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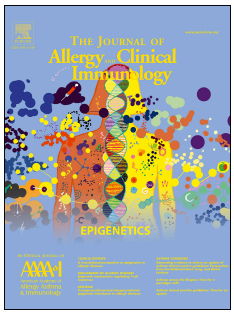

Exacerbation-Prone Asthma in the Context of Race and Ancestry in Asthma Clinical Research Network Trials

Nicole L. Grossman, MD, Victor E. Ortega, MD, PhD, Tonya S. King, PhD, Eugene R. Bleecker, MD, Elizabeth A. Ampleford, PhD, Leonard B. Bacharier, MD, Michael D. Cabana, MD, MPH, Juan C. Cardet, MD, MPH, Tara F. Carr, MD, Mario Castro, MD, Loren C. Denlinger, MD, Joshua L. Denson, MD, Nicolas Fandino, Anne M. Fitzpatrick, PhD, Gregory A. Hawkins, PhD, Fernando Holguin, MD, MPH, Jerry A. Krishnan, MD, PhD, Stephen C. Lazarus, MD, Sharmilee M. Nyenhuis, MD, Wanda Phipatanakul, MD, Sima K. Ramratnam, MD, Sally Wenzel, MD, Stephen P. Peters, MD, PhD, Deborah A. Meyers, PhD, Michael E. Wechsler, MD, MMSc, Elliot Israel, MD

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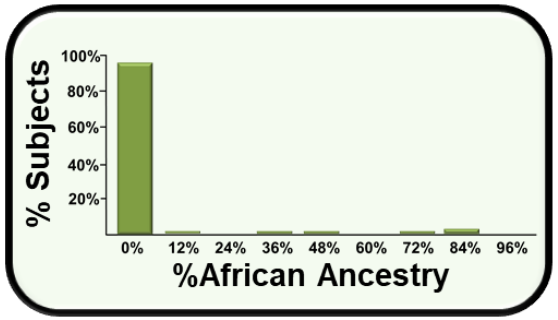
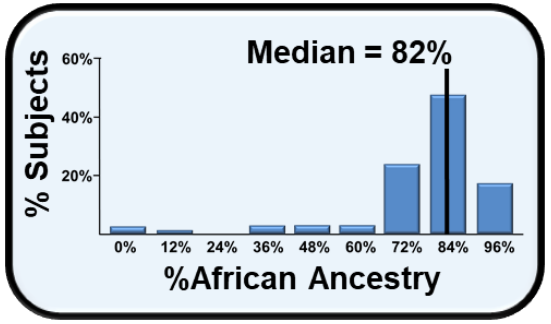
Exacerbation-Prone Asthma in the Context of Race and Ancestry in Asthma Clinical Research Network Trials

Exacerbations in 1,840 Asthma Subjects from 12 Clinical Trials (Median Observation Period=126-379 Days)

Whites (N=489)

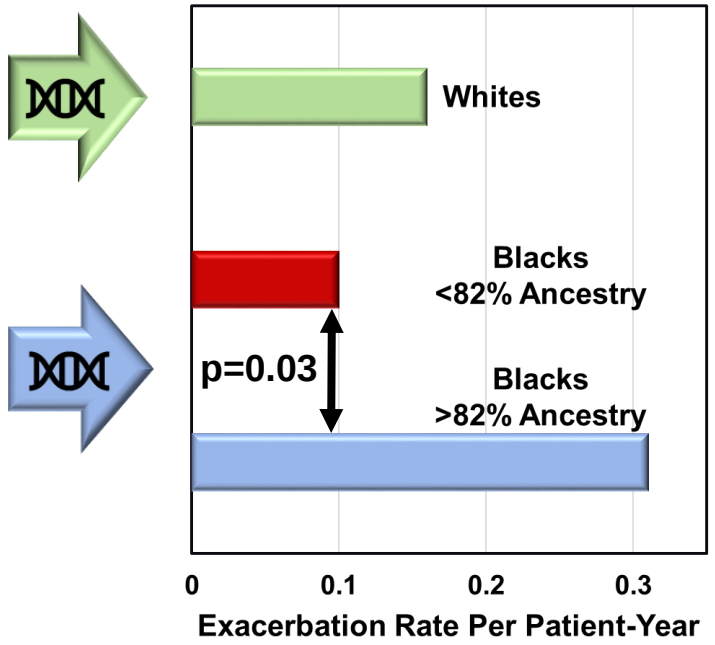
Blacks (N=161)





Whole-Genome Ancestry Estimated in Subgroup

Genetic African Ancestry >82% is a Risk Factor for Exacerbations in Self-Reported Blacks.



Exacerbation-Prone Asthma in the Context of Race and Ancestry in Asthma Clinical Research Network Trials

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ABSTRACT

Background: African descent minority groups experience disproportionately higher asthma morbidity compared to other racial groups suggesting that genetic variation from a common ancestry could influence exacerbation risk.

Objective: We evaluated clinical trial measures in the context of self-reported race and genetic ancestry to identify risk factors for asthma exacerbations.

Methods: 1,840 multi-ethnic individuals from 12 ACRN and AsthmaNet trials were analyzed for incident asthma exacerbations with Poisson regression models that included clinical measures, self-reported race (Black, non-Hispanic White, and other), and estimates of global genetic African ancestry in a subgroup (N=760).

Results: 24% of 1,840 individuals self-identified as Black. Blacks and Whites had common risk factors for exacerbations, including a history of ≥2 exacerbations in the previous year and FEV1%predicted, while chronic sinusitis, allergic rhinitis, and GERD were only associated with increased exacerbation risk in Blacks. In the combined, multi-ethnic cohort, neither race (p=0.30) nor percentage genetic African ancestry as a continuous variable associated with exacerbation risk (adjusted rate ratio [RR]=1.26, 95%CI=0.94-1.70, p=0.13; RR per one SD change [32% ancestry]=0.97, 95%CI=0.78-1.19, p=0.74). However, in 161 Blacks with genetic data, those with African ancestry greater than the median (≥82%) had a significantly greater risk of exacerbation (RR=3.06, 95%CI=1.09-8.6, p=0.03).

Conclusion: Blacks have unique risk factors for asthma exacerbations, of which global African genetic ancestry had the strongest effect.

Clinical Implications: The association between African ancestry and exacerbations in African descent groups demonstrates that self-reported race is insufficient to understanding the genetic or other factors that underlie racial differences in asthma morbidity.

Capsule Summary: This analysis of incident exacerbations in 1,840 unique individuals with asthma from 12 clinical trials identified risk factors unique to 450 self-identified Blacks, including genetic African ancestry in 161 Blacks.

