Title

Pharmacogenetic studies of long-acting beta agonist and inhaled corticosteroid responsiveness in randomised controlled trials of individuals of African descent with asthma

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Data Sharing Statement:

Inhaled Corticosteroid Response in Randomized Controlled Trials of African Descent Minorities with Asthma

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Immediately following publication, we will share de-identified individual participant clinical trial and genome-wide genotyping data that underlie the reported results for the BARD trial cohorts through the database of Genotypes and Phenotypes (dbGaP). dbGaP will provide controlled access to these data for investigators whose proposed use of the data has been approved by an independent data access committee. For more information on applying for access, please contact dbgap-sp-help@ncbi.nlm.nih.gov. The protocols and analytical methods for determining the hierarchal composite primary clinical outcome for the BARD clinical trials are provided in the parent manuscript.[7]

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Abstract

Background: Pharmacogenetic studies in asthma cohorts, primarily whites of European descent, have identified loci associated with response to inhaled beta agonists and corticosteroids (ICS). Because differences exist in how individuals from different ancestral backgrounds respond to long-acting beta agonist (LABA) and ICS, it is important to understand pharmacogenetic mechanisms regulating therapeutic responsiveness in African descent minorities.

Methods: We performed whole-genome admixture mapping in 249 children and 267 adolescents/adults from the Best African Response to Drug (BARD) trials based on the composite superior response outcome comparing step up from low-dose ICS to quintupling (5xICS) versus doubling ICS (2.5xICS) or 5xICS versus adding LABA (salmeterol) to fluticasone 100mcg twice daily (FP100SAL).

Findings: In children, we identified a significant admixture mapping peak for superior responsiveness to 5xICS versus FP100SAL on chromosome 12 ($OR_{local\ African}$=3.95, 95%, CI=2.02–7.72, p=6.1x10^{-5}) fine mapped to a locus adjacent to *RNFT2* and *NOS1* (rs73399224, $OR_{allele\ dose}$=0.17, 95%CI=0.07–0.42, p=8.4x10^{-5}). In adolescents/adults, we identified a peak for superior responsiveness to 5xICS versus 2.5xICS on chromosome 22 ($OR_{local\ African}$=3.35, 95% CI=1.98–5.67, p=6.8x10^{-6}) containing a locus adjacent to *TPST2* (rs5752429, $OR_{allele\ dose}$=0.21, 95%CI=0.09–0.52, p=5.7x10^{-4}). We replicated rs5752429 and nominally replicated rs73399224 in independent African American cohorts.

Interpretation: BARD is the first genome-wide pharmacogenetic study of LABA and ICS response in clinical trials of African descent minorities to detect and replicate genome-wide significant loci. Admixture mapping of the composite BARD trial outcome enabled the identification of novel pharmacogenetic variation accounting for differential therapeutic responses in African descent asthmatics.

Keywords

asthma; pharmacogenetics; genes; beta agonist; long-acting beta agonist; African American; ethnic group; ancestry; admixture mapping; single nucleotide polymorphism; corticosteroid

Introduction

Inhaled beta2-adrenergic receptor agonist (beta agonist) and corticosteroids (ICS) are the most commonly used inhaled medications for the management of asthma. Long-acting beta agonists (LABAs) were once suspected to increase the risk for asthma-related deaths in observational studies of salmeterol, but this safety concern has been refuted by a number of studies, including the international LABA safety study.[1,2] ICS are an effective first-line therapy for asthma, but there is high interindividual variability in therapeutic responsiveness with a significant proportion showing worsening of asthma control or a lack of preferential response compared to alternative therapies.[3,4] Nonetheless, while LABA and ICS are effective in most individuals with asthma, observational cohort studies and *post hoc* analyses of clinical trials suggest that individuals from different ethnic groups respond differently to these therapies.[2,3,5,6]

After an increased risk for LABA treatment failures in African Americans compared to non-Hispanic whites was observed in trials from two NHLBI-sponsored asthma networks,[5] the NHLBI-sponsored Best African Response to Asthma Drugs (BARD) trials were performed by AsthmaNet investigators to identify the optimal combination of a LABA and ICS in African descent asthmatic individuals not controlled on low-dose ICS therapy.[7] The BARD trials found that adolescents and adults showed a superior response to a LABA-containing therapy. By contrast, a similar proportion of children showed a superior responsiveness

to higher-dose ICS monotherapy versus LABA-containing therapies while a significantly larger proportion showed a superior response to quintupling versus doubling ICS dose. Whole-genome genotyping for estimating global African ancestry in BARD participants was not associated with the superiority therapeutic outcome.[7] However, a retrospective analysis of Asthma Clinical Research Network (ACRN) trials demonstrated that self-reported Blacks with higher African ancestry had an increased risk for exacerbation-prone asthma compared to both Blacks with lower African ancestry and whites implicating ancestry-specific risk factors for inter-ethnic differences in asthma severity, including pharmacogenetic factors.[8] In comparison to this analysis of parallel controlled trials, BARD was a crossover trial based on a composite outcome of three dimensions of asthma control.[7,8]

Global genetic ancestry strongly aligns with historical, geographic, cultural, and socio-economic factors which likely contribute to African ancestral associations previously reported for lung function and exacerbations.[8–10] Hence, the contrasting global ancestry associations reported in ACRN and BARD does not exclude ancestry-specific loci for asthma severity and drug response.[7,8,11] Pharmacogenetic studies in asthma cohorts have identified over 100 loci associated with therapeutic responsiveness to inhaled beta agonists or ICS primarily in whites of European descent.[12–22] Pharmacogenetic studies of short-acting beta agonist (SABA) response in African descent ethnic groups have identified novel beta agonist response loci with higher allele frequencies in ethnic groups and geographical regions with higher global African ancestry.[18–20] A smaller number of pharmacogenetic studies have identified ancestry-specific rare variants that could influence responsiveness to LABA.[19,23,24]

Admixture mapping maps ancestry in genomic regions throughout the genome to test for associations between local ancestry and phenotype. Admixture mapping is more powerful than allelic association (GWAS) especially when applied to phenotypes that vary in expression between groups from different ancestral backgrounds, such as asthma drug response and severity.[5,6,11,25] We hypothesize that while the parent BARD trials did not identify global ancestral associations with therapeutic responsiveness, genetic variants in specific loci throughout the genome from a common ancestry affect inter-individual LABA and ICS responsiveness in African descent individuals with asthma.

