

Pharmacogenetics: Implications of race and ethnicity on defining genetic profiles for personalized medicine

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Pharmacogenetics is being used to develop personalized therapies specific to subjects from different ethnic or racial groups. To date, pharmacogenetic studies have been primarily performed in trial cohorts consisting of non-Hispanic white subjects of European descent. A “bottleneck” or collapse of genetic diversity associated with the first human colonization of Europe during the Upper Paleolithic period, followed by the recent mixing of African, European, and Native American ancestries, has resulted in different ethnic groups with varying degrees of genetic diversity. Differences in genetic ancestry might introduce genetic variation, which has the potential to alter the therapeutic efficacy of commonly used asthma therapies, such as β_2 -adrenergic receptor agonists (β -agonists). Pharmacogenetic studies of admixed ethnic groups have been limited to small candidate gene association studies, of which the best example is the gene coding for the receptor target of β -agonist therapy, the β_2 -adrenergic receptor (*ADRB2*). Large consortium-based sequencing studies are using next-generation whole-genome sequencing to provide a diverse genome map of different admixed populations, which can be used for future pharmacogenetic studies. These studies will include candidate gene studies, genome-wide association studies, and whole-genome admixture-based approaches that account for ancestral genetic structure, complex haplotypes, gene-gene interactions, and rare variants to detect and replicate novel pharmacogenetic loci. (J Allergy Clin Immunol 2014;133:16-26.)

Key words: Asthma, genes, pharmacogenetics, response heterogeneity, single nucleotide polymorphism, admixture mapping, ethnic group

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It is well known that frequencies and even severities of disease can differ between *rac*es. Two simple examples include sickle cell

Abbreviations used

<i>ADRB1</i> :	β_1 -Adrenergic receptor
<i>ADRB2</i> :	β_2 -Adrenergic receptor
BADGER:	Best Add-on Therapy Giving Effective Response Trial
BARGE:	Beta Agonist Response by Genotype Trial
CAAPA:	Consortium on Asthma among African-ancestry Populations in the Americas
GALA:	Genetics of Asthma in Latino Americans
<i>GLCCI1</i> :	Glucocorticoid-induced transcript 1 gene
<i>GRK5</i> :	G protein receptor kinase 5
GWAS:	Genome-wide association study
ICS:	Inhaled corticosteroid
LABA:	Long-acting β -agonist
LARGE:	Long-acting Beta Agonist Response by Genotype
NHLBI:	National Heart, Lung, and Blood Institute
NIH:	National Institutes of Health
PEFR:	Peak expiratory flow rate
SABA:	Short-acting β -agonist
SMART:	Salmeterol Multicenter Asthma Research Trial
SNP:	Single nucleotide polymorphism
<i>SPATS2L</i> :	Spermatogenesis-associated, serine-rich 2-like gene

anemia, which is caused by a mutation that arose in Africa, whereas the mutations that cause cystic fibrosis primarily arose in subjects of European white descent. A key question is whether a person's ethnic or ancestral background determines individual response to a medication? There is robust evidence from clinical trials for different medical conditions showing that subjects from different *ethnic groups* experience variable responses to specific therapeutic agents. For instance, there is a class of antihypertensive drugs, β_1 -adrenergic receptor blockers or β -blockers, which might be less effective in a subgroup of African American subjects for the management of congestive heart failure.^{1,2} This reduced efficacy might be related to variants in 2 genes related to the G protein-coupled pathway of the β_1 -adrenergic receptor (*ADRB1*) and G protein receptor kinase 5 (*GRK5*) genes. These gene variants are overrepresented in African ancestral populations and result in changes in the amino acid code: a code change from arginine to a glycine in amino acid position 389 on *ADRB1* (Arg³⁸⁹Gly) and Gln⁴¹Leu on *GRK5*.³ The Arg³⁸⁹ allele in *ADRB1* and the Leu⁴¹ allele in *GRK5* have both been associated with a reduction in mortality in human subjects with heart failure and coronary ischemia treated with a β -blocker in different pharmacogenetic studies.^{3,4} These examples of variable drug responses in patients with cardiovascular disease illustrate the challenge of having personalized approaches not only through recognizing ethnic or racial subgroups, which show variable therapeutic drug responses, but also identifying

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Terms in boldface and italics are defined in the glossary on page 17.

them through pharmacogenetic biomarkers related to a common genetic ancestral origin.

