram strength per actuation and divided by the milligram strength of the lowest recommended dose as specified in National Asthma Education and Prevention Program Expert Panel Report 3 (ERP-3) guidelines.¹⁶ We then divided the calculated measure for each fill by the total number of days between fills (i.e., days until the next fill), so as to estimate the amount of ICS used each day. To derive a summary measure for the amount of use for the preceding 180 days, we summed each daily value for that time period and divided by 180. The summary measure could thus 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 an individual’s follow-up to create a moving window of ICS exposure for each day that represented average ICS exposure over the preceding 180 days. 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 combination with the ICS over the preceding 180 days. As time-updated proxy measures of asthma severity, we created separate variables for short-acting beta-agonist (SABA) MDI and nebulizer use. These were similar to the time-updated measures of ICS use with a measure for each day of follow-up. However, the SABA measures represented the sum of doses filled (e.g., 2 MDI canisters of 100 doses per canisters = 200 doses) divided by 90 days (the exposure window). In other words, these measures represented the average number of SABA doses per day used in the preceding 3-months. We have shown that these SABA measures predict subsequent asthma exacerbations.¹⁷ 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.*¹⁸ Genome-wide genotype data was generated using the Axiom AFR array (Affymetrix Inc.) and processed as described for the discovery group.

Additional validation was performed in 2 cross-sectional, case-control asthma cohort studies – GALA II and SAGE II.^{19, 20} In both cohorts, analyses were restricted to asthma cases. Participants from the GALA II cohort were recruited from 5 centers in the U.S. (Chicago, Illinois; New York City, New York; Houston, Texas; San Francisco, California; and San Juan, Puerto Rico). Subjects were aged 8 to 21 years and had four Latino grandparents. Cases had a physician diagnosis of asthma. Individuals were excluded from participation if they reported any of the following: ≥ 10 pack-years of smoking; any smoking within 1 year of the recruitment date; pregnant and in the third trimester; or a history of any one of the following: sickle cell disease, cystic fibrosis, sarcoidosis, cerebral palsy, or heart or chest surgery. Exacerbations were defined as the patient-reported occurrence of at least one of the following asthma-related events in the 12-month period preceding study enrollment: emergency care, hospitalization, or oral corticosteroids use. The SAGE II cohort recruited participants from the San Francisco Bay Area, California. Participants were aged 8-21 years at the time of enrollment and reported that all four grandparents were African American. The exclusion criteria and the definitions of asthma and exacerbations were the same as for GALA II. Treatment step was used as a proxy for disease severity in both cohorts.¹⁶ Both GALA II and SAGE II subjects were genotyped using the Axiom LAT1 array (Affymetrix Inc.); the quality control procedures for the genotyping data have been described elsewhere.^{21, 22}

Lastly, we had a group of European American individuals from the SAPHIRE 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 SAPHIRE participants had the same treatment regimen and follow-up duration when compared to African Americans in the discovery set, we could assess the change in ACT composite score as the outcome variable. The rs3827907 SNP was genotyped using a TaqMan SNP Genotyping Assay (Applied Biosystems).

Population Structure

Given the ancestral admixture in both the discovery and validation populations,²³⁻²⁵ we used the genome wide genotype data and the program EIGENSOFT to derive principal components (PCs) for each cohort (with the exception of European American SAPHIRE participants who did not have genome wide genotype data).^{26, 27} These PCs defined axes of variation in the genotype data, which, in turn, reflected the underlying structure attributable to ancestry.

In SAPHIRE, global ancestry (i.e., the overall proportion of continental group ancestry) was calculated by using the program RFMix to estimate local ancestry at each autosomal SNP using the Yoruba in Ibadan, Nigeria (YRI) and Utah Residents with Northern and Western European Ancestry (CEU) reference populations from the 1000 Genomes Project.²⁸ Genome-wide African ancestry was computed as the percentage of African alleles. Global ancestry in GALA II and SAGE II were estimated using the program ADMIXTURE.²⁹ Three ancestral population groups were assumed (i.e., African, European, and Native American) for Latino participants in GALA II, and 2 population groups (i.e., African and European) were assumed for African American participants in SAGE II. Reference populations included 71 Native American individuals genotyped in Dr. Esteban Burchard's lab using the Axiom LAT1 array,³⁰ and African and European individuals from HapMap phase II.³¹

Statistical Analysis

In the discovery analysis, we used an additive genetic model (i.e., individual genotypes coded 0, 1, and 2 "effect" alleles) 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 (PC1-3), 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 (heretofore referred to a *joint test*). Kraft *et al.* demonstrated that the joint test had the best combined characteristics for detecting a gene-environment interaction especially in the context of a marginal genetic main effect.³² We applied genomic control to provide conservative p-value estimates for the joint test results.

The primary validation analysis was performed in a separate set of African American SAPHIRE participants (i.e., those not involved in the 6-week ICS treatment trial). We carried forward SNP associations with $P < 5.0 \times 10^{-7}$ (strict Bonferroni correction $P < 0.05/574,370 = 8.71 \times 10^{-8}$) from the discovery set to assess for ICS x genotype interactions on

asthma exacerbations. In this analysis, ICS exposure was calculated for each day of follow-up, and this moving window of exposure reflected the level of ICS use over the preceding 6 months. A Cox proportional hazard model 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 included the following variables: 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 PCs, 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 model with all but the SNP term and the SNP x ICS interaction term.