Methods

Overview

The BARD trials randomized 574 individuals with asthma uncontrolled with low-dose ICS (1xICS: fluticasone propionate [FP] 50mcg in children, 100mcg in adolescents and adults) who reported at least one Black grandparent to four different step-up combinations therapies. These included the step-up to double (2–2.5xICS: 100mcg twice daily in children, 250mcg twice daily in adolescents and adults) or quintuple the dose of fluticasone (5xICS: 250mcg twice daily in children and 500mcg twice daily in adolescents and adults), or the addition of a LABA (salmeterol 50mcg twice daily) to an ICS (FP100SAL and FP250SAL) in a cross-over design. The BARD trials were registered (ClinicalTrials.gov NCT01967173) and the trial protocol, DNA collection, and genetic studies were approved by the AsthmaNet Steering Committee, an NHLBI-appointed protocol review committee, a data and safety

monitoring board, and institutional review boards at all sites. Study coordinators obtained informed consent from all subjects.

We performed ancestry-based pharmacogenetic studies of the BARD primary hierarchal composite outcome of pairwise superior responsiveness based on asthma exacerbations, a 31-day difference in annualized asthma-control days, or a five percent difference in percentage predicted FEV_1 in 249 unrelated children and 267 unrelated adults and adolescents genotyped with the Illumina Multi-Ethnic Global Array (MEGA) genotyping array (Figure 1).[7,26] Detailed genotyping methods and an illustrated summary of the analyses and key findings (Figure S1) are provided in the supplementary appendix.

Whole-Genome Admixture Mapping

For admixture mapping (Figure 1), we stratified by children and the pooled adolescent and adult groups and designated the step up to 5xICS versus 2–2.5xICS and 5xICS versus FP100SAL as our primary pairwise comparisons without adjustment for the multiple treatment arm comparisons similar to the parent study. These co-primary comparisons were based on the lack of discernible efficacy differences for medium-dose ICS compared to low or high-dose ICS.[27] These comparisons also revealed important preferences in children (5x versus 2xICS) and adolescents/adults (LABA versus 5xICS) in the parent study.[7] We inferred local ancestry using RFMix version 1.5.4 and the 1000 genomes CEU (n=99) and YRI (n=108) panels.[28,29] A total of 15,159 local ancestry segments were inferred by RFMix, and genome-wide African ancestry estimates were highly concordant with genome-wide ancestry estimates from ADMIXTURE (0.7% mean difference).[30] Ordinal regression models tested for associations between the number of copies of local African ancestry (0, 1 or 2 copies) and the composite categorical outcome (inferior, equivalent, and superior response) for each pairwise treatment comparison and included AsthmaNet partnership, age, sex, season, and global genetic African ancestry as covariates.[7] The genome-wide significance threshold was a Bonferroni adjustment for the number of effective tests calculated for all autosomes based on 312 independent ancestral regions resulting in a $p<1.6x10^{-4}$ (details in the supplementary appendix).[31] We combined the children and adolescent/adult age groups using inverse-variance meta-analysis.

Fine Mapping and Local Ancestral Segments Interactions

Fine mapping of genome-wide significant peaks was performed using the Local Ancestry Adjusted Allelic Association (LAAA) model (Figure 1) after imputation to the TOPMed reference panel on the Michigan imputation server and re-calling of local ancestry at imputed variants with high imputation accuracy (Rsq ≥ 0.9).[32,33] Within admixture mapping peaks, we used the ordinal regression approach for admixture mapping fit for LAAA to jointly model allele dose, local African ancestry dose, and allele-African interaction dose for single nucleotide polymorphisms (SNP) with a minor allele frequency (MAF) ≥0.03 (details in the supplementary appendix).[32] Top associated SNPs with $p_{\text{allele dose}}<10^{-3}$ within genome-wide significant peaks were carried forward for replication.

Replication

For children, we tested for replication in two independent African descent minority asthma cohorts with retrospective data on exacerbations and medication use over the past year in adolescents and children: African Americans from the Study of Asthma, Genes and the Environment (SAGE, 170 treated with LABA+ICS, 379 treated with ICS) and Puerto Ricans from the Genes-environments and Admixture in Latino Americans (GALA II, 134 treated with LABA+ICS, 230 treated with ICS).[16,34] For adolescents/adults, we tested for replication in a 12-month randomized, parallel clinical trial of adult and adolescent African American asthmatics (AAAA trial) treated with high-dose budesonide (N =222) or low-dose budesonide with the LABA, formoterol, (N=218) characterized for exacerbations.[35] Logistic regression models tested for associations with exacerbations (defined in the supplementary appendix) adjusted for age, sex, BMI and genome-wide African ancestry in the AAAA trial cohort; and age, sex, obesity, first two principal components of genotype data in SAGE and GALA II. Replication models were stratified by treatment in SAGE/GALA II (ICS only versus LABA+ICS) and by trial arm in AAAA. Replication p-values achieving region-wide significance are <0.05 divided by the number of independent tests within an admixture mapping segment (details provided in supplementary appendix), otherwise nominal p-values were <0.05.[36]

Candidate Loci Studies

Candidate loci analyses were performed independent of admixture mapping to confirm whether 101 independent pharmacogenetic loci previously associated with corticosteroid or beta agonist response determine therapeutic responsiveness in African descent individuals. Due to the lower frequency of many candidate loci in African descent populations, we applied a dominant genetic mixed-effects model with multinomial logistic regression adjusting for the same co-variates as admixture mapping.[7,26]

Reporting Guideline Compliance

Our reporting abides by the STrengthening the Reporting Of Pharmacogenetic Studies (STROPS) guidelines (checklist in supplementary appendix).[37]

Role of the funding source

The sponsors of the study, including the NHLBI, had no involvement in study design; data collection, data analysis, and data interpretation; or the preparation of the report and the decision to submit for publication. Victor E. Ortega, MD, PhD; Michelle Daya, PhD; Eugene R. Bleecker, MD; Deborah A. Meyers, PhD; Elliot Israel, MD; and Michael E. Wechsler, MD had full access to all the data in the study and accepted final responsibility for the decision to submit for publication.

