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Integrative approach identifies corticosteroid response variant in diverse populations with asthma

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Abstract

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

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

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

Results: One variant, rs3827907, was significantly associated with ICS-mediated changes in asthma control in the discovery set ($P=7.79\times10^{-8}$) and was jointly associated with asthma exacerbations in three validation cohorts (P=0.023, P=0.029, and P=0.041). RNA-seq analysis found the rs3827907 C-allele to be associated with lower *RNASE2* expression ($P=6.10\times10^{-4}$). *RNASE2* encodes eosinophil-derived neurotoxin (EDN), and the rs3827907 C-allele appeared to particularly influence ICS treatment response in the presence of eosinophilic inflammation (i.e., 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.

Keywords

pharmacogenetics; *EDDM3B*; *RNASE2*; eosinophil-derived neurotoxin; transcriptome; eosinophils

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 populationspecific 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 (320μg 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 ≥50% of individuals.

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

Measurements—Blood and serum samples were collected from SAPPHIRE 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-value $<8.71\times10^{-8}$ (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 $<1.56\times10^{-3}$ (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., ≤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 SAPPHIRE participants (n=803), Latino patients from GALA II patients (n=1,461), African American patients from SAGE II (n=563), and European American SAPPHIRE participants (n=98). As can be seen in Table 1, SAPPHIRE 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 (± 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 (λ=1.09), we adjusted our Pvalues 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 $<5.0\times10^{-7}$ 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 – $P=7.79\times10^{-8}$ (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., ≤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 ($P=8.57\times10^{-8}$ after genomic control).

Based on the regression models (Table 2), a similar pattern of association was seen for SNP rs73906251 on chromosome 20 ($P=3.85\times10^{-7}$, 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 ($P=5.25\times10^{-8}$, 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 ($P=1.16\times10^{-6}$ for rs73906251 and $P=1.86\times10^{-7}$ 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 ($P=6.51\times10^{-7}$, 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 SAPPHIRE 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 crosssectional 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 SAPPHIRE (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 SAPPHIRE 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 SAPPHIRE participants (408 with asthma and 405 without asthma). Expression of *EDDM3B* in whole blood was low and did not meet our criterion for evaluation (≥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 ($P=6.10\times10^{-4}$) and *RNASE1* expression ($P=7.92\times10^{-4}$) using a conservative Bonferroni threshold ($P<0.05/32=1.56\times10^{-3}$). 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/μL). 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 SAPPHIRE 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 SAPPHIRE 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/μL), 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 FEV_1 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 SAPPHIRE 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 SAPPHIRE 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS:

ACT	Asthma Control Test
CHR	chromosome
ECP	eosinophil cationic protein
EDN	eosinophil derived neurotoxin
eQTL	expression quantitative trait locus (or loci)
FEV_1	forced expiratory volume at one second
GALA II	Genes-environments & Admixture in Latino Americans Study
ICS	inhaled corticosteroid

LABA	long-acting beta-agonist
MDI	metered dose inhaler
SABA	short-acting beta-agonist
SAGE II	Study of African Americans, Asthma, Genes and Environments
SAPPHIRE	Study of Asthma Phenotypes and Pharmacogenomic Interactions by Raceethnicity
SNP	single nucleotide polymorphism

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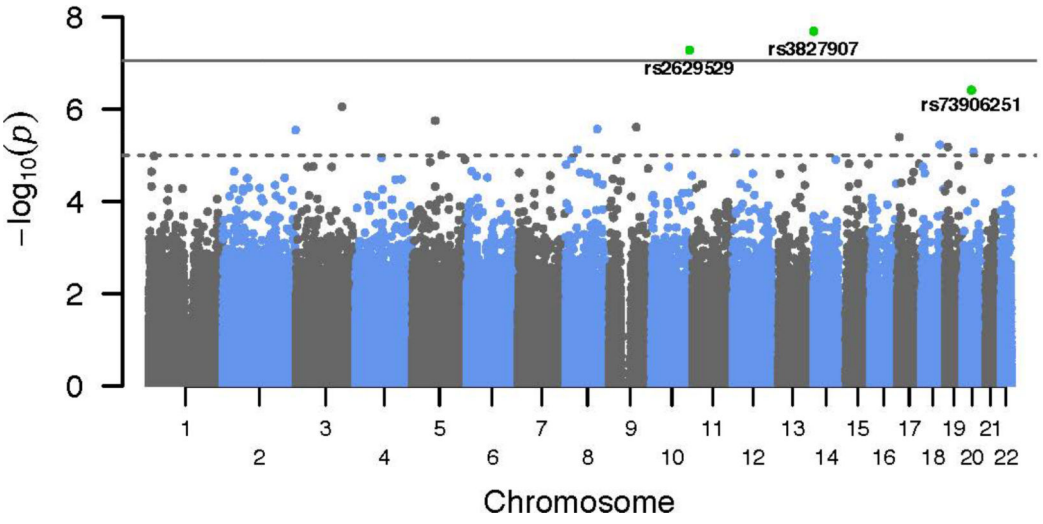

Figure 1.