For the validation analyses in SAGE II and GALA II, we used logistic regression to model the dichotomous outcome of exacerbation in the 12-months preceding enrollment. These models also adjusted for the following participant characteristics measured at the time of enrollment: age, sex, BMI percentiles, environmental tobacco smoke exposure, ICS use, and asthma treatment category.¹⁶ BMI percentiles were based on age- and sex-specific growth charts (<http://www.cdc.gov/nccdphp/dnpao/growthcharts/resources/sas.htm>) relating height to weight. A 2-degree of freedom likelihood ratio test was used to evaluate the significance for difference in fit for a model with all variables as compared with a model with the covariates but lacking both the SNP term and SNP x ICS interaction term.

To demonstrate the pharmacogenomic interactions identified in discovery, we plotted the actual change in ACT score among the discovery set stratified by both genotype and ICS adherence. We dichotomized ICS adherence into low ($\leq 75\%$ adherence) and high use ($> 75\%$ adherence) based on previous work by our group showing that this cutoff was useful in distinguishing those who would benefit from treatment.¹³ Since individuals in the discovery set received the same dose of beclomethasone HFA daily (i.e., 320 μ g per day), the adherence cutoff of 75% could be interpreted as taking on average $\leq 240\mu$ g daily versus $> 240\mu$ g daily. We performed the same plots after restricting the discovery set to individuals whose asthma was not controlled prior to treatment (i.e., a composite ACT score ≤ 19), and we also plotted the results for European American SAPHIRE participants who completed 6-weeks of ICS treatment. Analysis of variance (ANOVA) was used to assess the overall trend in ACT change across the 3 rs3827907 genotype groups (i.e., TT, CT, and CC) within ICS adherence strata.

The dependent variable for the expression quantitative trait loci (eQTL) analyses was gene expression (i.e., mapped reads from the whole blood RNA-seq results in SAPHIRE participants). Since we were interested in nearby genes (i.e., “*cis*–” genes), whose expression was potentially regulated by rs3827907, we restricted our analysis to genes whose transcription start site (TSS) is located within 1 megabase (Mb) upstream and downstream of the genomic location of the rs3827907 SNP. This genomic distance is commonly used when defining *cis*-eQTL (as opposed to *trans*-eQTL which are more distantly located [> 5 Mb]).^{33, 34} We identified 32 genes whose expression in blood met our inclusion criteria (≥ 1 read count in $\geq 50\%$ of individuals) and whose TSS is within 1 Mb of rs3827907. We used linear regression to model gene expression as a function of rs3827907 genotype (i.e., using an additive genetic model coding TT=0, CT=1, and TT=2). Our models included variables for participant 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), and sequence batch. Lastly, we included 30 factors derived using the Probabilistic

Estimation of Expression Residuals (PEER) software package to adjust for additional hidden structure in the RNA-seq data.³⁵

To assess the potential effects of eosinophilic inflammation on ICS response among rs3827907 C-allele carriers and non-carriers, we separately stratified our discovery set by the median serum eosinophil-derived neurotoxin (EDN) level (i.e., ≤ 2.34 ng/mL and > 2.34 ng/mL). We then separately assessed the effect of low vs. high ICS exposure (i.e., $\leq 75\%$ adherence and $> 75\%$ adherence) on the change in ACT score among C-allele carriers and non-carriers. Mean differences in ACT score change between low and high ICS exposure were compared using a t-test. The above analysis was repeated using blood eosinophil counts as the indicator of eosinophilic inflammation. The discovery set was first stratified by the median blood eosinophil count prior to ICS treatment (i.e., ≤ 222 cells/ μ L and > 222 cells/ μ L). We again separately assessed the effect of low vs. high ICS exposure (i.e., $\leq 75\%$ adherence and $> 75\%$ adherence) on the change in ACT score among C-allele carriers and non-carriers using a t-test. Multivariable linear regression was used to model the relationship between the dichotomously defined rs3827907 C-allele carrier status (i.e., dominant model), ICS use (i.e., low vs. high adherence) and the interaction between these two variables on change in asthma control (i.e., change in the ACT composite score over 6 weeks of ICS treatment). These models adjusted for patient age, sex, BMI, smoking status, baseline ACT score, and first 3 PCs. We also stratified these analyses by C-allele carrier status, so that the variables used included ICS use, patient age, sex, BMI, smoking status, baseline ACT score, and first 3 PCs. Lastly, we repeated the plot analyses in the 98 European American SAPHIRE participants who underwent 6 weeks of ICS treatment. We stratified using the same cut-points that were used for the African American participants (i.e., an EDN level of 2.34 ng/mL and a blood eosinophil count of 222 cells/ μ L). Differences in the mean ACT score change between groups were again compared using t-tests.