Results

Baseline Characteristics:

The baseline characteristics for the 249 unrelated children and 267 adolescents and adults evaluated in BARD pharmacogenetic studies are summarized in Table 1. The number of subjects for each pairwise treatment arm comparison is summarized in Table S1.

Admixture Mapping and Fine Mapping of Peaks for the co-Primary Outcomes in Children:

In children, there were two genome-wide significant admixture mapping peaks associated with superior responsiveness to 5xICS versus FP100SAL on chromosome 6 (hg19 nucleotide positions [pos] 10,546,283–11,089,096, odds ratio for the local African ancestral effect [$OR_{local\ African}$]=0.26 [95%CI=0.13–0.51], p=8.2x10^{-5}, Table 2, Figure S2A) and on chromosome 12 (pos 117,349,857–119,305,282, $OR_{local\ African}$=3.95 [2.02–7.72], p=6.1x10^{-5}, Table 2, Figure S2A). The chromosome 6 admixture mapping peak segment was fine mapped to intronic SNPs within *SYCP2L* (rs58273381/rs75872959, MAF=0.05, $OR_{allele\ dose}$=10.6 [95%CI=2.73–41.1], p=6.4x10^{-4}, Table 3, Figure S3A). The top allelic associations in the chromosome 12 admixture mapping peak segment were fine mapped to SNPs adjacent to *RNFT2* (rs73399232, MAF=0.12, OR_{allele} dose=0.19 [95%CI=0.09–0.41], p=2.4x10^{-5}; rs73399224, MAF=0.08, $OR_{allele\ dose}$=0.17 [0.07–0.42], p=8.4x10^{-5}, Table 3, Figure S4A) and *NOS1* (rs9658447, MAF=0.06, $OR_{allele\ dose}$=0.20 [92%CI=0.08–0.52], p=2.4x10^{-5}, Table 3, Figure S4A). We also identified a genome-wide significant admixture mapping peak for superior responsiveness to 5x versus 2xICS on chromosome 6 (pos 12,882,674–13,111,241, $OR_{local\ African}$=0.31 [95%CI=0.17–0.56], p=1.1x10^{-4}, Table 2, Figure S2A) that was independent of that mapped for 5xICS versus FP100SAL. Fine mapping of this peak showed a top associated SNP, rs112596714, between *EDN1* and *PHACTR1* (MAF=0.03, $OR_{allele\ dose}$=0.09 [95%CI=0.03–0.34], p=3.7x10^{-4}, Table 3, Figure S3B).

Admixture Mapping and Fine Mapping for the co-Primary Outcomes in Adolescents and Adults:

In adolescents and adults, we identified a peak for superior responsiveness to 5xICS versus 2.5xICS on chromosome 22 (pos 27,429,052–27,896,369, $OR_{local\ African}$=3.35 [95%CI=1.98–5.67], p=6.8x10^{-6}, Table 2, Figure S2B). This peak segment was fine mapped to SNPs adjacent to *TPST2* (rs142225730/rs145267568, MAF=0.03, $OR_{allele\ dose}$=0.09 [95%CI=0.02–0.32], p=2.0x10^{-4}; rs5752429, MAF=0.36, $OR_{allele\ dose}$=0.21 [95%CI=0.09–0.52], p=5.7x10^{-4}, Table 3, Figure 2A).

Replication between BARD age groups and meta-analyses:

The top admixture mapping peak chromosomal segments across the children and adolescent/adult age groups did not replicate between age groups (Table S2). Meta-analyses combining age groups by similar treatment groups did not identify admixture mapping peaks reaching genome-wide significance.

Replication in Children:

The results of replication analyses for the co-primary outcome comparisons are shown in the supplementary appendix (Tables S3–S6). In BARD children, we found nominal evidence for replication of rs73399224 associated with superior responsiveness to 5xICS versus FP100SAL. 38% of BARD children homozygote for the rs73399224 common allele (AA, N=167) and 61% of minor allele heterozygotes (AG, N=31) showed a superior response to FP100SAL (FP100SAL superior, Figure S4B) while 49% of AA homozygotes and 23% of AG heterozygotes showed a superior response to quintupling ICS (5xICS superior, Figure S4B). Hence, there was a preferential response to FP100SAL for rs73399224 minor allele genotypes nominally replicated in ICS-treated SAGE participants where a higher proportion of African Americans with exacerbations was found among minor allele heterozygote and homozygotes (OR=1.97 [95%CI=1.07–3.64], p=0.03 [p>0.017 threshold for three independent tests], Figure S4C, Table S5). rs73399224 was not replicated in LABA-treated SAGE participants (OR=0.74 [95%CI=0.27–2.07], p=0.57, Table S5) consistent with an ICS-specific pharmacogenetic effect.

Replication in Adolescents and Adults:

In BARD adolescents and adults, we found evidence for replication of rs5752429 on chromosome 22 associated with superior responsiveness to 5xICS versus 2.5xICS. 23% BARD homozygotes for the rs5752429 common allele (GG, N=83), 42% of minor allele heterozygotes (AG, N=100) and 42% of AA minor allele homozygotes (N=24) showed a superior response to doubling ICS (2.5xICS superior, Figure 2B) while 41% of GG homozygotes, 32% of AG heterozygotes, and 29% of AA minor allele homozygotes showed a superior response to quintupling ICS (5xICS superior, Figure 2B). Hence, there was a preferential response to 2.5xICS rather than 5xICS for rs5752429 minor allele genotypes. In high-dose ICS-randomized AAAA trial participants, a higher proportion of African Americans with exacerbations was found among minor allele heterozygote and homozygotes (OR=2.28, [95%CI=1.33– 3.90], p=0.003 [p<0.017 threshold for three independent tests], Figure 2C, Table S6). rs5752429 was not replicated in LABA-randomized AAAA participants (OR=1.30 [95%CI=0.68–2.50], p=0.43, Table S6).