Pharmacogenetics is the study of the role of genetic variability in determining interindividual (between-subject) variability in responses to a pharmacologic therapy. Pharmacogenetics represents a gene-environment interaction whereby variation in a gene interacts with an exposure to a drug (the “environment”) to alter a measurable *phenotype* related to drug efficacy or toxicity. The ultimate goal of pharmacogenetic research is the development of personalized medicine through genetic markers (individual genetic profiles), which would accurately predict which subjects with a particular condition would respond to a specific medical therapy, not respond to a therapy, or experience adverse effects.

RATIONALE FOR PHARMACOGENETICS IN THE MANAGEMENT OF ASTHMA IN DIFFERENT ETHNIC GROUPS

Asthma is a complex, chronic inflammatory disease of the airways that results from the interaction of multiple genetic and environmental factors. Asthma is a heterogeneous disease with variability in its phenotype expression and variability in interindividual therapeutic responses to different pharmacologic therapies.⁵⁻⁷ Variability in therapeutic responses might result from the interaction of multiple genes from different biologic pathways and even shared environmental influences.⁸⁻¹⁰ Persons with shared ancestry and physical traits are most commonly categorized by ethnic or racial groups; however, this designation also implies common genetic ancestral backgrounds, which might affect therapeutic responses.¹¹

To date, pharmacogenetic studies have been primarily performed in trial cohorts consisting of non-Hispanic asthmatic

subjects of European descent; however, a small number of studies have also evaluated study cohorts of recently admixed ethnic groups, such as African American or Hispanic subjects. There are inherent challenges related to the genetic study of recently admixed ethnic or racial subgroups from varying ancestries. These challenges have been primarily related to sample size, complex ancestral population structures, and genotype data primarily based on a genetic background from populations of European descent. In this review article we will summarize the genetic and epidemiologic basis for the variable genetic backgrounds observed between different recently admixed ethnic groups, outline the rationale for pharmacogenetic research in these ethnic groups, discuss the contribution of pharmacogenetic studies in identifying ethnic group-specific genetic variants for therapeutic responses in asthmatic subjects, and outline how admixture-based analytic methods and next-generation sequencing will contribute to future pharmacogenetic studies.

WHY GENETIC DIVERSITY VARIES BETWEEN SUBJECTS OF DIFFERENT ANCESTRIES

Information about the demographic history of our species can be evaluated through the distribution of gene variants located throughout the human genome. Initially, most publicly available databases contained mostly common gene variants, such as *single nucleotide polymorphisms* (SNPs) based on the Human Genome Project.^{12,13} The early ascertainment strategies of the Human Genome Project favored the identification of common variants, which were of particular interest based on the “common disease–common allele hypothesis,” which states that multiple common gene variation with mild-to-moderate effects influences susceptibility to complex common phenotypes (Fig 1).¹⁴⁻¹⁸

GLOSSARY

ETHNIC GROUPS: A socially defined category of persons based on shared social experience (eg, language, dressing style, and cuisine) or ancestry. The only ethnic category queried in the US Census is whether respondents are of Hispanic or Latino origin.

EXOSITE: The β_2 -adrenergic receptor has 7 transmembrane spanning domains (TMDs). Conventional β -agonists have critical contact points at TMDs 3, 5, and 6. The long duration of salmeterol's action involves its side chain binding to TMDs. In contrast to most β agonists, salmeterol binding to a nontraditional site on the fourth TMD is termed an exosite. Exosite binding is critical to the long duration of action of salmeterol by anchoring the drug to the receptor to provide for repetitive binding events. Such binding to a nontraditional receptor-binding site is termed exosite binding because it lies outside the critical contact points.