Except where noted, study analyses were performed using the R statistical programming language.³⁶

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 ($\lambda=1.09$); 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 ($\leq 75\%$ 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 ($\leq 75\%$ 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 ($\leq 75\%$ 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 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 ($\geq 50\%$ 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 ($\leq 75\%$ 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 ($\leq 75\%$ 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/ μ L and > 222 cells/ μ L, 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 rs3827907, 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/HEAT R4 (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

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 SAPHIRE 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 SAPHIRE 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	5.38x10 ⁻⁴	0.097	0.851	4.405	1.52x10 ⁻⁵
High EDN‡	121	-3.838	9.87x10 ⁻⁵	-0.014	0.984	5.485	1.35x10 ⁻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	4.61x10 ⁻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	--	--

SAPHIRE 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 SAPHIRE participants from the discovery set with available data and who underwent 6 weeks of observed ICS treatment. Change in 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.

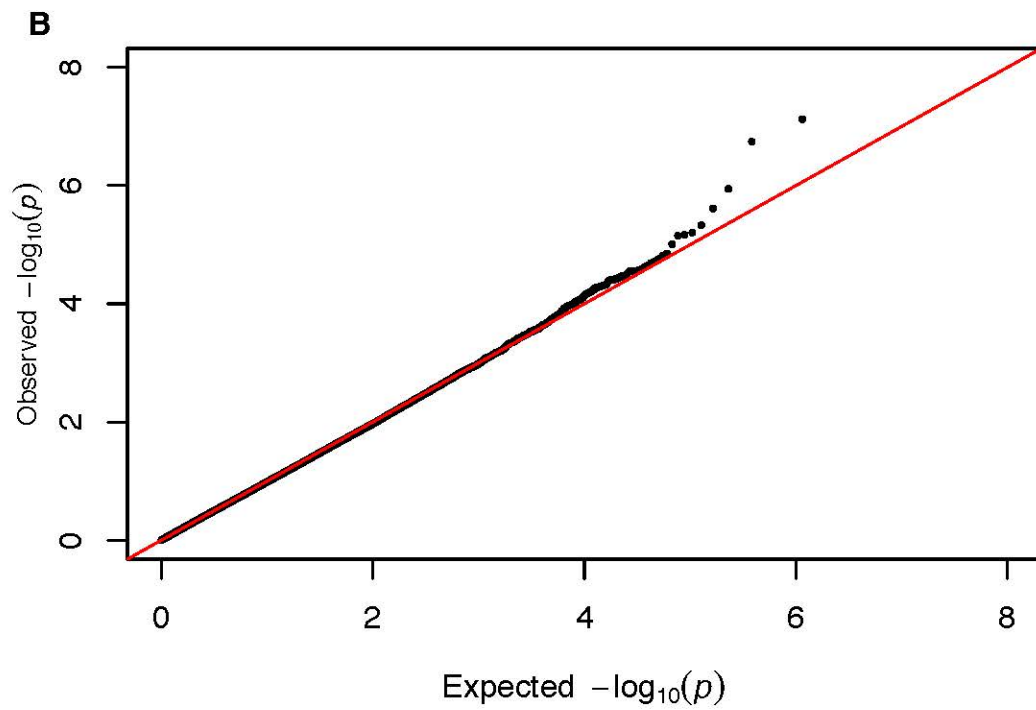
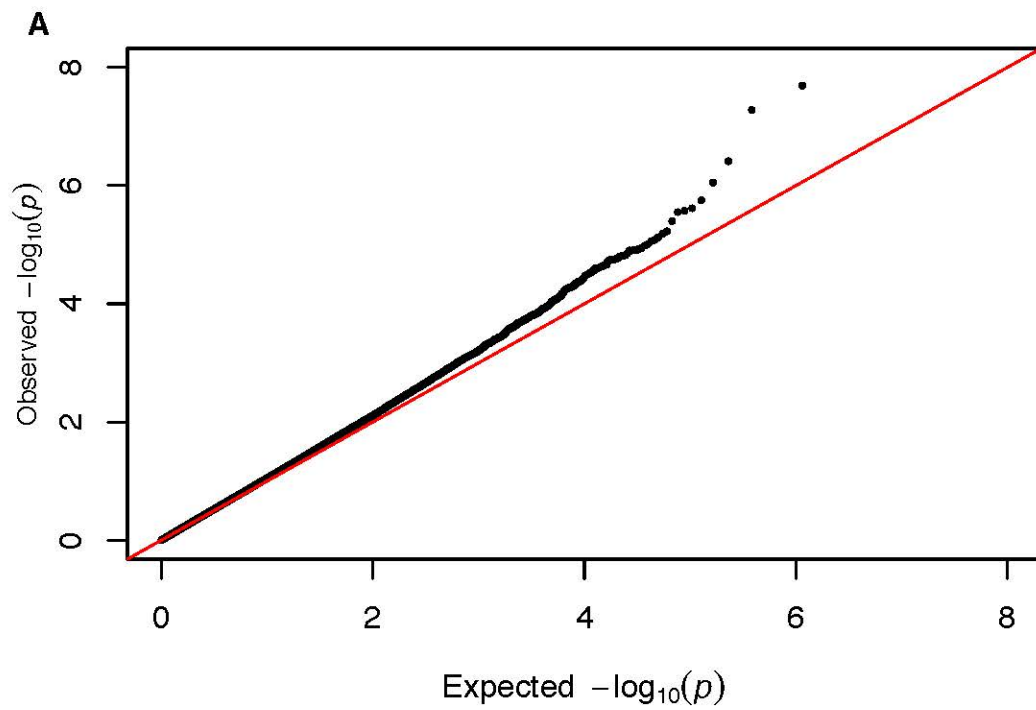
§The study population was also stratified based on the median blood eosinophil for the entire group with these data (n=133) prior to initiating ICS treatment. A baseline blood eosinophil count ≤ 222 cells/ μ L was considered low and a count > 222 cells/ μ L was considered high.