For replicated loci, significant differences in the BARD composite superiority outcome across genotypes were driven by differences in exacerbations (rs73399224, $OR_{allele\ dose}$=0.25 [95%CI=0.09–0.68], p=6.2x10^{-3}) and asthma control days in children ($OR_{allele\ dose}$=0.21 [95%CI=0.08–0.51], p=6.0x10^{-4}) and asthma control days in adolescents and adults (rs5752429, $OR_{allele\ dose}$=0.31, [95%CI=0.12–0.76], p=0.01, Table 4). For both SNPs, differences in the remaining components of the composite outcome showed a similar direction of effect (Table 4).

Admixture Mapping and Fine Mapping for Secondary Outcomes:

We summarized results from admixture mapping (Table S7, Figures S5–S6), fine mapping (Tables S8–S9), and replication (Tables S10–13) in the supplementary appendix. The only replicated genome-wide significant admixture mapping peak was for response to 2xICS vs FP100SAL in children which fine mapped to rs35922748 (Table S8, Figures S7A–S7B) and replicated in ICS-treated Puerto Ricans from GALA II (Table S11a, Figure S7C).

Candidate Loci Studies:

Candidate pharmacogenetic loci tested for the co-primary outcomes are shown in Tables S14–S19. The top associations in adults and adolescents were for 5xICS versus 1xICS+LABA and included rs17834628 in *DNAH5* (OR=0.27 [95%CI=0.11–0.62], p=0.002, Table S14a).[18] For children, the top association was for 5xICS versus 2xICS at rs6467778 in *TRIM24* (OR=0.34 [95%CI=0.16–0.72, p=0.005, Table S19b).[28]

Discussion

The BARD trials were designed to identify the optimal combination asthma therapy for African descent individuals with uncontrolled asthma. In contrast to prior studies, the drug response outcome in BARD was not based on group mean or proportional response phenotypes, but leveraged a hierarchal, composite outcome to demonstrate the heterogeneity of inter-individual preferential response across treatment strategies.[7] This outcome was combined with the power of admixture mapping to identify novel pharmacogenetic loci for preferential response to the addition of either LABA or high-dose ICS therapy to low-dose ICS.[26] Pharmacogenetic studies for ICS and beta agonist response in asthma have been based on unidimensional outcomes from a small number of randomized, parallel clinical trials of primarily European whites and primarily SABA response in African descent individuals.[12–22] The ability to account for multiple dimensions of therapeutic response with a composite outcome enabled detection of pharmacogenetic loci with varying effects on exacerbations, symptom control, and lung function cumulatively resulting in genome-wide significant discoveries (Table 4).

In BARD adolescents and adults, our approaches identified a variant adjacent to *TPST2* (Table 3, Figures 2A–B) determining superior responsiveness to quintupling versus doubling ICS therapy which replicated in African Americans randomized to high-dose ICS therapy (Figure 2C).[35] *TPST2* encodes tyrosyl-protein sulfotransferase-2 which regulates the tyrosine sulfation of chemokine receptors, including CCR8, implicated in T2 inflammation in asthma.[38,39] We also confirmed loci from prior studies, including a locus for SABA response previously found in GALA II and SAGE cohorts (Table S14a, *DNAH5*), confirming the robustness of this approach.[18]

In children, we identified a locus for quintupling ICS therapy versus adding a LABA in children (Table 3, Figures S4A–S4C) close to *RNFT2*. *RNFT2* negatively regulates IL-3/IL-3Rα signaling, a signaling pathway that induces TNF-α and is suppressed by corticosteroids and LABA.[40,41] Another neighboring gene encodes nitric oxide synthase (*NOS1*), a locus associated with asthma risk and airways inflammation.[42] *NOS1* is a nitric oxide biosynthetic pathway gene of which several have been associated with ICS and beta agonist response.[17] We did not replicate this locus at a regional-level of significance, but SAGE reported exacerbations and medication based on self-reported recollection. Despite the limitations of retrospective data, we previously identified a rare LABA response locus using this approach that was independently confirmed.[23,24] We also replicated associations for one of the secondary comparisons in BARD children in Puerto Ricans at rs35922748 adjacent to *ADAMTS2* (Table S11a), a GWAS locus for FEV_1/FVC ratio.[43] Glucocorticoids

increase *ADAMTS2* expression in CD14+ monocytes and alveolar macrophages providing a plausible pharmacogenetic link.[44]

We assessed for replication of our adolescent and adult findings in the AAAA trial, but there was no available pediatric asthma clinical trial cohort of African descent with genotyping data to confirm rs73399224.[35] Admixture mapping detects loci from a common ancestry that vary in frequency between individuals from different ancestral backgrounds, including low frequency variants.[11] The minor allele of rs73399224 is primarily found in individuals of African descent (BARD MAF=0.08, Table 3) but is exceedingly rare in European whites (1000 Genomes MAF=0.001, Table 3) making replication in a European white pediatric cohort improbable.[11,28] A low European allele frequency also limited our ability for tissue eQTL mapping due to the paucity of RNA expression datasets from African descent individuals. In addition, pharmacogenomic eQTL relate to drug exposures and most RNA expression datasets are not from therapeutic studies.[22,45]

Replication across BARD age groups was unsuccessful which raises the question of the application of pharmacogenetic discoveries across the ages (Table S2). From a trial design perspective, baseline and step-up doses of fluticasone propionate across BARD treatment arms were lower in children compared to adolescents and adults, and the lowest ICS dose-LABA combination therapy was a step-up in ICS dose in children. These issues might have influenced genetic associations between age groups. In addition, the BARD trials found differences across the ages in superior responsiveness to the step up to higher-dose ICS monotherapy and LABA-containing therapies.[7] Children also showed higher baseline lung function, blood eosinophils, and serum IgE compared to adolescents and adults (Table 1). Similarly, in the NHLBI-sponsored Severe Asthma Research Program (SARP), lung function impairment was more frequent in adult-onset asthma where LABA-induced bronchodilation is relevant while early-onset and childhood asthma is more likely to be triggered by T2 inflammation, the therapeutic target of ICS.[46] These differences might have influenced age-specific differences in the pharmacogenetic loci discoveries we report.