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 ($p=8.72\times10^{-8}$) is shown as a solid gray line, and a pvalue threshold of 10^{-5} is shown as a dashed gray line.

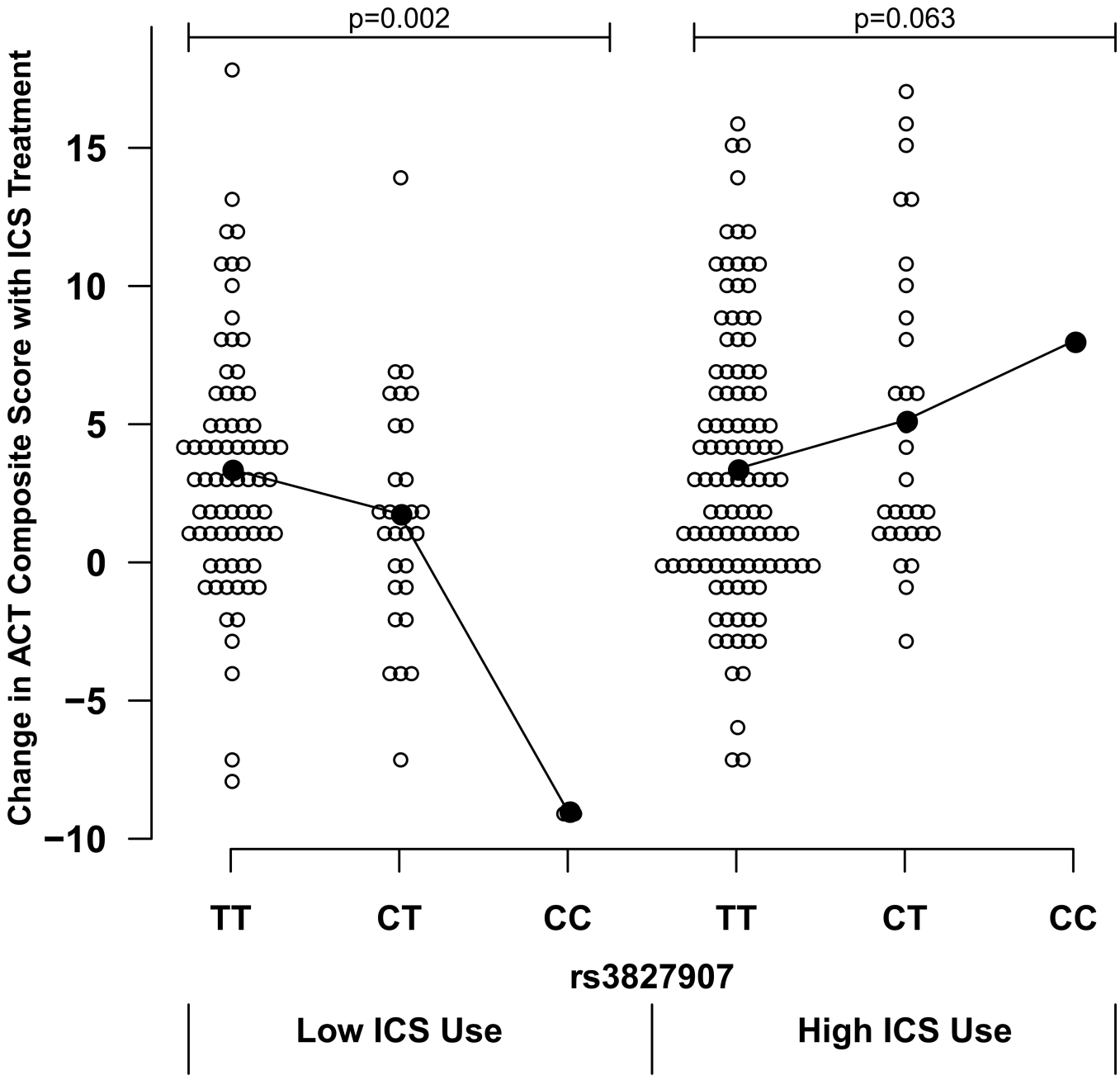

Figure 2.

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 (≤75% adherence was considered low use and >75% adherence was considered high use). Black lines connect the means for each group.