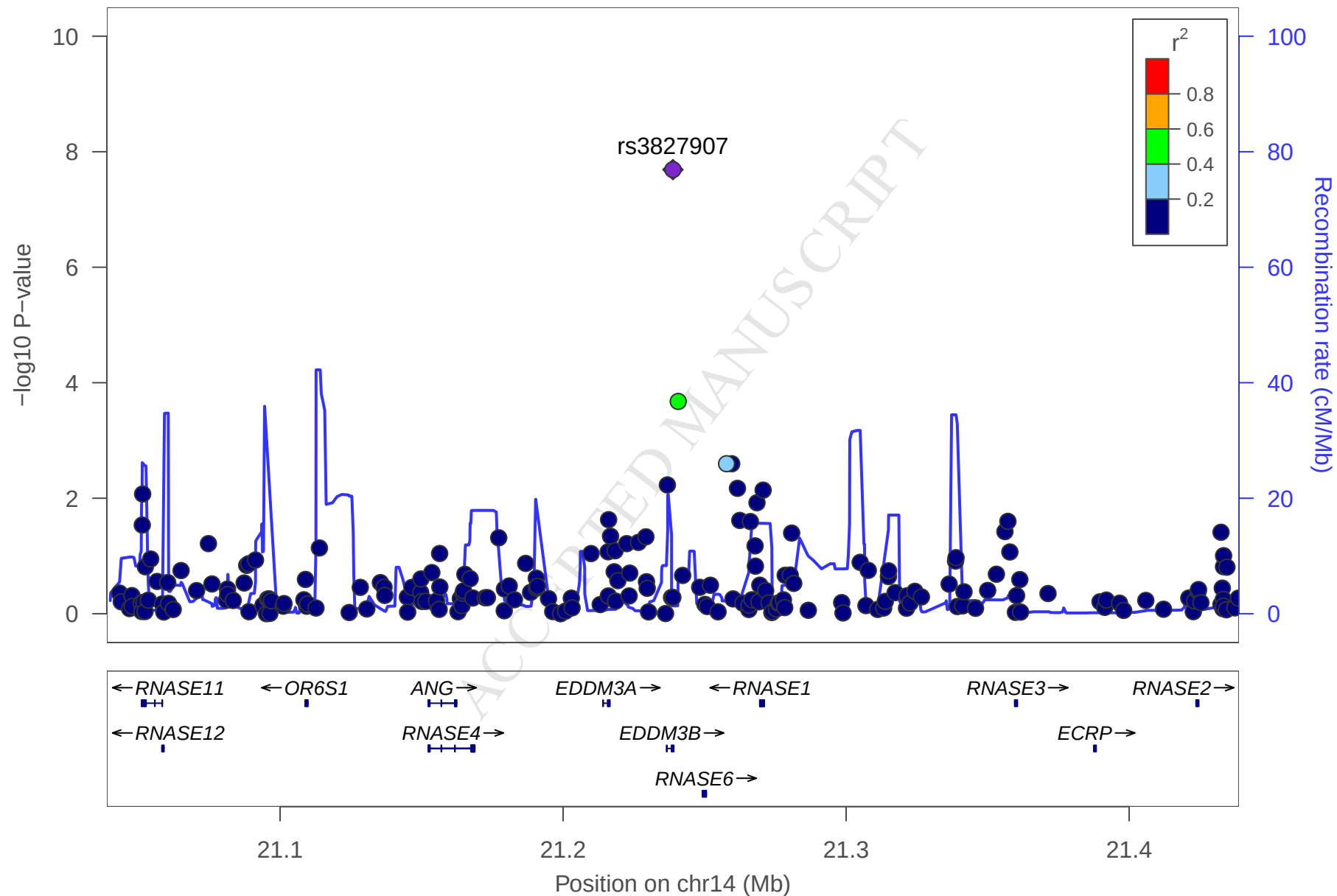
Table E4. Relationship between both rs3827907 and rs12891518 genotype and *RNASE2* gene expression in peripheral blood among African American participants with and without asthma from the SAPHIRE cohort.