The discovery of unique pharmacogenetic loci to individual age groups is not unique to our study. For example, we previously identified a locus for SABA bronchodilator response in African American children with asthma unique to African ancestry which had an opposite effect in SARP, a predominantly adult cohort.[20] The first GWAS for ICS response discovered the *GLCCI1* locus in pediatric asthma trial cohorts that was not confirmed in adults.[12,47] Age-related differences in the genetic architecture of therapeutic responsiveness likely relate to the variable roles of environmental exposures, viral response, innate immunity, and allergic inflammation across the lifespan, a phenomenon also relevant to GWAS loci for asthma susceptibility.[12,47,48]

Since BARD did not include whites, it remains to be determined if the novelty of the loci identified in this African descent cohort is related to the outcome or differences in genetic architecture between ethnic groups in which clinical differences have been described.[2,3,5,6] Due to BARD inclusion criteria, BARD participants had a broad range of global genetic African ancestry with a mean of 76–79% (Table 1). On average, African ancestry in BARD was less than reported in African American, Afro-Caribbean, and continental

African populations, but higher compared to Puerto Ricans due to varying admixture histories.[49] Hence, allele frequency differences across varying ancestral backgrounds limit the applicability of less frequent, African descent variants across all populations.[11]

The parent trial recruited a sample size achieving 90% power to detect a treatment preference of 0.2 assuming 25% loss to follow-up.[7] Post-hoc power estimates simulating our ordinal regression admixture mapping model and correction of 312 independent ancestral segments demonstrate 42% power to detect the reported effect sizes ($OR_{local\ African}$=3.3, Table 3). Factors not considered in this power estimate include the BARD cross-over trial design that monitored access and compliance to different controller therapies in the same individuals.[7] A cross-over design adjusted for geographic site and season minimized confounding by environmental and socioeconomic factors. Despite these strengths, the resulting pairwise comparisons across treatment arms increase the likelihood for false positive findings which we addressed by focusing on co-primary outcomes and demonstrating independent confirmation.

BARD is the first pharmacogenetic study of LABA and ICS response based on clinical trials of African descent minorities to detect and replicate genome-wide significant loci. Our variant discoveries partially explain the heterogeneity of preferential therapeutic response as described in BARD and are examples of risk variants that predict a lack of response to high-dose ICS therapy. Individuals with the risk rs73399224 and rs5752429 genotypes are a subgroup for which alternative step-up strategies should be considered, including the addition of a LABA. These approaches are applicable to future pharmacogenetic studies of diseases variably expressed across ethnic groups, especially as whole-genome data becomes increasingly available in diverse populations. These BARD pharmacogenetic studies demonstrate a rationale for prioritizing ancestral diversity and inclusion across the ages for the identification of more complete precision medicine profiles of drug response and disease severity.[23]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Research in Context Sidebar:

Evidence before this study:

We searched PubMed for articles published in any language before March 1, 2021 using the terms “asthma,” “beta agonist,” “inhaled corticosteroids,” “long-acting beta agonist,” ‘pharmacogenetics,” “genetics,” “admixture mapping,” and “genome-wide association study” or “GWAS.” This search identified candidate gene association studies and GWAS of asthma cohorts which assessed short-acting beta agonist (SABA) bronchodilator response and asthma clinical trials randomizing patients to inhaled corticosteroids (ICS). All pharmacogenetic studies of ICS response were based on clinical trials of whites of European descent. There were two discovery GWAS meta-analyses of long-acting beta agonist (LABA) and ICS response and two admixture mapping studies of SABA response that included Puerto Ricans and African Americans. The two GWAS meta-analyses of LABA and ICS response that included African Americans and Puerto Ricans were based on retrospective self-report of medication use and exacerbations but did not identify genome-wide significant loci. Therapeutic response phenotypes across all studies were based on unidimensional phenotypes.

Added value to this study:

To the best of our knowledge, our pharmacogenetic study of the BARD clinical trials is the first to identify genome-wide significant and independently confirmed genetic variants associated with drug response in a randomized clinical trial of African descent minorities. Novel pharmacogenetic loci discoveries for LABA and ICS response were possible because we analyzed the BARD hierarchal composite outcome across different age groups using admixture mapping, a more powerful method compared to GWAS for recently admixed ethnic groups.

Implications of all available evidence:

We demonstrate the critical importance of diversity with respect to age and ancestry in pharmacogenetic studies for the development of complete precision medicine profiles for all people.