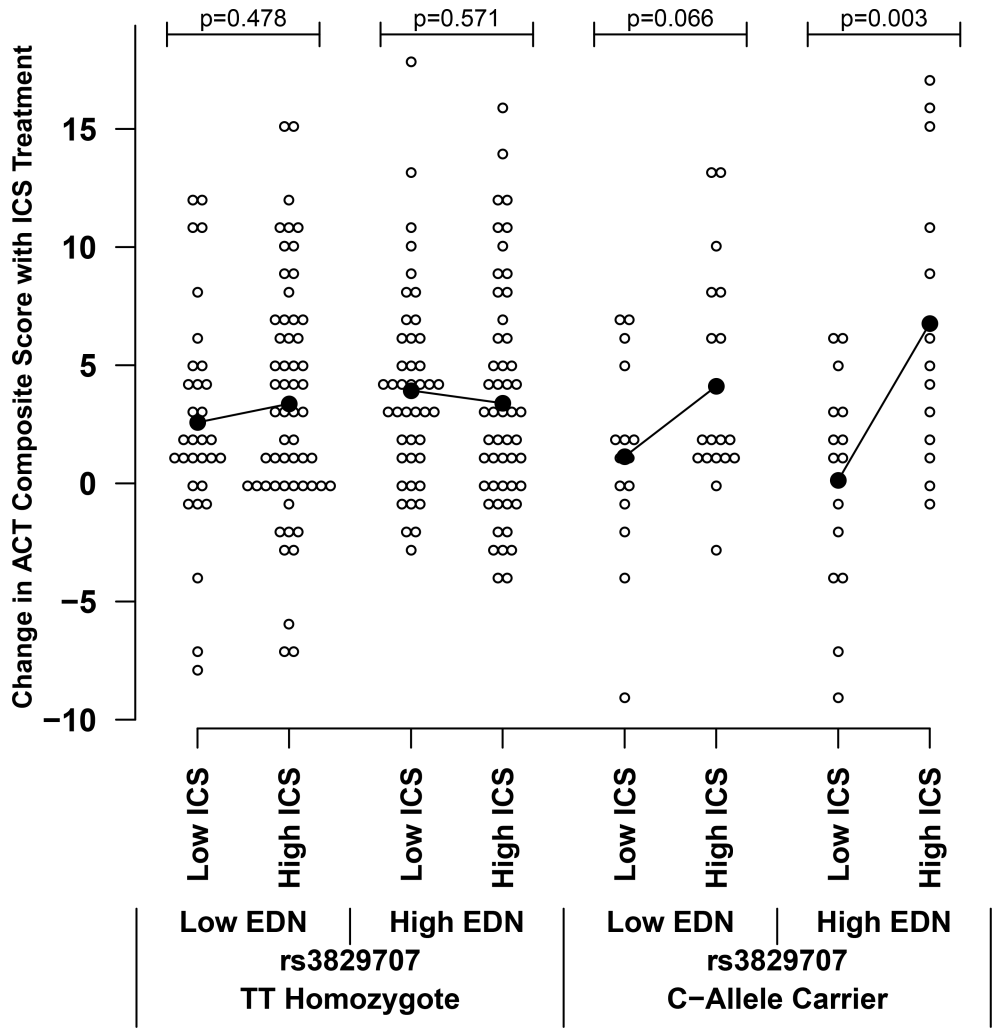

Figure 3.

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 (≤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), respectively. Black lines connect the means for each group.