FIRST MODERN HUMANS: Also referred to as anatomically modern humans and members of *Homo sapiens*, with an appearance consistent with the range of phenotypes in modern humans. The subspecies *Homo sapiens sapiens* constitutes anatomically modern humans. Features include a less prominent brow ridge, a more vertical forehead, and a more prominent chin compared with their predecessors.

LINKAGE DISEQUILIBRIUM (LD): In population genetics LD is the nonrandom association of alleles at 2 or more genetic loci derived from similar or different ancestral chromosomes. LD occurs along varying genomic distances and is defined as the following. *Admixture LD* arises when 2 ancestral populations with divergent allele frequencies

mix, is weak at all distances, and decays slowly. *Background LD* is used for conventional association mapping, is strong at short distances, and decays rapidly. *Mixture LD* occurs between unlinked markers because of varying ancestral proportions among subjects.

PHENOTYPE: The observable properties of an organism that are produced by the interaction of the genotype and environment.

POPULATION GENETIC EQUILIBRIUM: The frequency of an allele in a population does not change from generation to generation. It is a theoretic state taking into account ideal conditions. The most common conditions arise from Hardy-Weinberg equilibrium and include random mating, equal survival, no migration, no mutation, no inbreeding, large population sizes, and gene frequencies equal in male and female subjects.

RACE: A category of self-identification used as a data item by government. Self-reported race takes into account both ancestry and social and cultural characteristics. The 5 official US government racial categories are as follows: white, black or African American, Asian, American Indian/Alaska Native, and Pacific Islander. The US Census Bureau allows respondents to mark more than one race, if desired. Race is defined medically as a category of humankind that shares certain distinctive physical traits.

SINGLE NUCLEOTIDE POLYMORPHISM: One of 2 or more variants of a particular single base pair of DNA.

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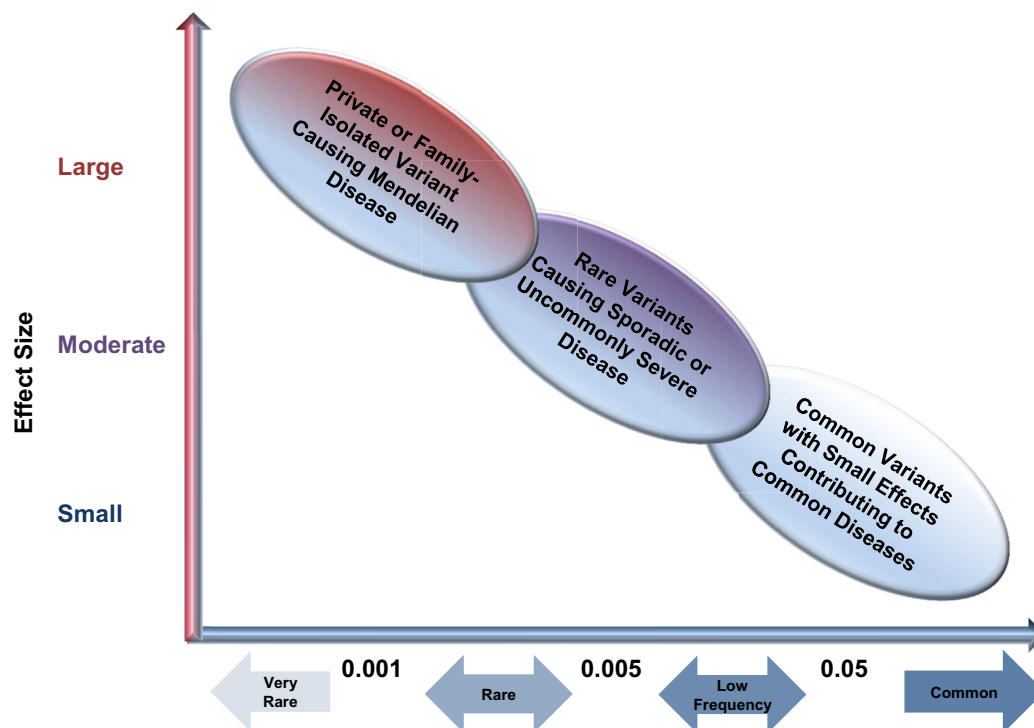