Key Words: exacerbations, race, ancestry, admixture, lung function, genetics, asthma, Black, African Americans, ethnic group

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Abbreviations:

95% CI - 95% confidence intervals

ACRN - Asthma Clinical Research Network

dbGaP - Database of Genotypes and Phenotypes

FEV_1- forced expiratory volume in 1 second

PC20 – provocative dose of methacholine resulting in a 20% decline in FEV1

NHLBI National Heart, Lung, and Blood Institute

RR – Rate Ratio

SHARP - SNP Health Association Resource [SHARe] Asthma Resource Project

SD - standard deviation

SNP – Single Nucleotide Polymorphism

INTRODUCTION

Non-Hispanic Blacks (i.e. African Americans) and Puerto Ricans, a Hispanic group with significant African ancestry, experience substantially higher asthma-related morbidity and mortality compared to non-Hispanic Whites and Hispanic subgroups of lower overall African ancestry [1-3]. Multiple studies have identified Black race as a risk factor for more frequent asthma exacerbations, even when accounting for socioeconomic factors [1, 4-9]. We recently identified a Black, exacerbation-prone asthma subgroup from a randomized trial cohort, best characterized by individuals with an asthma exacerbation in the prior year and lower lung function, factors also associated with exacerbations in other cohorts [10-13]. *Post hoc* analyses of NHLBI-sponsored Asthma Clinical Research Network (ACRN) and AsthmaNet clinical trial cohorts have shown that African Americans have lower lung function, a greater proportion of uncontrolled asthma, and a greater likelihood of treatment failure compared to their White counterparts [14, 15].

Race and ethnic designations do not sufficiently capture the ancestral genetic, cultural, geographic, or socio-economic contexts which underlie differences in asthma severity between racial groups [16]. Ancestry-based studies leveraging genome-wide genotyping technologies have demonstrated whole-genome African ancestry is associated with higher risk of asthma, lower lung function, and poor symptom control in African Americans and African descent Hispanic groups [17-23]. In a previous study of 392 adolescent and adult African Americans with mild asthma, higher African ancestry was associated with an increased risk of exacerbations requiring a glucocorticoid burst, hospitalization, or emergency department visit based on prescription and health care visit records [22]. These findings were not replicated in other Hispanic or African American asthma cohorts suggesting that the risk factors which underlie inter-ethnic differences in asthma exacerbations are complex [21, 24, 25]. It remains plausible

that risk variants from a common ancestry could be enriched in a specific ethnic or racial group and contribute to differences in exacerbation risk [25].

Risk factors associated with inter-ethnic differences in exacerbation risk have not been previously investigated in the context of genetic ancestry in a randomized, controlled, longitudinal clinical trial setting. Hence, we performed a study to identify the determinants of exacerbations in 12 multi-ethnic, NHLBI-sponsored asthma clinical trial cohorts consisting of mild-to-moderate asthma subjects and to evaluate the effects of genetic ancestry on exacerbation risk in a subgroup of trials where genetic data was available. This study tested the hypothesis that self-reported Black race and African ancestry are determinants of asthma exacerbation risk due to genetic factors inherited from a common ancestry in self-reported Blacks and that, in Blacks, genetic ancestry will serve as a stronger predictor due to the complex factors underlying the designation of race.

METHODS

Study Populations

NHLBI-sponsored ACRN and AsthmaNet studies with data on exacerbations requiring systemic glucocorticoid therapy and a minimum of 125 days per study were included to ensure sufficient time to observe exacerbations. The full methodology and results of all 12 clinical trials which randomized asthma subjects to different therapeutic interventions have been previously published and are described in the online repository [26-38]. Each study was approved by the appropriate institutional review boards, and all subjects signed written informed consent.

Study outcomes

The primary outcome of this analysis was the number of asthma exacerbations in a unique individual over the course of participation in one or more ACRN and AsthmaNet trials. Exacerbations were defined as any worsening of asthma symptoms requiring the initiation of treatment with systemic corticosteroids documented with patient and coordinator-completed questionnaires and medication data collected for each trial. Self-identified race and ethnicity (Hispanic versus non-Hispanic was not collected independent of race in all cohorts) were classified as Caucasian or non-Hispanic White (NHW), Black (Hispanic and non-Hispanic), and other (which included Hispanics who did not identify as Black or White).

Estimating Global Genetic Ancestry

Genome-wide genotyping data from the TALC/BASALT cohorts (290 NHW, 110 Blacks, and 75 other) and the SLIC/SOCS/IMPACT cohorts from the NHLBI Database of Genotypes and Phenotypes (dbGaP) SNP Health Association Resource (SHARe) Asthma Resource Project (SHARP: 218 NHW, 54 Blacks, and 37 other) were used to estimate whole-genome ancestral admixture (dbGaP project number 12153). The TALC/BASALT and

SLIC/SOCS/IMPACT genotyping datasets contained 60,117 and 53,826 single nucleotide polymorphisms (SNP's) meeting Hardy-Weinberg expectations ($p<10^{-4}$), respectively, after pruning ($r^2 \geq 0.1$) to estimate whole-genome ancestral admixture. Percentage European, African, and Native American ancestry was estimated using the ADMIXTURE program and genetic data from 113 European descent Whites (CEU, HapMap), 113 Yorubans (YRI, HapMap), and 43 Native Americans [39, 40].

Statistical analysis

Poisson regression models evaluated the associations between predictors and number of exacerbations accounting for the duration of time that each participant was observed in each trial and accommodated individual participation in multiple trials using generalized estimating equations. Potential predictors were assessed individually in bivariate models adjusted for protocol in all subjects from the multi-ethnic cohorts (N=1,840) and stratified by race. Bivariate models in the subgroup with genetic data (N=760) evaluated for the same potential predictors and for associations with percentage African ancestry as a continuous and categorical (above and below median) variable due to the narrow distribution of African ancestry in Blacks (interquartile range=71-87%, Figure Ib) as consistent with prior ancestry-based studies [20, 23].

Significant predictors of exacerbation rate ($p<0.05$) were combined in multivariable models in the combined multi-ethnic cohorts and by individual race. Multivariable models in the combined multi-ethnic subgroup with genetic data analyzed African ancestry above and below median values as a nested effect within race group and in individual race groups. Results are reported as exacerbation rates per person-year for each group, rate ratios (RR), and 95% confidence intervals (95%CI) between groups. To internally validate the association between median African ancestry and exacerbation risk in Blacks, multivariable models were independently performed in trial cohorts genotyped through dbGaP SHARe (51 Blacks from

SOCS/SLIC/IMPACT) and with the Illumina OmniExpress HumanExome BeadChip ("Exome Chip," 51 from TALC and 59 from BASALT, Table E1).

RESULTS

Baseline characteristics

A total of 1,840 unique subjects from 12 ACRN and AsthmaNet studies with median observation periods ranging from 126 to 379 days were included in the analyses (Table E1). The mean exacerbation rate for all subjects was 0.20 exacerbations per person-year (95%CI=0.18-0.23, Table E1). 24% of subjects self-identified as Black, 60% as non-Hispanic White, and 15% as other. The baseline characteristics of the combined multi-ethnic cohorts, Whites, and Blacks are summarized in Table I (data for "other" racial or ethnic groups are in Table E2). The mean FEV_1 was 82 percent of predicted (80% in Blacks and 84% in Whites, Table I). 760 unique subjects had whole-genome genotyping data available for ancestry-based analyses, including 161 Blacks. Individual trial-specific data for mean exacerbation rates and genotyping data are in Table E1. Across the whole population, percent African ancestry had a bimodal distribution reflecting known distributions in Black and non-Black ethnic groups (Figure 1a) [20, 23]. Self-identified Blacks had a median African genetic ancestry of 82% (interquartile range=71-87%, Figure Ib) while the majority of non-Black subjects had <25% African ancestry (Figure 1c).