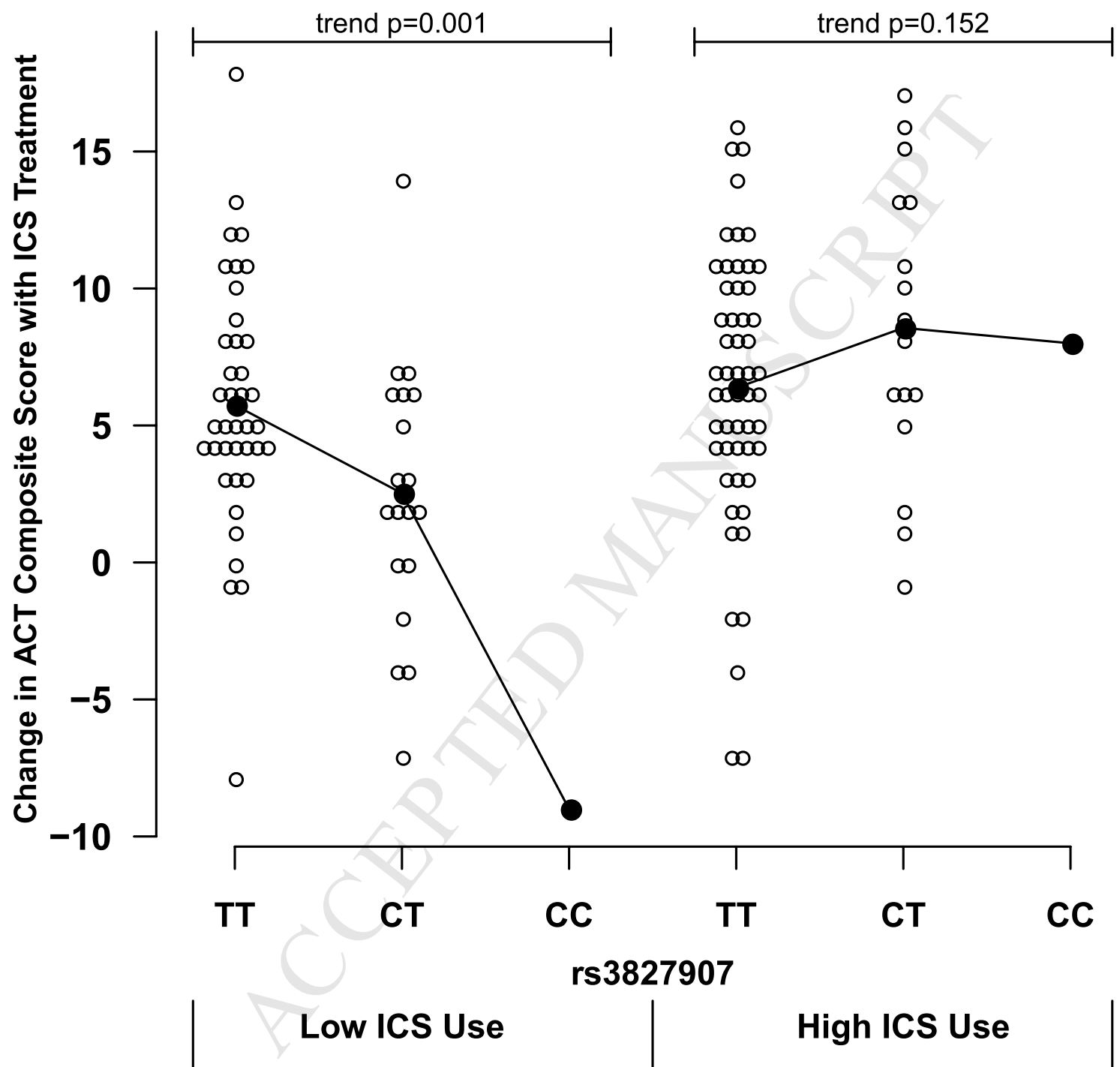
Groups	rs3827907 variant		rs12891518 variant	
	Parameter estimate*	P-value	Parameter estimate*	P-value
All participants (n=597)	-0.237	0.004	-0.235	0.008
Asthma cases (n=408)	-0.243	0.018	-0.220	0.047
Controls (n=189)	-0.224	0.103	-0.267	0.069

SAPHIRE 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.