FIG 1. Common and rare genetic variants in human disease. On the basis of the “common disease–common allele” hypothesis, multiple common genetic polymorphisms (alleles or variants) with small-to-moderate effect sizes contribute additively to a common disease. GWASs have detected multiple common variants associated with risk for common diseases, such as hypertension or asthma. The “common disease–rare allele hypothesis” states that rare genetic variants with a large effect size contribute to risk for common diseases. Rare genetic variants cannot be detected with classic GWASs and have been evaluated with family-based genetic studies, admixture mapping, and DNA sequencing. Adapted from Tsuji.¹⁴

SNPs are the most common form of gene variation in the human genome and can act as heritable landmarks for genetic diversity and population structure. On the basis of the diversity of these common variants, different recombination models have estimated that the human population has experienced a “bottleneck” history.^{18,19} This bottleneck history likely began nearly 40,000 years or 1,600 generations ago when the *first modern humans* migrated from sub-Saharan Africa to colonize Europe.¹⁸ This initial colonization of Europe by modern humans was accompanied by a loss or “collapse” of genetic diversity, followed by modest growth of the human population during the Upper Paleolithic period (Fig 2).¹⁹ An important consequence of this bottleneck event is that populations of primarily European ancestry have gene variants that remain coinherited through linkage equilibrium (often because of being physically close together on a chromosome) as a result of fewer recombination events since the last “collapse.” In contrast, populations with a primarily ancient African ancestry have genomic regions with a greater diversity of gene variation, resulting from a greater number of recombination events and shorter regions of *linkage disequilibrium*.¹⁸

Over the past 400 generations, the human population has experienced a marked increase in growth by at least 3 orders of magnitude.²⁰ This accelerated growth has resulted in departures from *population genetic equilibrium* and a recent growth of rare variation because of recent mutations.²¹ The phenomenon of increased rare variant load has only been recently appreciated

with the advent of next-generation sequencing technologies that have allowed for whole-genome or whole-exome sequencing. Large-scale whole-genome sequencing projects have already provided insight into the load of rare variants in different populations through multicenter studies, which include the 1000 Genomes Project Consortium; the National Institutes of Health (NIH)/National Heart, Lung, and Blood Institute (NHLBI) GO Exome Sequencing Project; and the NIH/NHLBI Consortium on Asthma among African-ancestry Populations in the Americas (CAAPA).^{22,23} For instance, whole-genome sequencing data from 1,097 individual genomes from 14 different populations from the 1000 Genomes Project Consortium recently identified an enrichment of rare variants defined as an allele frequency of less than 0.005 in subjects, which is consistent with the recent explosive expansion of the human population.^{21,22}

The genetic diversity of different ancestral populations has been shown to have implications for the frequency of rare genetic variants. For instance, the 1000 Genomes Project investigators reported that each subject had at least 240 rare coding variants (allele frequency, <0.05) and hundreds of noncoding variants at conserved motifs, such as transcription factor binding sites, and thus the total number of individual rare variants was greater than that of common variants in the total 1000 Genomes Project study population.²² In addition, these investigators demonstrated that populations of African ancestry had 3 times as many rare variants compared with those of European and Asian origin, reflecting the ancestral bottlenecks of the descendant non-African populations