Bivariate Models of Exacerbations Excluding Genetic Ancestry (All 1,840 subjects: 450 Blacks, 1088 Whites)

Self-reported Black race was not associated with a statistically significant difference in exacerbation rate compared with Whites (RR=1.26, 95%CI=0.94-1.70, p=0.13, Table II). Across

all races, significant bivariate risk factors for exacerbations included a previous history of 1 and ≥2 exacerbations, female sex, lower % predicted FEV1, methacholine bronchial hyperresponsiveness, sputum eosinophils, chronic sinusitis, GERD, nasal polyps, allergic rhinitis, and household income ($p<0.05$, Table III).

In Blacks, increased exacerbation risk significantly associated with history of ≥2 steroid-requiring exacerbations in the prior year versus none (RR=2.2, 95%CI=1.2-4.0, p=0.01), lower % predicted FEV1 (RR=1.4 per standard deviation [SD=15%] decrease, 95%CI=1.1-1.9, p = 0.02), chronic sinusitis (RR=2.8, 95%CI=1.5-5.1), p=0.001), GERD (RR=2.2, 95%CI=1.2-4.1, p=0.01), and allergic rhinitis (RR=2.2, 95%CI=1.0-4.7, p=0.04, Table III). In Whites, bivariate risk factors common to Blacks included a history of ≥2 exacerbations (RR=2.51, 95%CI=1.49-4.24, $p<0.001$) and lower % predicted FEV1 (RR=0.76 per SD (15%) increase, 95%CI=0.62-0.93, p = 0.008). Risk factors unique to Whites included a history of one steroid-requiring exacerbations in the prior year versus none (RR=1.94, 95%CI=1.27-2.99, $p<0.001$), female sex (RR=1.48, 95%CI=1.01-2.16), BMI (p=0.04), and methacholine bronchial hyperresponsiveness (RR=0.82 per 1.86 unit increase in logPC20, 95%CI=0.68-0.99, p=0.04, Table III).

Bivariate Models of Exacerbations Including Genetic Ancestry (760 Subjects with Genetic Data: 161 Blacks, 489 Whites)

In the subgroup of all races with genetic data and non-missing outcome data necessary for the Poisson regression models, African ancestry as a continuous variable was not associated with exacerbation rate (RR per SD [32%] 0.97, 95%CI=0.78-1.19, p=0.74, Table III). European and Native American ancestry also did not associate (Table E3). In Blacks, African ancestry as a continuous variable (RR per SD [18%] 2.1, 95%CI=0.85-5.02, p=0.11, Table III) and in 20% increments (to confirm a prior study) was not associated with exacerbation risk (RR=2.2 for every 20% increase in African ancestry, 95%CI=0.84-6.00, p=0.11) [22].

Due to the remarkably different distributions of genetic ancestries between racial groups (Table I, Figures 1b-d) and the narrow range of African ancestry in Blacks (Table I, Figure 1b) consistent with previous studies, African ancestry was evaluated for individuals from all racial groups with ancestry above and below median values as a nested effect within race group [20]. In a simple model with median African ancestry nested within race adjusted for protocol, Blacks with ≥82% African ancestry had a three-fold higher rate of exacerbations per patient-year compared with those below the median 82% (RR 3.06, 95%CI=1.09, 8.60, p=0.03, Table IV). Blacks with ≥82% African ancestry had a 1.5-fold higher rate of exacerbations compared to Whites (0.31 versus 0.16 exacerbations per patient-year, p=0.19), but the difference was also 3-fold higher than other Blacks with ancestry <82% (0.10 exacerbations per patient-year, p=0.03, Table IV, Figure 2). Median African ancestry was not associated with exacerbations in non-Hispanic Whites (RR=1.43, 95%CI=0.86, 2.37, p=0.17, Table IV) who had a low median African ancestry of 0% within a narrow range (interquartile range=1-2%, Table I, Figure Ic).

Multivariable Models Excluding (1,840 Total Subjects) and Including Ancestry (760 Subjects with Genetic Data)

In the multi-ethnic Poisson regression models in all subjects which included protocol, age, sex, FEV_1, race, and exacerbation history; significant predictors of future exacerbations across all races continued to be a history of 1 and ≥2 exacerbations in the last year (RR=1.73, 95%CI=1.26-2.37, p<0.001 and RR=2.55, 95%CI=1.80-3.61, p<0.001, Table E4), female sex (RR 1.61, 95%CI=1.17-2.20, p=0.003, Table E4), and lower percentage predicted FEV_1 (RR=0.73 for each 15% increase, 95%CI=0.62-0.86, p<0.001, Table E4). When median African ancestry was nested by race in the combined cohort of 161 Blacks, 489 Whites, and 110 other with genetic ancestry data, a history of ≥2 exacerbations in the last year (RR=2.09,

95%CI=1.11-3.94, p=0.02) and median African ancestry in Blacks (RR=2.84, 95%CI=1.00-8.04, p=0.0495, Table E4) were the only significant risk factors for future exacerbations.

When the multivariable models were stratified by race in all subjects with and without genetic data, a history of ≥2 exacerbations in the past year and lower percentage predicted FEV_1 remained significant risk factors for exacerbations in both Blacks and Whites (Table V). However, in Blacks with genetic ancestry data, African ancestry ≥82% was the only significant risk factor for exacerbations (RR=3.4, 95%CI=1.15-9.81, p=0.027, Table VI) while a prior history of ≥2 exacerbations (p=0.56), FEV_1 (p=0.86, Table VI) and chronic sinusitis (p=0.77, data not shown for the subgroup [N=254]) were not significantly associated with exacerbations. When Blacks were stratified for internal validation, (baseline characteristics for groups are shown on Table E5), Blacks with higher African ancestry ≥82% across dbGaP SHARe (SOCS/SLIC/IMPACT, RR=7.34, 95%CI=0.86-62.8, p=0.07) and Exome Chip-genotyped TALC (RR=2.82, 95%CI=0.61-13.0, p=0.18) and BASALT (RR=1.34, 95%CI=0.12-15.1, p=0.81) cohorts had comparatively higher rates of exacerbations compared to those below the median. The only significant difference between Blacks with African ancestry above versus below the median was that Blacks with African ancestry ≥82% had a higher rate of chronic sinusitis (25% vs 9%, p=0.02, Table E6).

DISCUSSION

In the United States, self-identified Blacks and Puerto Ricans with asthma experience a disproportionate burden of asthma compared to Whites, with more frequent exacerbations requiring urgent outpatient visits, hospitalizations, and death [1-9]. Multiple epidemiologic studies have demonstrated racial differences in asthma-related morbidity which persist even after statistical adjustments for different socioeconomic surrogates [1, 4-9]. The racial designations used in these studies are determined by a complex interplay between genetic, geographic, cultural, and socioeconomic factors. Even after adjustment for surrogate markers for socioeconomic status, self-reported race and ancestry are insufficient to achieve an understanding of the mechanisms underlying differences in disease expression between racial groups [16, 41]. Few studies have compared risk factors for asthma exacerbations between similarly recruited racial or ethnic groups in a longitudinal cohort, and only two evaluated genetic ancestry and longitudinal risk for incident exacerbations [5, 6, 8, 9, 21, 22].