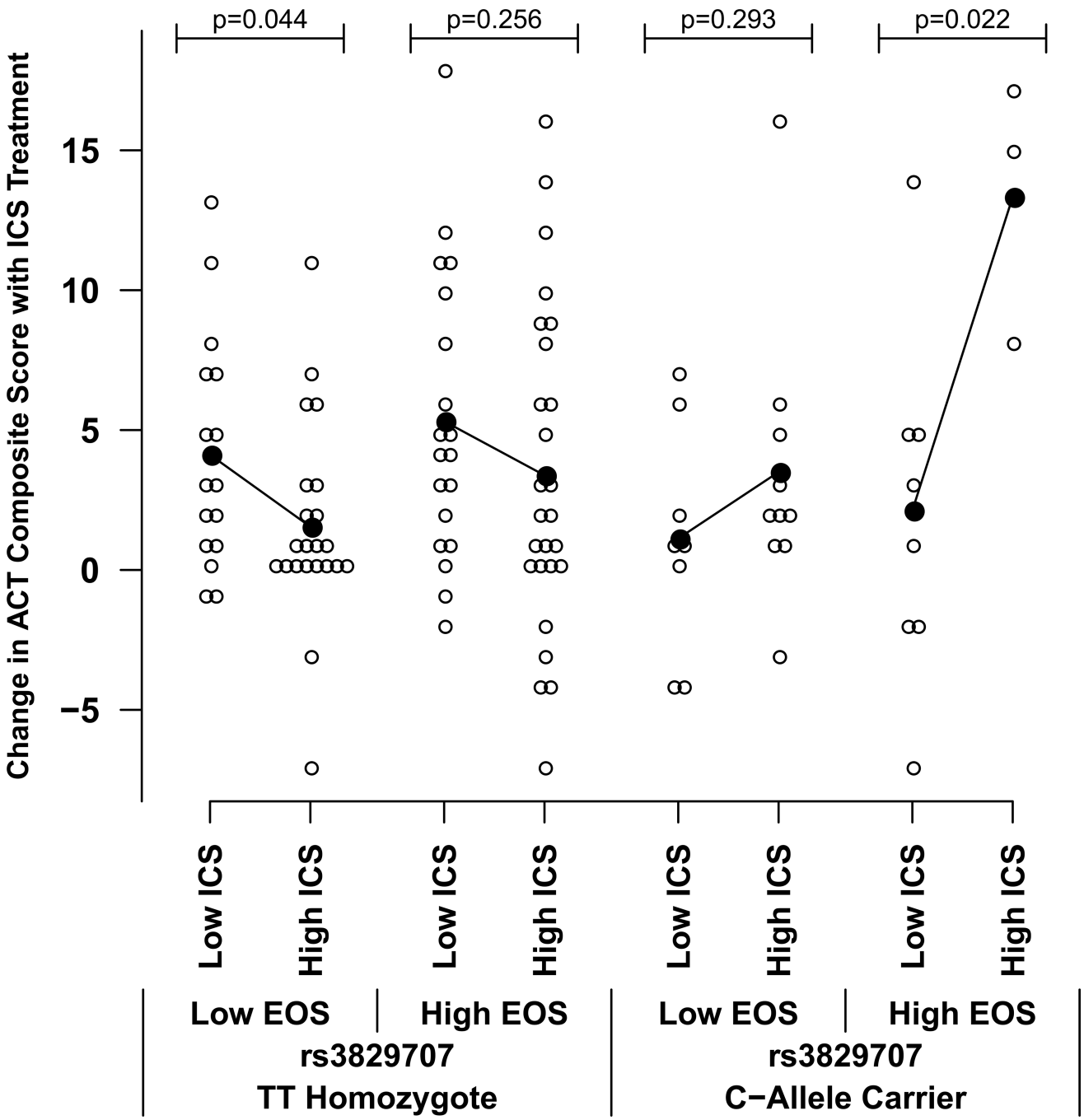

Figure 4.

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 (≤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), respectively. Black lines connect the means for each group.

Table 1.

Characteristics of subjects 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/m^2, weight in kilograms per height in meters squared; ACT, asthma control test; and FEV_1, 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 interaction term[§]	P-value for joint test[‖]	Adjusted P-value for joint test[¶]
rs3827907	14	21238798	C/T	0.13	0.59	*EDDM3B*	−0.51	0.641	−9.67	3.06×10^{-9}	12.35	5.98×10^{-9}	2.04×10^{-8}	7.79×10^{-8}
rs2629529	10	126534415	C/A	0.06	1.00	--	3.43	0.001	2.07	0.357	−7.74	7.40×10^{-3}	5.25×10^{-8}	1.86×10^{-7}
rs73906251	20	29991274	T/C	0.05	0.48	--	1.89	0.065	−14.51	1.13×10^{-5}	14.60	2.77×10^{-4}	3.85×10^{-7}	1.16×10^{-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 (minor 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 × ICS interaction variables are shown for the model simultaneously including these variables, as well as adjusting for patient age, sex, ACT score at 660 baseline (i.e., time of study enrollment), smoking status (coded as never, past, and present), the first three principal components, and 661 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 × ICS interaction‡	P-value for interaction‡	P-value for joint test§
rs3827907	14	21238798	C/T	0.13	*EDDM3B*	−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 (minor 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 ICSLABA 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	*EDDM3B*	−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	*EDDM3B*	−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 (minor 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 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 [National Asthma Education and Prevention Program. Expert Panel Report 3 (EPR-3): Guidelines for the Diagnosis and Management of Asthma-Summary Report 2007. J Allergy Clin Immunol. 120, 2007, S94-S138.]), 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 5.

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

Gene	Start	End	Distance to rs3827907 (bp) †	Rank	Cases (n=408) Parameter estimate ‡	P-value	Rank	Controls (n=405) Parameter estimate ‡	P-Value	Rank	Total (n=81 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	7.92×10^{-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	6.10×10^{-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

Gene	Start	End	Distance to rs3827907 (bp)†	Rank	Cases (n=408) Parameter estimate‡	P-value	Rank	Controls (n=405) Parameter estimate‡	P-Value	Rank	Total (n=81 Parameter estimate‡	P-value
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

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

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