p=0.022

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p=0.074

Change in ACT Composite Score with ICS Treatment

15

10

5

0

-5

-10

TT

C-

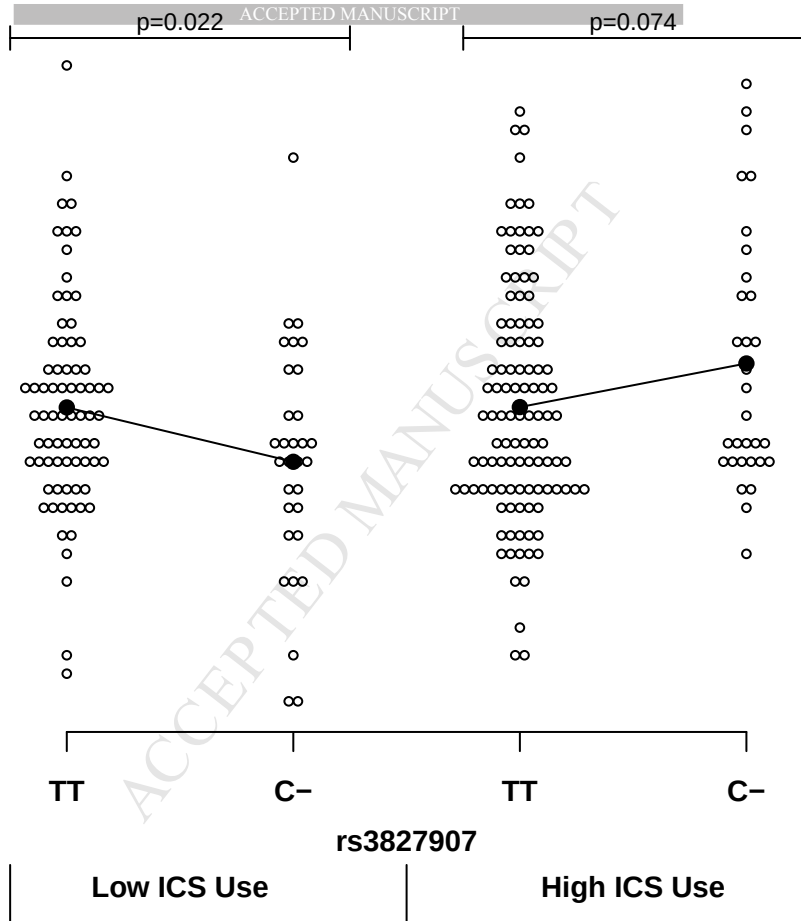
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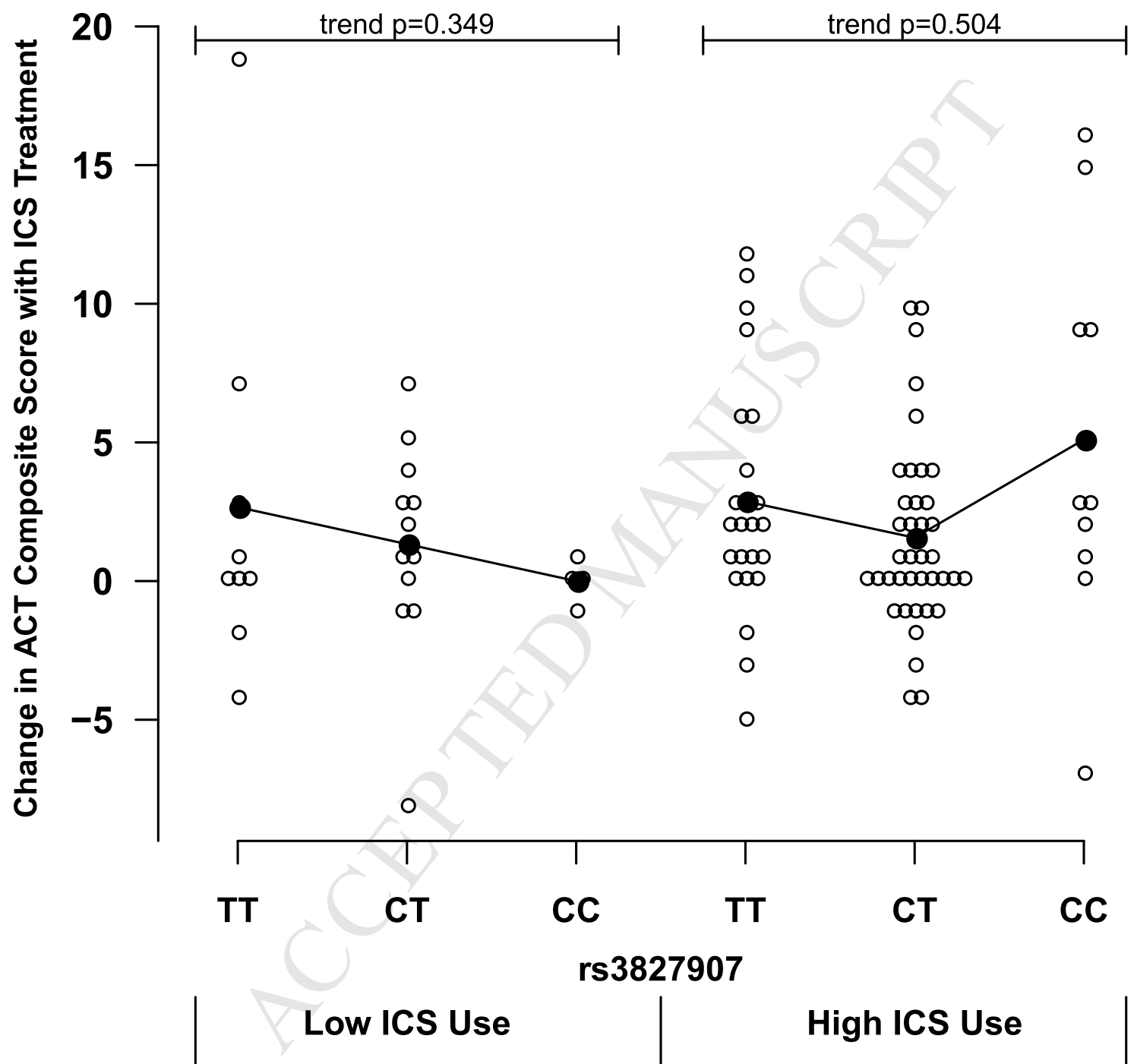
C-