In ACRN and AsthmaNet trial cohorts, we found that a history of prior exacerbations and lower lung function were associated with incident exacerbations across all races, while chronic sinusitis, allergic rhinitis, and GERD were only associated in Blacks. Most importantly, we were unable to detect statistically significant differences in exacerbation rates between individuals of self-reported Black race compared to Whites (Table II). The absence of significant inter-racial differences could relate, at least in part, to a sample size underpowered to detect the complex effects of race, but ancestry-based genetic analyses in a smaller subgroup of Blacks with genetic ancestry detected significant differences between Blacks with a higher African ancestry above the median of 82% who had a higher exacerbation rate compared to those with lower ancestry who were similar to Whites (Figure 2). The relative exacerbation rate associated with African ancestry was highest among the risk factors we identified, including exacerbation history

and was also associated with risk for chronic sinusitis. This demonstrates that exacerbation risk is better predicted by genetic variation from a common ancestry than race or even exacerbation history, of which the latter is a well-recognized, strong risk factor for exacerbations in this and other studies [8, 10, 11].

The study of clinical trial cohorts allowed for the study of individuals from different ethnic groups with objectively diagnosed asthma continually monitored for compliance and outcomes such as exacerbations requiring glucocorticoid therapy as a pre-defined outcome. We identified multiple risk factors for exacerbations, including baseline lung function, history of prior exacerbations, chronic sinusitis, and GERD, all of which have been associated with exacerbation risk in previous studies, including cross-sectional and longitudinal studies from different phases of the NHLBI-sponsored Severe Asthma Research Program [1, 4-8, 42]. Both sinus disease and GERD were unique risk factors in Blacks, but have consistently been associated with exacerbation risk in multiple asthma cohorts [6, 12, 42, 43]. The role of these co-morbid conditions in determining asthma exacerbations in Blacks is unclear, but genetic African ancestry has been associated with IgE levels in different African descent Hispanic asthma cohorts suggesting mechanisms related to allergic inflammation in African descent individuals [17, 18, 24].

High-throughput genotyping has provided an unprecedented opportunity for ancestry-based genetic studies in diverse cohorts to precisely and objectively define genetic ancestry in order to improve our understanding of how genetic variation from a common ancestry associates with disease outcomes [16]. This analysis of global African genetic ancestry was based on the hypothesis that risk alleles from a common ancestry are enriched in ethnic groups that experience a disproportionate burden of disease. There are variants in multiple genetic pathways known to influence measures of clinical severity (lung function, atopic measures, comorbidities) and therapeutic responsiveness (pharmacogenetic loci) in asthma cohorts [25,

44-46]. Like most variation throughout the genome, these genetic risk loci have varying allele frequencies between individuals from different ancestral backgrounds that could influence asthma severity and therapeutic responsiveness to commonly used asthma therapies in African descent individuals [5, 14, 15, 25, 44-48]. In African Americans and Puerto Rican asthma cohorts, higher African ancestry has consistently been shown to be inversely associated with baseline lung function measures consistent with observations from large, general populations [20, 23, 45, 49].

Three prior ancestry-based genetic studies have tested for associations between global genetic ancestry and asthma exacerbations in African descent cohorts, two in African Americans and one in Puerto Ricans [21, 22, 24]. Of these, a single-center urban study of 392 self-reported Blacks ages 12-56 years with physician-diagnosed mild asthma from the Study of Asthma Phenotypes and Pharmacogenomic Interactions by Race-Ethnicity (SAPPHIRE) was the only to find an association between genetic African ancestry and exacerbation risk. In SAPPHIRE, African genetic ancestry was estimated using 59 ancestry-informative markers only for African and European ancestry resulting in a mean African ancestry estimate of 76.1%, which was lower than Blacks from our trial cohorts (82%). This difference reflects the known differences in ancestral proportions in Blacks across the United States in addition to the fact that our study did not include adolescents [22, 23, 50]. SAPPHIRE found a 4.3-fold increased risk of exacerbation with each 20% increase in African ancestry only in male subjects. We did not find an interaction between ancestry and sex on exacerbation risk as sex was only associated with exacerbation rates in Whites (Table I) [22]. The basis for the differences in associations identified between SAPPHIRE and ours could relate to the variability of risk factors in adolescents versus adults or mild versus mild-to-moderate asthma.

Our study demonstrates several novel aspects as it relates to the association between African ancestry and asthma exacerbations which distinguishes it from prior studies, particularly

444 from the SAPPHIRE cohort [21, 22, 24]. First, our study is the first to demonstrate that higher
445 African ancestry associated with risk for asthma exacerbations in all Blacks, independent of sex.
446 Second, this study is the first to evaluate African ancestry in the context of race-specific
447 predictors of asthma exacerbations across multiple ethnic groups. Prior studies were limited to
448 specific minority groups without similarly recruited subjects from other ethnic groups [21, 22,
449 24]. This is important because non-Hispanic Whites had a similar risk for exacerbations (0.16)
450 compared to Blacks with an African ancestry <82% (0.16 versus 0.10 per patient-year) who
451 were both lower than Blacks with higher African ancestry (0.31 per patient-year, Figure 2).
452 These unique comparisons based on ancestry in Blacks versus Whites resulted in further
453 supportive evidence of protective genetic factors from higher European versus lower African
454 ancestry as a rationale for known inter-racial differences in asthma morbidity. Finally, this study
455 is the first to leverage longitudinal clinical trial data to evaluate African ancestry in a setting
456 where an objective asthma diagnosis, baseline asthma severity at enrollment, exacerbation
457 events, availability of chronic asthma therapies, and compliance are precisely defined,
458 documented systematically, and monitored with in-person visits over narrow time intervals. A
459 clinical trial design distinguishes us from prior studies based on a physician's diagnosis of
460 asthma that might not have fully accounted for socioeconomic factors determining asthma
461 severity or availability of chronic therapies [22].

462 We were unable to fully account for socioeconomic status, stress, environmental, or
463 cultural factors that track with ancestry to influence exacerbation risk. While socioeconomic
464 factors have been associated with increased exacerbation risk, they alone do not account for all
465 of the increased asthma risk amongst Blacks and other minority groups and the usual
466 surrogates for socioeconomic status do not fully account for the life-long social experiences
467 implicit to self-reported race [1, 5, 16, 41]. Unfortunately, socioeconomic data was not collected
468 uniformly across ACRN and AsthmaNet trials; however, medication compliance was rigorously

monitored throughout the course of these clinical trials with close clinical follow-up mitigating, at least in part, the adverse effects of lower income on access and adherence to treatment.

In conclusion, in Whites and Blacks with asthma, lower baseline lung function and a history of prior exacerbation associated with an increased exacerbation rate requiring systemic glucocorticoid therapy while in self-identified Blacks these and additional unique race-specific factors were no longer associated when the stronger effects of African ancestry was considered (Table V). These findings suggest that factors tracking with African ancestry mediated associations between lung function, allergic sinus disease, and exacerbation frequency in Blacks. Hence, studies based on self-reported race alone are insufficient to improve our understanding of genetic and environmental factors that track with ancestry which could underlie racial differences in asthma morbidity [25]. Additional studies, including whole-genome admixture mapping or GWAS complemented by multi-omic studies in longitudinal, comprehensively-characterized, diverse asthma cohorts will be required to identify the ancestry-specific genomic and environmental factors that influence exacerbation risk.