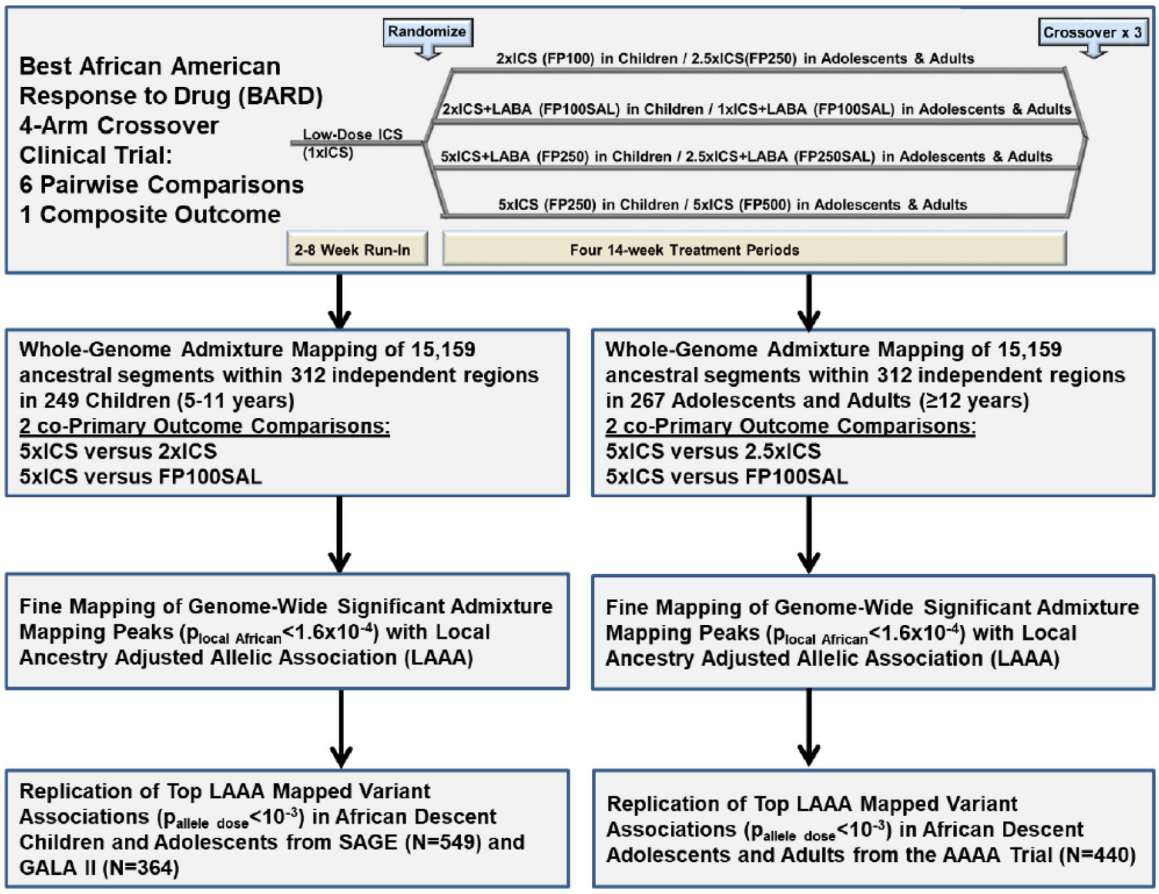
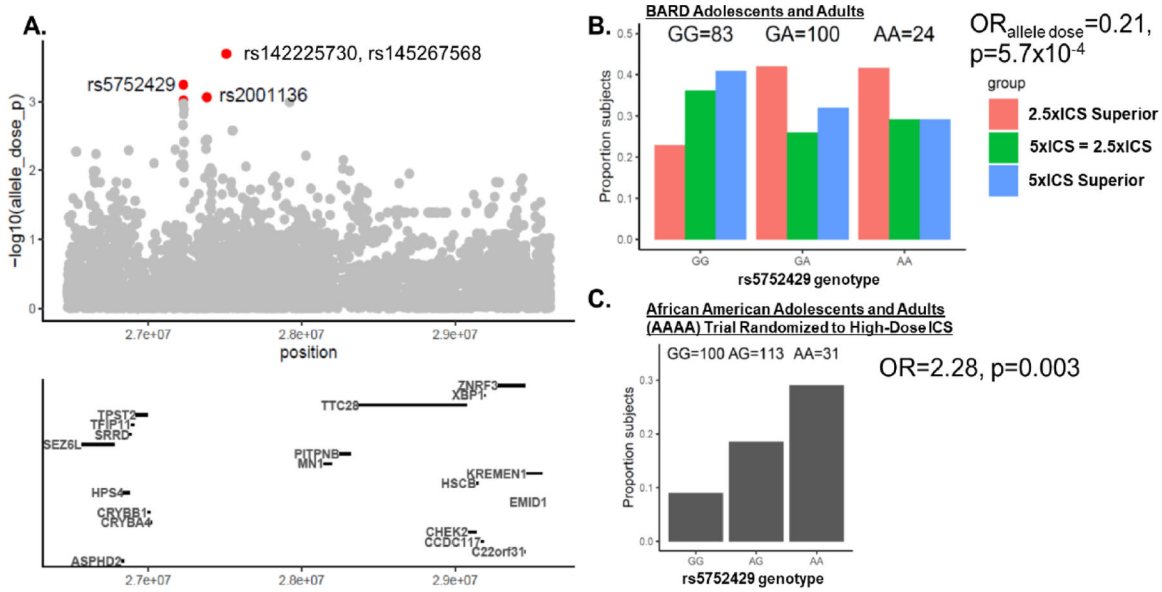


Figure 1: BARD Pharmacogenetic Study Flow Diagram.

This flow diagram summarizes the sequential analytical steps of the pharmacogenetic studies of the BARD four-arm cross-over trials in children and in adolescent and adult groups. The first series of studies was whole-genome admixture mapping to identifying ancestral chromosomal segments having genome-wide significant associations ($p_{local\ African}<1.6x10^{-4}$ based on 15,159 ancestral segments within 312 independent regions) for superior response to the co-primary treatment arm comparisons of (1) quintupling inhaled corticosteroid dose (5xICS) versus doubling corticosteroid dose (2–2.5xICS) and (2) 5xICS versus the addition of LABA to low-dose ICS (FP100SAL). Second, we performed fine mapping of the ancestral segments reaching genome-wide significance in each age group to identify individual variants using Local Ancestry Adjusted Allelic Association (LAAA) models. Finally, we evaluated each of the top SNP allelic associations ($p_{allele\ dose}<10^{-3}$) for replication in independent African American and Puerto Rican adolescents and children (SAGE and GALA II) and an African American Adolescent and Adult (AAAA) clinical trial cohort.



Figures 2A, 2B, 2C: (A) Fine mapping of the chromosome 22 admixture mapping peak for 5xICS versus 2.5xICS in adolescents/adults from BARD, (B) proportion of adolescents/adults with superior/equivalent responsiveness to 5xICS versus 2.5xICS based on rs5752429 genotype, and (C) independent replication based on proportion of exacerbations in African American Adolescents and Adults (AAAA trial) randomized to high-dose ICS.

(A) The fine mapping of the chromosome 22 ancestral segments reaching genome-wide significance for superior response to 5xICS versus 2.5xICS in adolescents and adults is shown as a locus zoom plot with negative log-transformed p-values. SNPs having an allele dose p-value $<10^{-3}$ are highlighted in red. (B) The proportion of BARD adolescents and adults with superior response to 5xICS, 2.5xICS, or no preference based on the BARD composite outcomes is shown by rs5752429 genotype. (C) The proportion of ICS-randomized African American adolescents and adults from the AAAA trial who experienced the primary outcome of exacerbation is shown by rs5752429 genotype.