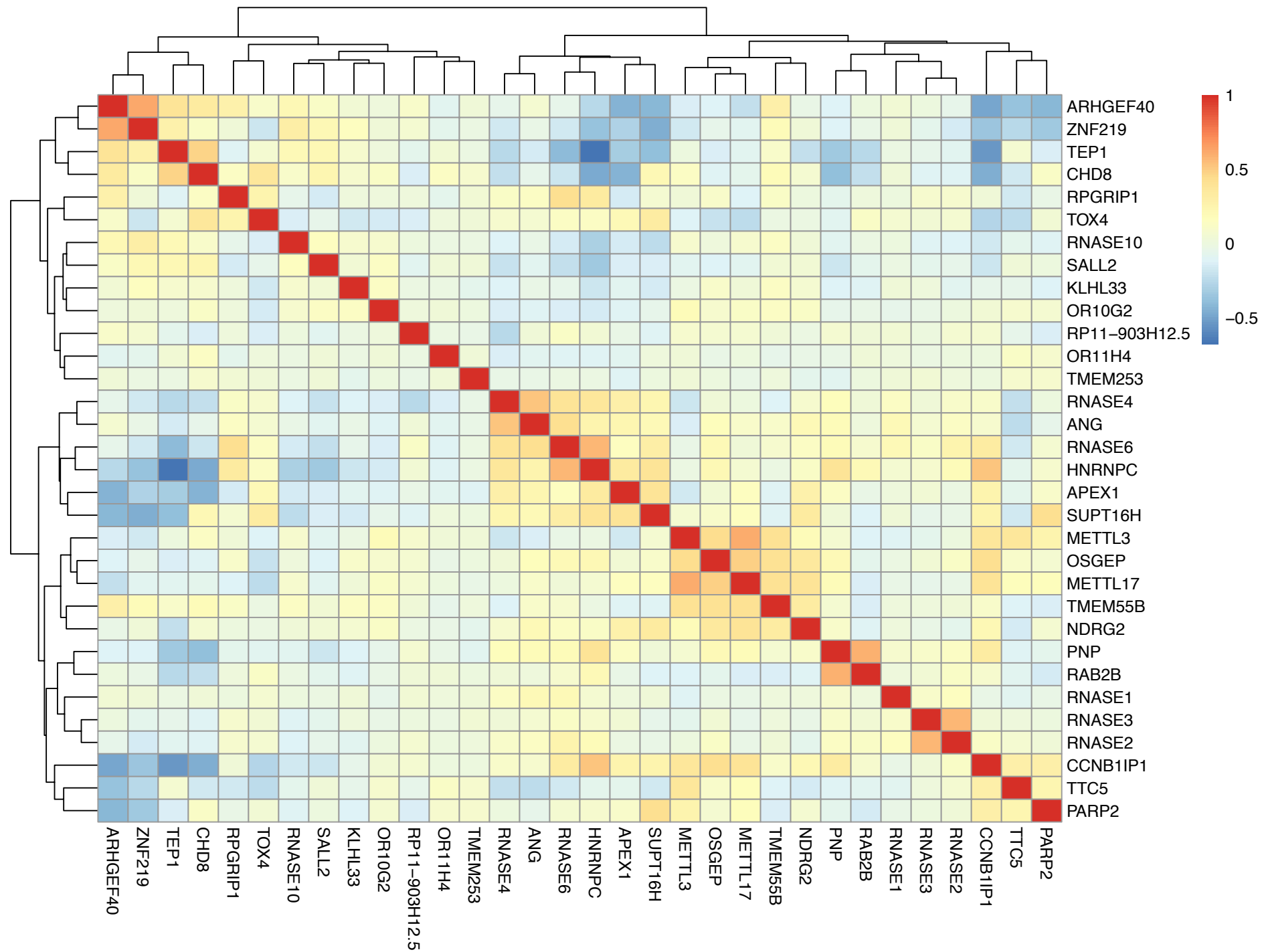
rs3827907

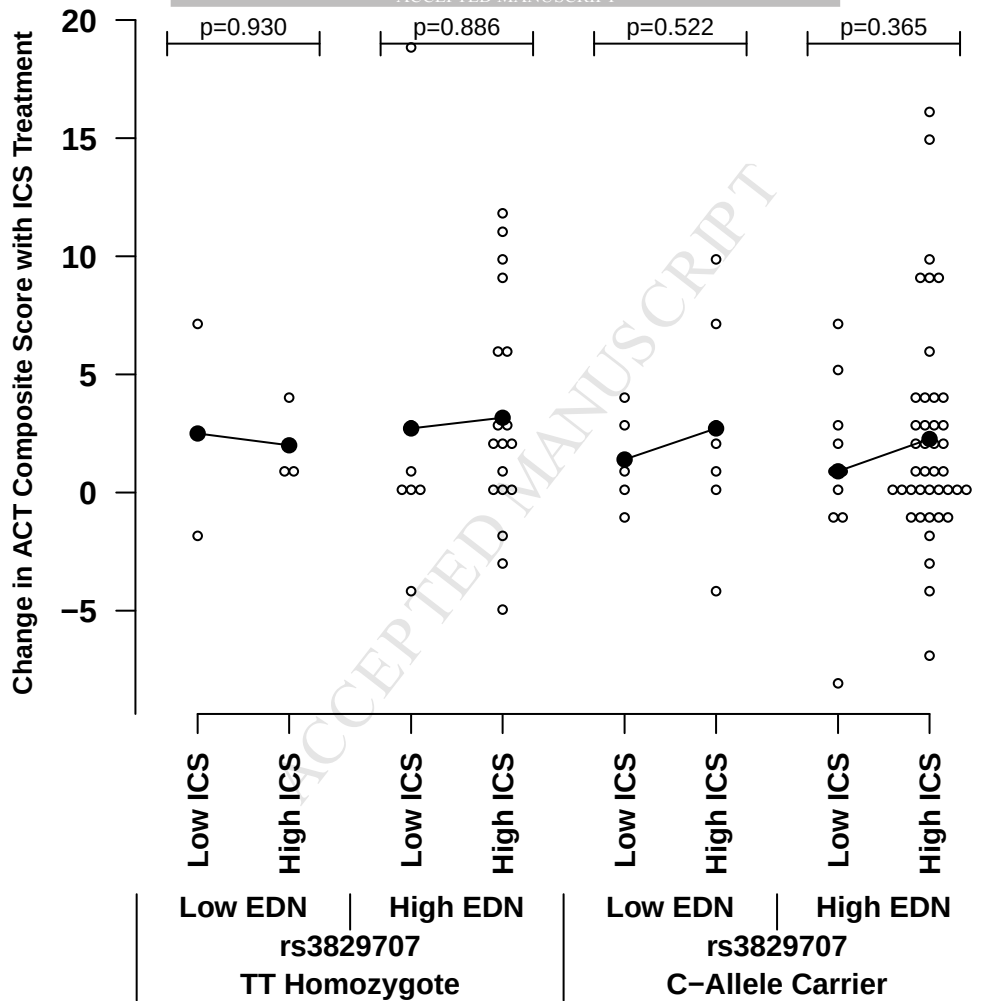