TABLE AND FIGURE LEGENDS:

Table I: Baseline Characteristics. [a]Baseline characteristics expression as a mean with standard deviation (SD) unless otherwise stated, including medians with interquartile ranges (Q1, Q3). [b]Methacholine PC20 available on 1,604 subjects (392 Blacks, 970 Whites). [c]Bronchodilator (BD) reversibility of FEV_1 in response to four puffs of albuterol available on 1,363 subjects (450 Blacks, 807 Whites). [d]Asthma Control Questionairre-6 (ACQ-6) available on 1,035 subjects (233 Blacks, 640 Whites). [e]Blood eosinophil (eos) counts available on 967 subjects (216 Blacks, 587 Whites). [f]Sputum eosinophil (eos) percentages available on 1,291 subjects (286 Blacks, 808 Whites). [g]IgE Available on 983 subjects (212 Blacks, 605 Whites).

Table II: Effects of Self-Identified Race on Exacerbation Rate in the Multi-Ethnic Trial Cohorts. Models adjusted for protocol.

Table III: Bivariate Associations with Exacerbations in the Multi-Ethnic Cohorts, Self-Identified Blacks, and Non-Hispanic Whites. [a]Relative rates (RR) for continuous variables expressed per change by one standard deviation. Models were based on repeated measures, poisson regression models adjusted for protocol. Variables not listed were not statistically significant.

Table IV: Mean exacerbation rates Above and Below Median Percentage African Genetic Ancestry by Self-Identified Race. Models adjusted for protocol.

Table V: Multivariable Models for Exacerbations in Self-Identified Blacks and Non-Hispanic Whites. Relative rates (RR) for continuous variables expressed per change by one standard deviation. Models include median percentage African genetic ancestry (AA) which varied between race dues to marked differences in distribution of Native American, European, and African ancestries. Models also include protocol, age, sex, FEV1, race, and exacerbation history. [a]Poisson regression, repeated measures.

Table VI: Multivariable Models Stratified by Self-Reported Race for Exacerbations in Trial Cohorts with Genetic Ancestry Data. Relative rates (RR) for continuous variables expressed per change by one standard deviation. Models include median percentage African genetic ancestry (AA) which varied between races due to marked differences in distribution of Native American, European, and African ancestries. Models also include protocol, age, sex, FEV1, race, and exacerbation history. [a]Poisson regression.

Figures 1a-d: Distribution of Percentage African Genetic Ancestry in the Multi-Ethnic Cohort and by Self-identified Race. Frequency histograms demonstrate the distribution of percentage whole-genome, global African ancestry determined by genetic variants in the combined multi-ethnic cohort and by racial group.

Figure 2: Effects of Genetic African Ancestry on Exacerbation Rates in Blacks and Compared to Whites. Exacerbation rates per patient-year shown for 161 Blacks above and below a median African ancestry of 82% compared to 489 non-Hispanic Whites. Models adjusted for protocol.

553 **TABLES:**

554

555 **Table I: Baseline Characteristics**

Variable[a]	All Subjects (N=1840)	Blacks (N=450)	Non-Hispanic Whites (N=1108)
Age (years)	36 (12)	37 (12)	36 (12)
Sex (n [%male])	665 (36%)	110 (24%)	667 (60%)
BMI	29 (7.7)	32 (9.2)	27 (6.6)
Subgroup with genotyping	787	165	510
Ancestry median (Q1,Q3): **% African** **% European** **% Native American**	2% (0.3%, 14.7%); 97% (54.7%, 99.1%); 1% (0.2%, 1.9%)	82% (71%, 87%); 16% (11%, 26%); 1% (1%, 2%)	0% (0%, 2%); 99% (98%, 99%); 0%(0%, 1%)
oral steroid bursts in prior 12 months	0.36 (0.82)	0.61 (1.1)	0.27 (0.65)
FEV1%predicted	82% (15%)	80% (14%)	84% (15%)
PC20, geometric mean (CV)[b]	1.54 (1.31)	1.35 (1.36)	1.60 (1.29)
%BD reversibility post 4 albuterol puffs[c]	12% (9.4%)	13% (11%)	12% (9.0%)
ACQ6[d]	0.96 (0.74)	1.02 (0.88)	0.95 (0.68)
Blood eos (absolute count/mm3), median (Q1, Q3)[e]	198 (100, 300)	178 (100, 295)	200 (100, 300)
%Sputum eos, median (Q1, Q3)[f]	0.4 (0.0, 1.7)	0.4 (0.0, 1.7)	0.4 (0.0, 1.6)
Serum IgE, median (Q1, Q3)[g]	136 (51, 333)	201 (83, 496)	107 (43, 256)

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558 **Table II: Effects of Self-Identified Race on Exacerbation Rate in the Multi-Ethnic Trial**
559 **Cohorts.**

Self-identified race (n=1,840)	Mean exacerbation rate, per person-year (SE)	RR (95% CI)	P-value
			0.30
Black (n=440)	0.21 (0.03)	1.26 (0.94,1.70)	0.13
White non-Hispanic (n=1108)	0.17 (0.02)	ref	ref
Other (n=282)	0.19 (0.04)	1.14 (0.78, 1.66)	0.50

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563 **Table III: Bivariate Associations with Exacerbations in the Multi-Ethnic Cohorts, Self-**
564 **Identified Blacks, and Non-Hispanic Whites**

Variable, per SD change for cont variables	Entire population (all races)			Self-identified Black			Non-Hispanic Whites		
	N	RR[a] (95% CI)	p-value	N	RR (95% CI)	p-value	N	RR (95% CI)	p-value
Hx steroid-requiring exacerbation: ≥2 vs 0 1 vs 0	1840	 2.9 (2.03, 4.12) 1.9 (1.37, 2.56)	 <0.001 <0.001	450	 2.2 (1.17, 4.01) 1.7 (0.97, 3.16)	 0.014 0.075	1108	 2.51 (1.49, 4.24) 1.94 (1.27, 2.99)	 <0.001 <0.001
Sex (F vs M)	1840	1.6 (1.19, 2.22)	0.002	450	1.1 (0.65, 1.86)	0.720	1108	1.48 (1.01, 2.16)	0.04
BMI per 7.7 increase (9.2 in Blacks, 6.6 in Whites)	1840	1.0 (0.90, 1.18)	0.64	450	1.1 (0.91, 1.42)	0.26	1108	0.82 (0.67, 0.99)	0.04
% pred FEV1, per 14.65% increase (13.99 in Blacks, 14.62 in Whites)	1839	0.74 (0.63, 0.87)	<0.001	450	0.73 (0.55, 0.95)	0.021	1107	0.76 (0.62, 0.93)	0.008
Log2(PC20), per 1.89 unit increase (1.94 in Blacks, 1.86 in Whites)	1604	0.85 (0.74, 0.98)	0.030	382	1.0 (0.81, 1.34)	0.742	952	0.82 (0.68, 0.99)	0.04
Sputum Eos, per 4.5% increase (4.2% in Blacks, 4.7% in Whites)	991	1.1 (1.03, 1.22)	0.009	279	1.0 (0.97, 1.06)	0.669	793	1.07 (0.92, 1.25)	0.35
Chronic sinusitis (Y vs N)	855	1.9 (1.28, 2.85)	0.002	254	2.8 (1.51, 5.07)	0.001	493	1.53 (0.83, 2.80)	0.17
GERD (Y vs N)	841	1.8 (1.20, 2.61)	0.004	250	2.2 (1.17, 4.13)	0.014	486	1.72 (0.97, 3.07)	0.06
Nasal polyps (Y vs N)	838	1.7 (1.00, 2.91)	0.049	243	2.0 (0.89, 4.55)	0.093	488	1.30 (0.56, 3.03)	0.55
Allergic rhinitis (Y vs N)	336	1.7 (1.01, 2.96)	0.045	121	2.2 (1.03, 4.68)	0.042	168	1.49 (0.63, 3.51)	0.37
Household income, per each increase in income bracket	333	0.66 (0.50, 0.85)	0.002	120	0.78 (0.50, 1.22)	0.278	168	NA	0.09
%African Ancestry, per 32% change in all (18% change in Blacks, 7% in NHW)	760	0.97 (0.78, 1.19)	0.745	161	2.1 (0.85, 5.02)	0.109	497	0.86 (0.53, 1.40)	0.55

Table IV: Mean exacerbation rates Above and Below Median Percentage African Genetic Ancestry by Self-Identified Race: Model Adjusted for Protocol.