Table 1:

Baseline Characteristics

Baseline Characteristics	Children (5–11 years)	Adolescent/Adults (≥12 years)
N	249	267 (49 adolescents/ 218 adults)
Age (years)	8.53 (1.84)	37.5 (16.3)
Female Sex (%)	99.0 (39.8%)	180 (67.4%)
BMI	19.1 (4.38)	32.6 (8.75)
Percentage Genetic African Ancestry		
Mean (SD)	75.6 (15.6)	79.4 (13.0)
Median [Min, Max]	81.0 [19.1, 100]	81.9 [25.4, 100]
Unscheduled asthma outpatient or emergency room visit past 12 months (%)	190 (76.3%)	118 (44.2%)
Overnight asthma hospitalization past 12 months (%)	37 (14.9%)	12 (4.5%)
Systemic glucocorticoids treatment past 12 months (%)	155 (62.2%)	88 (33.0%)
Inhaled glucocorticoid/long-acting beta agonist combination therapy	0 (0%)	0 (0%)
Inhaled or nebulized glucocorticoid monotherapy	209 (83.9%)	174 (65.2%)
Leukotriene receptor antagonist or 5lipooxygenase inhibitor	100 (40.2%)	45.0 (16.9%)
FEV1 percent of predicted[a]	95.5 (16.9)	83.7 (17.6)
Bronchodilator Response to 4 puffs albuterol (relative % change)	14.0 (14.8)	12.8 (12.7)
Total IgE (IU/ml)[b,c]		
Mean (SD)	590 (801)	439 (834)
Median [Min, Max]	286 [1.00, 5000]	181 [1.00, 8990]
Blood Eosinophil Count (cells/mm^3)[a,c]		
Mean (SD)	412 (317)	243 (195)
Median [Min, Max]	358 [0, 2430]	200 [0, 1200]
Sputum Eosinophil Percentage[d]		
Mean (SD)	NA	1.26 (4.10)
Median [Min, Max]	NA	0.20 [0, 43.2]

Baseline characteristics expressed in means and standard deviation (SD) unless specified otherwise.

[a] p<0.001 for comparison between children and adolescents/adults.

[b] p=0.01 for comparison between children and adolescents/adults.

[c] log-transformed for statistical comparisons.

[d] Sputum not collected in children and collected in 202 adolescents and adults.

Table 2:

Significant admixture mapping peaks detected for the primary outcomes.

Treatment Arm Comparison	BARD Age Group	Hg19 begin-end chromosomal (Chr) position	Genes within locus	$OR_{local\ African}$ [95%CI]	p-value
5xICS vs 2xICS	Children	chr6:12,882,674–13,111,241	*PHACTR1*	0.307 [0.17–0.56]	$1.1x10^{-4}$
5xICS vs FP100SAL	Children	chr6:10,546,283–11,089,096	*TMEM14B, GCM2, CYCP2L, ELOVL2*	0.259 [0.13–0.51]	$8.2x10^{-5}$
5xICS vs FP100SAL	Children	chr12:117,349,857–119,305,282	*RNFT2, TESC, NOS1, KSR2*	3.95 [2.02–7.72]	$6.1x10^{-5}$
5xICS vs 2.5xICS	Adolescents/ Adults	chr22:27,429,052–27,896,369	*TPST2, CRYBB1, CRYBA4*	3.35 [1.98–5.67]	$6.8x10^{-6}$

Admixture mapping peaks shown for associations with the co-primary comparisons reaching genome-wide significance ($p<1.6x10^{-4}$ for 15,159 ancestral segments within 312 independent regions). The odds ratio ($OR_{local\ African}$) shown with the 95% confidence intervals (95%CI) for the effect of local African ancestry for superior response in the first treatment arm versus (vs) the second treatment arm as shown in the first column.

Table 3:

Top Associations from Fine Mapping of Admixture Mapping Peaks for the Co-Primary Outcomes.

Comparisons	rs number	Chr	Position	Ref Allele	Alt Allele	BARD Freq	EUR Freq	AFR Freq	N	Allele dose OR [95%CI]	Allele dose p-value	Allele-African Dose OR [95%CI]	Allele-african dose p-value
Children													
5xICS vs 2xICS	rs112596714	6	12633469	T	C	0.03	0	0.06	202	0.09 [0.03–0.34]	$3.7x10^{-4}$	NA	NA
5xICS vs 2xICS	NA	6	12633763	G	A	0.03	NA	NA	202	0.09 [0.03–0.34]	$3.7x10^{-4}$	NA	NA
5xICS vs 100FP/SAL	rs59177473	6	10912455	T	G	0.05	0.0005	0.08	198	14.5 [2.98–70.4]	$9.3x10^{-4}$	NA	NA
5xICS vs 100FP/SAL	rs58273381	6	10928330	C	T	0.05	0.0005	0.09	198	10.6 [2.73–41.1]	$6.4x10^{-4}$	NA	NA
5xICS vs 100FP/SAL	rs75872959	6	10938783	T	C	0.05	0.0005	0.09	198	10.6 [2.73–41.1]	$6.4x10^{-4}$	NA	NA
5xICS vs 100FP/SAL	rs1234816814	12	116381296	G	C	0.84	NA	NA	198	0.19 [0.07–0.48]	$5.5x10^{-4}$	7.26 [1.97–26.8]	$2.9x10^{-3}$
5xICS vs 100FP/SAL	rs73399224	12	117084238	A	G	0.08	0.001	0.09	198	0.17 [0.07–0.42]	$8.4x10^{-5}$	NA	NA
5xICS vs 100FP/SAL	rs73399232	12	117087099	G	T	0.12	0.002	0.13	198	0.19 [0.09–0.41]	$2.4x10^{-5}$	NA	NA
5xICS vs 100FP/SAL	NA	12	117680155	C	A	0.06	NA	NA	198	0.17 [0.06–0.45]	$4.5x10^{-4}$	NA	NA
5xICS vs 100FP/SAL	rs9658447	12	117690860	C	T	0.06	0.0006	0.09	198	0.2 [0.08–0.52]	$9.7x10^{-4}$	NA	NA
Adolescents/Adults													
5xICS vs 2.5xICS	rs5752429	22	27228977	G	A	0.36	0.48	0.34	207	0.21 [0.09–0.52]	$5.7x10^{-4}$	4.67 [1.74–12.5]	$2.2x10^{-3}$
5xICS vs 2.5xICS	NA	22	27229346	A	C	0.46	NA	NA	207	0.24 [0.1–0.56]	$9.6x10^{-4}$	4.26 [1.7–10.7]	$2.0x10^{-3}$
5xICS vs 2.5xICS	rs2001136	22	27381136	A	G	0.34	0.2	0.39	207	6.26 [2.13–18.4]	$8.7x10^{-4}$	0.15 [0.04–0.48]	$1.6x10^{-3}$
5xICS vs 2.5xICS	NA	22	27381193	C	A	0.34	NA	NA	207	6.26 [2.13–18.4]	$8.7x10^{-4}$	0.15 [0.04–0.48]	$1.6x10^{-3}$
5xICS vs 2.5xICS	rs142225730	22	27506447	C	T	0.03	0	0.03	207	0.09 [0.02–0.32]	$2.0x10^{-4}$	NA	NA
5xICS vs 2.5xICS	rs145267568	22	27510343	C	T	0.03	0	0.03	207	0.09 [0.02–0.32]	$2.0x10^{-4}$	NA	NA