Low ICS Use

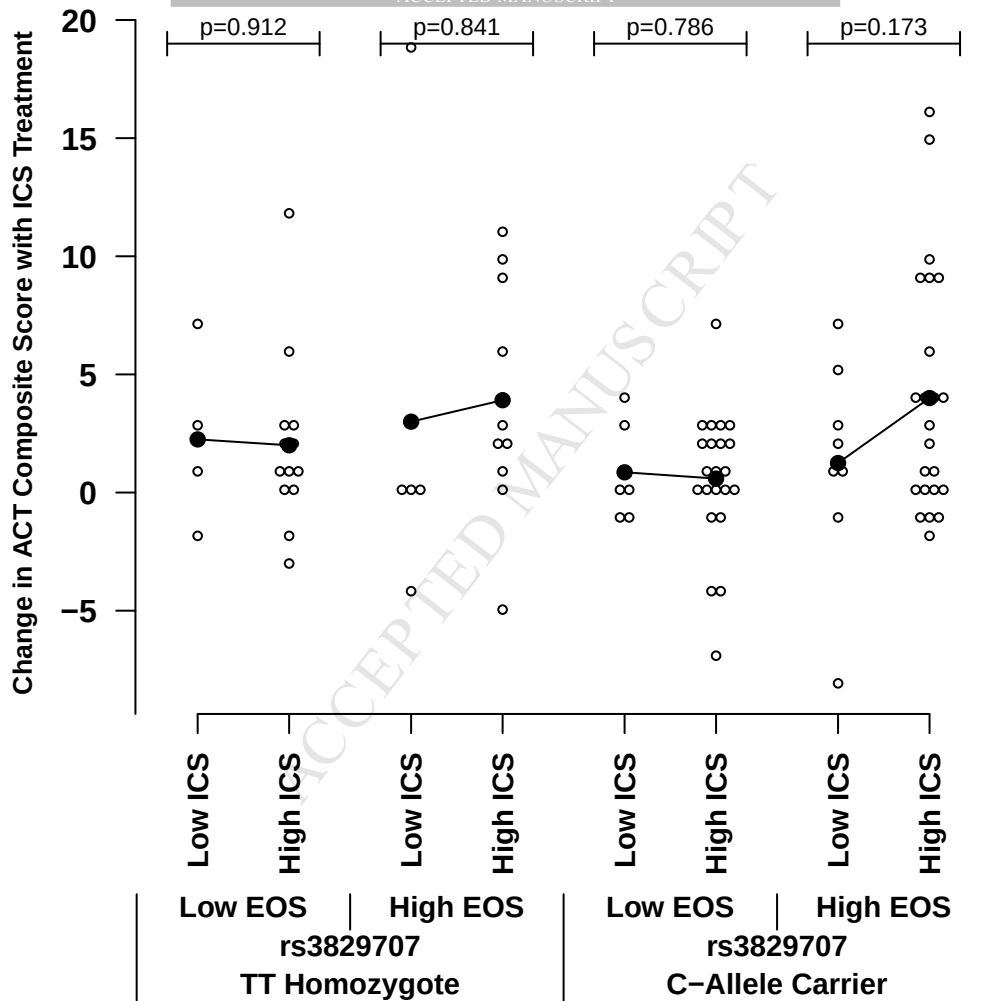
High ICS Use











Plotted SNPs