Self-identified race	Mean exacerbation rate per person-year			
	Above Median	Below Median	RR (95%CI)	P-value
Black (n=161) Median %AA = 82%	0.315	0.103	3.06 (1.09, 8.60)	0.034
White non-Hispanic (n=489) Median %AA = 0.5%	0.263	0.190	1.43 (0.86, 2.37)	0.167
Other (n=110) Median %AA = 9.3%	0.220	0.336	0.65 (0.25, 1.73)	0.391

Table V: Multivariable Models for Exacerbations in Self-Identified Blacks and Non-Hispanic Whites.

Significant exacerbation Predictors	All Blacks (n=450)[a]		All non-Hispanic Whites (n=1108)[a]	
	RR (95% CI)	P-value	RR (95% CI)	P-value
Exacerbation history		0.053		<0.001
≥2 vs 0	2.1 (1.10-3.84)	0.024	2.5 (1.45-4.18)	<0.001
1 vs 0	1.7 (0.92-3.03)	0.091	1.8 (1.20-2.84)	0.005
Sex (Females versus Males)	1.1 (0.63-1.83)	0.79	0.7 (0.47-1.01)	0.054
% predicted FEV1, per 14% increase	0.7 (0.54-0.95)	0.022	0.8 (0.62-0.93)	0.008

Table VI: Multivariable Models Stratified by Self-Reported Race for Exacerbations in Trial Cohorts with Genetic Ancestry Data.

Significant exacerbation Predictors	Blacks, with ancestry data available (n=161)[a]		Whites, with ancestry data available (n=489)[a]	
	RR (95% CI)	P-value	RR (95%CI)	P-value
Exacerbation history		0.83		0.19
≥2 vs 0	1.5 (0.37-6.34)	0.56	1.4 (0.48-3.83)	0.57
1 vs 0	1.2 (0.35-4.33)	0.75	1.7 (0.96-3.13)	0.07
Sex (Females versus Males)	0.9 (0.31-2.52)	0.82	0.7 (0.41-1.28)	0.27
% pred FEV1, per 14% increase	1.1 (0.59-1.89)	0.86	0.8 (0.64-1.07)	0.16
%AA (median cutpoint)	3.4 (1.15-9.81)	0.027	1.5 (0.92, 2.51)	0.10

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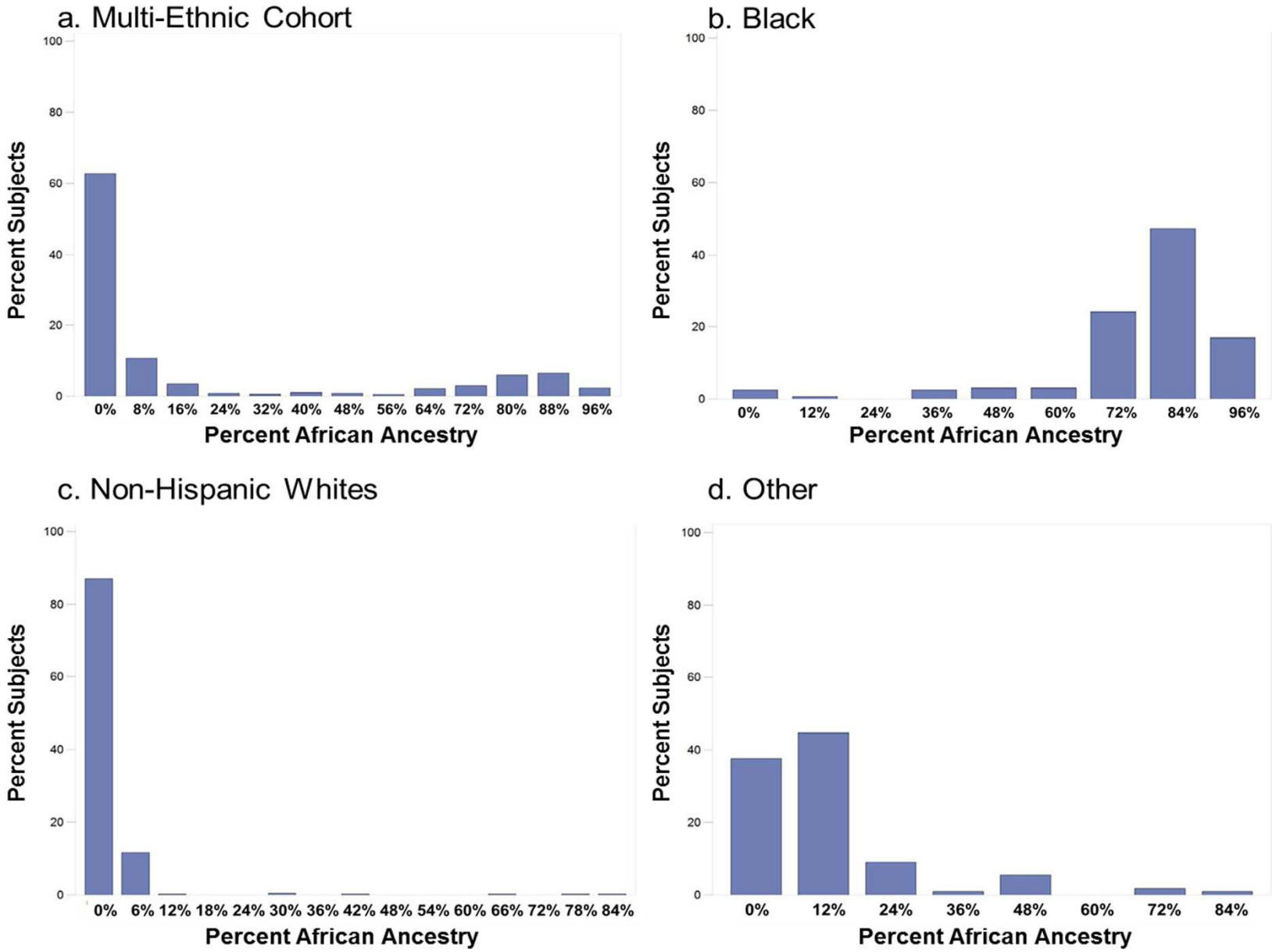

Figures 1a-d: Distribution of Percentage African Genetic Ancestry in the Multi-Ethnic Cohort and by Self-identified Race.