Results with p-value $<10^{-3}$ are shown for the Local Ancestry Adjusted Allelic (LAAA) Models for ancestral segments within admixture mapping peaks reaching genome-wide significance for the co-primary outcomes with reference sequence (rs) numbers, (ref) and alternative (Alt), the alt allele frequency (Freq) in the BARD cohort and European (EUR) and African (AFR) ancestral populations based on the 1,000 Genomes Project database.[28] For chromosome 6, the fine mapped SNPs for the comparison of 5xICS vs 2xICS and 5xICS vs 100FP/SAL are located in distant, independent admixture mapping peaks. Sample sizes for each of the trial arm comparison LAAA models shown with allelic dose odds ratios ($OR_{allele\ dose}$) and p-values. Results with p-value $<10^{-3}$ are shown in this table. The allele dose OR reflects the effect of the alternate (Alt) allele for being in the first treatment group vs the second treatment group. For example, an $OR_{allele\ dose}>1$ for comparison 5xICS vs 2.5xICS

means that the alternate allele is associated with an improved outcome on 5xICS rather than 2.5xICS. The allele-African dose OR reflects the effect direction of the combination an alternate (Alt) allele and African local ancestry. P-values shown for the allele dose and the interaction between allele dose and local African ancestry based on the LAAA method. Lower frequency SNPs more likely occur on a single ancestral allele background resulting in no variability in the allele-African dose (NA=not available).

Table 4:

Deconvolution of the BARD composite co-primary trial arm comparisons for top and replicated SNP associations from fine mapping.

Comparison	Group	rs number	Ref/Alt	Alt Frq	Allele dose OR [95% CI][a]	Allele dose p-value	Allele-African dose OR [95% CI][a]	Allele-African dose p-value
5xICS vs FP100SAL Chromosome 12 Peak in Children								
Exacerbations	**Children**	**rs73399224**	**G/T**	**0.08**	**0.25 [0.09–0.68]**	**0.006**	NA	NA
Asthma control days	**Children**	**rs73399224**	G/T	**0.08**	**0.21 [0.08–0.51]**	**0.0006**	NA	NA
FEV1	Children	rs73399224	G/T	0.09	0.69 [0.31–1.52]	0.36	NA	NA
5xICS vs FP100SAL Chromosome 6 Peak in Children								
Exacerbations	Children	rs58273381	C/T	0.05	2.94 [0.85–10.2]	0.09	NA	NA
Asthma control days	**Children**	**rs58273381**	**C/T**	**0.05**	**2.86 [1.07–7.64]**	**0.04**	NA	NA
FEV1	**Children**	**rs58273381**	**C/T**	**0.05**	**4.71 [1.32–16.8]**	**0.02**	NA	NA
5xICS vs 2xICS Chromosome 6 Peak in Children								
Exacerbations	**Children**	**rs112596714**	**T/C**	**0.03**	**0.08 [0.02–0.29]**	**0.0001**	**NA**	**NA**
Asthma control days	Children	rs112596714	T/C	0.03	0.36 [0.10–1.27]	0.11	NA	NA
FEV1	Children	rs112596714	T/C	0.03	0.40 [0.12–1.28]	0.12	NA	NA
5xICS vs 2.5xICS Chromosome 22 Peak in Adolescents and Adults								
Exacerbations	Adolescents/Adults	rs5752429	G/A	0.36	0.59 [0.17–2.09]	0.41	1.39 [0.34–5.7]	0.65
Asthma control days	**Adolescents/Adults**	**rs5752429**	**G/A**	**0.35**	**0.31 [0.12–0.76]**	**0.01**	**3.65 [1.3–10.3]**	**0.01**
FEV1	Adolescents/Adults	rs5752429	G/A	0.34	0.76 [0.3–1.96]	0.57	1.62 [0.57–4.55]	0.36

Results of the components of the BARD co-primary hierarchal composite clinical outcomes are shown for the SNP associations from fine mapping that were independently replicated.

[a]Odds ratios (OR) are the odds of having superior responsiveness based on asthma exacerbations, a 31-day difference in annualized asthma-control days, or a five percent difference in percentage predicted FEV_1 by SNP allele or African ancestry allele dose. For a given SNP and the comparison between drug X versus Y, an $OR_{allele\ dose}>1$ indicates that the alternative or minor SNP allele is associated with a greater likelihood of having a superior response to drug X. P-values shown for the allele dose and the interaction between allele dose and local African ancestry based on the LAAA method. Significant associations for the allele dose are in bold. Lower frequency SNPs more likely occur on a single ancestral allele background resulting in no variability in the allele-African dose (NA=not available).