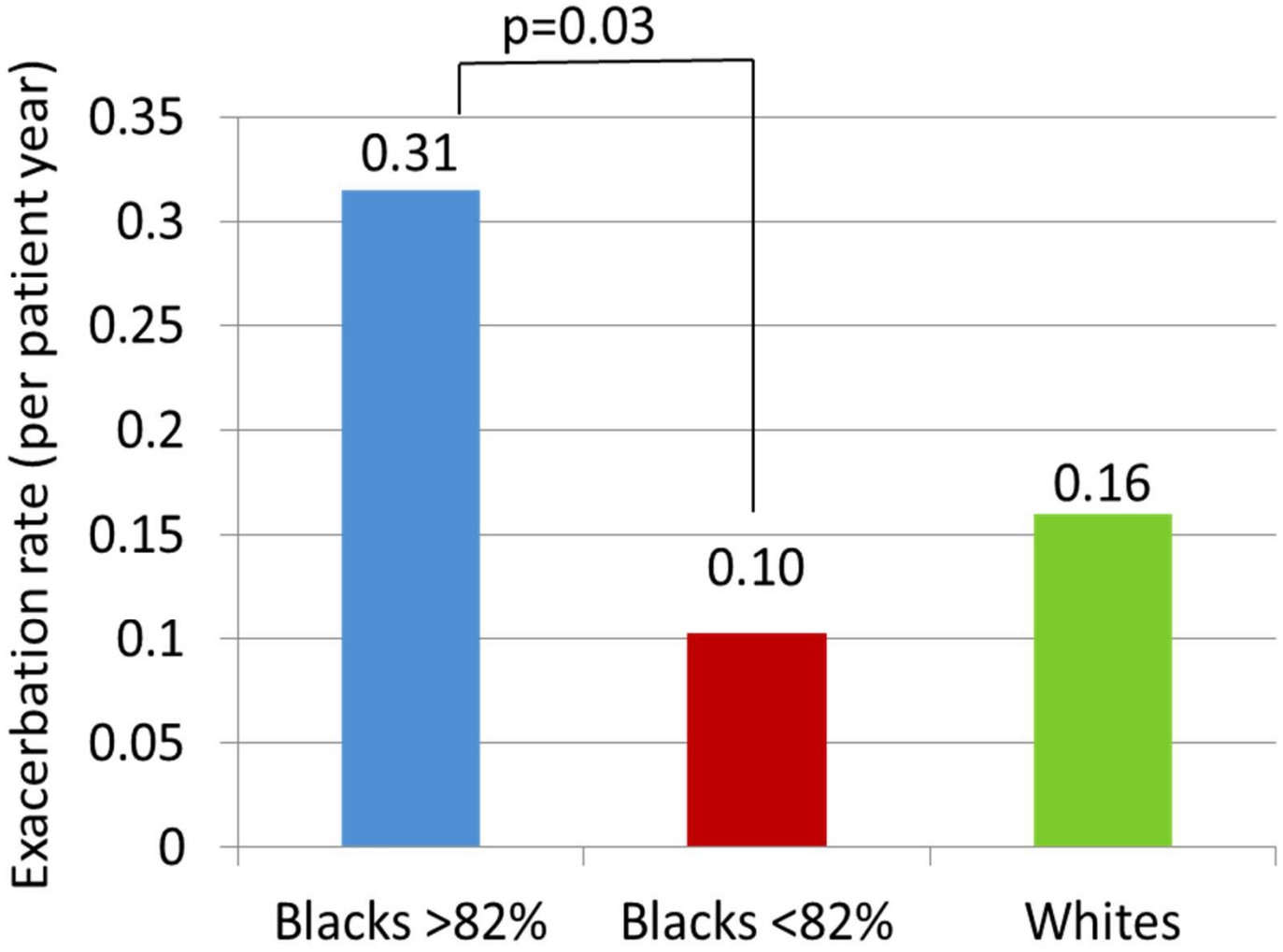

Figure 2: Effects of Genetic African Ancestry on Exacerbation Rates in Blacks and Compared to Whites

ONLINE REPOSITORY MATERIALS

Exacerbation-Prone Asthma in the Context of Race and Ancestry in Asthma Clinical Research Network Trials

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Description of the Clinical Trials Cohorts [1-13]

BASALT was a parallel, placebo-controlled trial of 342 adults with mild to moderate controlled asthma who were randomized from 2007-2010 to low-dose inhaled corticosteroid adjustment based on physician assessment, biomarkers, or symptoms. TALC was a crossover trial of 210 adults with moderate uncontrolled asthma who were randomized from 2008-2010 to one of six treatment sequences that involved permutations of tiotropium / single dose inhaled corticosteroid (ICS), long acting beta agonist (LABA) / single dose ICS, and double dose ICS. IMPACT was a parallel trial of 225 adults with mild, persistent asthma who were randomized from 2000-2003 to budesonide, zafirlukast, or placebo. SLIC was a parallel trial of 175 subjects aged 12-65 with persistent, suboptimally controlled asthma who were randomized from 1997-1999 to placebo / triamcinalone or salmeterol xinafoate / triamcinalone, with additional randomization to continue or taper the triamcinalone.SOCS was a parallel trial of 164 moderate, controlled asthmatics ages 12-65 years who were randomized from 1997-1999 to triamcinolone, salmeterol, or placebo. VIDA was a parallel trial from 2011-2014 of 408 patients with symptomatic asthma and vitamin D deficiency who had their inhaled corticosteroid tapered after randomization to vitamin D or placebo supplementation. MIA was a parallel trial of 92 uncontrolled, adult asthmatics with and without chronic mycoplasma or chlamydia airway infection who were randomized from 2006-2009 to clarithromycin / fluticasone or placebo / fluticasone.LARGE was a crossover trial of 90 subjects with the B16 Arg/Arg genotype or B16 Gly/Gly genotype who were randomized from 2004-2008 to ICS / LABA versus ICS / placebo. SLIMSiT was a crossover trial of 192 subjects from age 12-65 with moderate asthma who were randomized from 2002-2004 to montelukast / salmeterol versus beclomethasone / salmeterol. SMOG was a crossover trial of 83 smokers and non-smokers who were randomized between 2002-2004 to ICS and montelukast. BARGE was a crossover trial of 78 mild asthmatics with the Arg16Arg or Gly16Gly ADRB2 genotypes who were randomized between 1999-2002 to

scheduled treatment with albuterol or placebo. Lastly, MICE was an open-label trial of 30 subjects with persistent asthma who were randomized between 1999-2000 to budesonide or fluticasone.

Table E1: Protocol-specific information

Protocol name	N Obs Total	Median days in study	Mean exacerbation rate, per person-year (SE)	% Self-identified Black	N Obs w GWAS data available	GWAS chip[a]
SOCS	**164**	154	0.42 (0.08)	15%	75	Affy_6.0
SLIC	**175**	126	0.35 (0.07)	23%	96	Affy_6.0
MICE	**30**	147	0.17 (0.12)	17%	0	n/a
BARGE	**78**	336	0.09 (0.05)	19%	0	n/a
IMPACT	**225**	375	0.16 (0.03)	15%	148	Affy_6.0
SMOG	**83**	154	0.06 (0.04)	23%	0	n/a
SLIMSIT	**192**	221	0.26 (0.06)	29%	0	n/a
LARGE	**90**	379	0.11 (0.03)	19%	0	n/a
MIA	**92**	169	0.24 (0.07)	27%	0	n/a
BASALT	**342**	252	0.11 (0.02)	20%	297	Illumina Exome Chip
TALC	**210**	341	0.18 (0.04)	29%	185	Illumina Exome Chip
VIDA	**408**	197	0.33 (0.04)	33%	0	n/a
Total unique individuals with complete exacerbation data	**1840**	n/a	**0.20 (0.01)**	**24%**	**760**	n/a

[a]Affy_6.0=Affymetrix Chip Affy_6.0, Illumina Exome Chip= Illumina OmniExpress HumanExome BeadChip

Table E2: Baseline Characteristics of the Other Ethnic and Racial Groups who did not Identify as Primarily Black or White, including Hispanics.

Variable[a]	Other (N=282)
Age (years)	34 (11)
Sex (n [%male])	43 (15%)
BMI	28.1 (6.7)
Subgroup with genotyping	111
Ancestry median (Q1,Q3): % African % European % Native American	9% (4%, 15%); 64% (43%, 73%); 23% (10%, 39%)
oral steroid bursts in prior 12 months	0.30 (0.65)
FEV1%predicted	80% (15)
PC20, geometric mean (CV)[b]	1.63 (1.29)
%BD reversibility post 4 albuterol puffs[c]	12% (8.4%)
ACQ6[d]	0.92 (0.74)
Blood eos (absolute count/mm3), median (Q1, Q3)[e]	200 (106, 300)
%Sputum eos, median (Q1, Q3)[f]	0.4 (0.0, 2.4)
Serum IgE, median (Q1, Q3)[g]	178 (75, 419)

[a]Baseline characteristics expression as a mean with standard deviation (SD) unless otherwise stated, including medians with interquartile ranges (Q1, Q3). [b]Methacholine PC20 available on 242 subjects. [c]Bronchodilator (BD) reversibility of FEV_1 in response to four puffs of albuterol available on 106 subjects. [d]Asthma Control Questionairre-6 (ACQ-6) available on 162 subjects. [e]Blood eosinophil (eos) counts available on 164 subjects. [f]Sputum eosinophil (eos) percentages available on 197 subjects. [g]IgE Available on 166 subjects.

Table E3: Effects of African, European, and Native American Genetic Ancestry on Exacerbation Rate in the Multi-Ethnic Trial Cohorts.

Percentage Genetic Ancestry (n=760 with Genetic Data)	RR, per 1SD increase (95%CL)	p-value
% African, per 32% increase	0.97 (0.78, 1.19)	0.74
% European, per 34% increase	0.98 (0.80, 1.20)	0.83
% Native American, per 13% increase	1.10 (0.95, 1.29)	0.21

Table E4: Median African Ancestry-Nested Multivariable Model for Exacerbations in the Multi-Ethnic Trial Cohorts

Significant exacerbation predictors	**All races (n=1840)[a]**		**All races, subjects with ancestry data available (n= 760)[b]**		**All races, ancestry nested within race (n=760)[b]**	
	RR (95% CI)	P-value	RR (95% CI)	P-value	RR (95% CI)	P-value
Exacerbation history		<0.001		0.044		0.043
≥2 vs 0	2.55 (1.80, 3.61)	<0.001	2.04 (1.09, 3.83)	0.026	2.09 (1.11, 3.94)	0.022
1 vs 0	1.73 (1.26, 2.37)	<0.001	1.54 (0.93, 2.56)	0.097	1.51 (0.91, 2.52)	0.112
Sex (Females versus Males)	1.61 (1.17, 2.20)	0.003	1.52 (0.96, 2.40)	0.074	1.52 (0.96, 2.41)	0.072
% pred FEV1, per 15% increase	0.73 (0.62, 0.86)	<0.001	0.81 (0.63, 1.05)	0.116	0.83 (0.64, 1.07)	0.155
Race		0.865		0.622		0.757
Black vs. Non-Hispanic White	1.07 (0.79, 1.45)	0.645	0.84 (0.49, 1.44)	0.534	1.08 (0.56, 2.07)	0.815
Other vs Non-Hispanic White	1.07 (0.75, 0.54)	0.698	1.18 (0.68, 2.04)	0.557	0.77 (0.33, 1.75)	0.527
%AA nested within race (median cutpoint)	n/a	n/a	n/a	n/a		0.083
Blacks with ≥82% AA vs <82% AA (N=161)	n/a	n/a	n/a	n/a	2.84 (1.00,8.04)	0.0495
Others with ≥9.3% AA vs <9.3% AA (N=110)	n/a	n/a	n/a	n/a	0.59 (0.22, 1.56)	0.287
White Non-Hispanic with ≥0.5% AA vs <0.5% AA (N=489)	n/a	n/a	n/a	n/a	1.40 (0.84, 2.32)	0.196

Relative rates (RR) for continuous variables expressed per change by one standard deviation. Models include median percentage African genetic ancestry (AA) nested within race due to marked differences in distribution of Native American, European, and African ancestries between self-reported races. Models also include protocol, age, sex, FEV1, race, and exacerbation history. [a]Poisson regression, repeated measures. [b]Poisson regression.

Table E5: Baseline Characteristics of the 161 Blacks with Genetic Data by Internal Validation Groupings Based on Trial Cohort and Genotyping Platform.

Black Cohort Grouping	SOCS/SLIC/IMPACT (N=51)	TALC (N=51)	BASALT (N=59)
Genotyping Platform[a]	Affy_6.0	Exome Chip	Exome Chip
Age	33.3 (11.1)	41.8 (12.1)	37.3 (11.1)
Sex (% Male)	24 (47%)	14 (27.4%)	21 (35.6%)
BMI	28.9 (8.19)	35.5 (9.98)	32.6 (8.97)
FEV1, %Predicted	81.3 (13.0)	70.5 (13.2)	83.9 (11.7)
oral steroid bursts in prior 12 months	0.45 (0.92)	0.76 (1.58)	0.47 (1.09)
Blacks with ≥82% AA (% Blacks ≥82% AA)[b]	24 (47.1%)	19 (37.2%)	36 (61.0%)

Data shown as mean with standard deviations, unless otherwise stated. [a]Affy_6.0=Affymetrix Chip Affy_6.0, Illumina Exome Chip= Illumina OmniExpress HumanExome BeadChip.
[b]AA=African genetic ancestry.

Table E6: Blacks with ≥82% AA vs those with <82% AA

	AA <82%		AA ≥ 82%		p-value
Variable	**N**	**Mean (SD), unless otherwise stated**	**N**	**Mean (SD), unless otherwise stated**	
Age	85	38 (12)	80	36 (11)	0.24
Sex (% Male)	85	36%	80	35%	0.84
BMI	85	32 (10)	80	32 (8.8)	0.86
ACQ6	73	1.0 (0.89)	65	0.93 (0.89)	0.51
Blood eos, median	65	180	62	105	0.10
% sputum eos, median	57	0.4	48	0.5	0.90
Serum IgE, median	66	134	60	197	0.21
FEV1, Liters	85	2.42 (0.68)	80	2.31 (0.59)	0.27
lnPC20	70	0.38 (1.23)	63	0.36 (1.14)	0.93
% with Chronic sinusitis	55	9%	55	25%	0.02
% with Nasal polyps	51	8%	53	13%	0.37
% with GERD	55	16%	55	16%	1.00

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