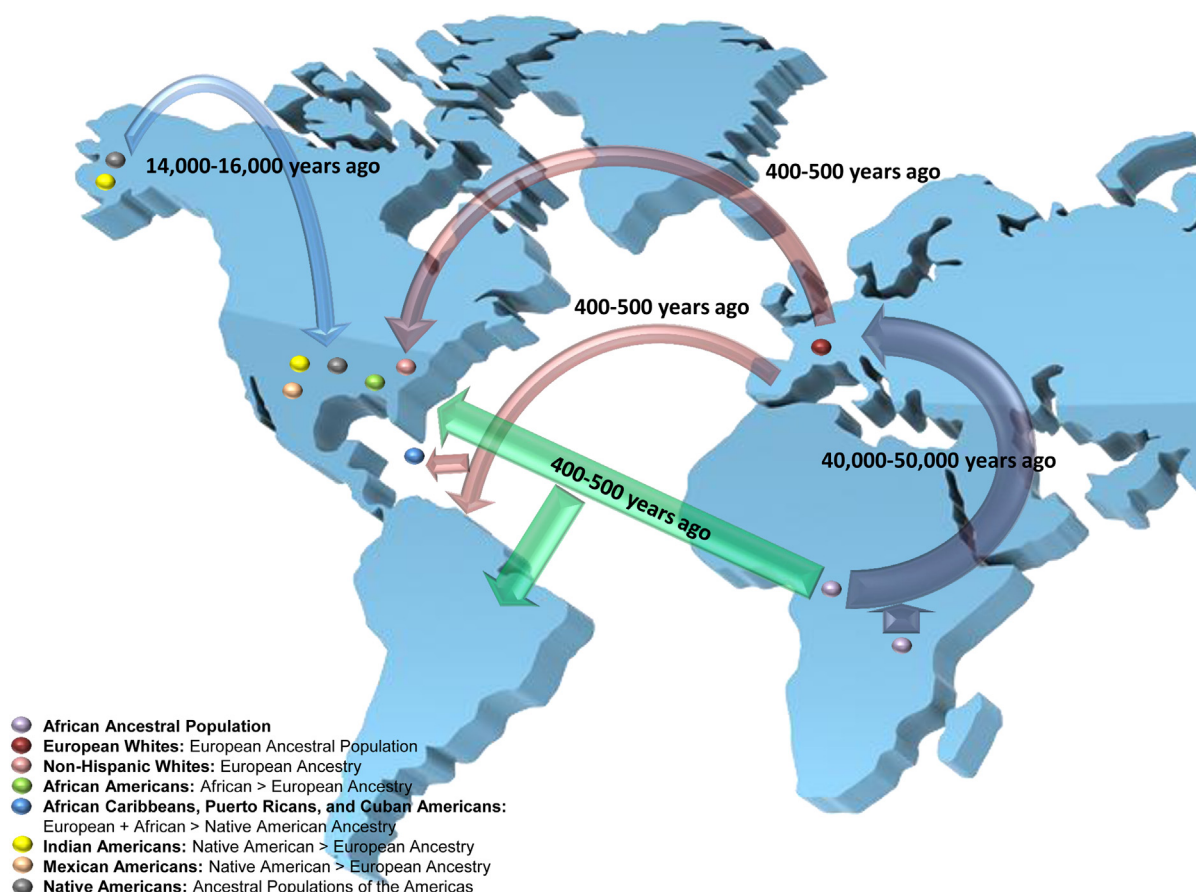


FIG 2. Ancestries of recently admixed ethnic groups in the United States. The first human colonization of Europe during the Upper Paleolithic period (purple arrow) was accompanied by a “bottleneck” or collapse of genetic diversity in the resulting European white ancestral population. Recent mixing between more genetically diverse ancient African ancestral populations with European white and Native American subjects (blue arrow represents the first human colonization of the Americas) during the European colonization of the Americas (red arrows) and the African slave trade (green arrows) resulted in different recently admixed ethnic groups with varying degrees of genetic diversity. The flow of genetic diversity is represented by the thickness of the arrows, with thicker arrows reflecting greater genetic diversity (ie, resulting from a greater number of recombination events, shorter genomic regions of linkage disequilibrium, and a greater frequency of rare variants).

(Fig 2).^{18,22} The availability of high-throughput sequencing, the recognition that rare genetic variants are frequent in the human population, and the potential for strong rare variant genetic effects on common diseases has resulted in a recent increase in the study of rare variants in common complex disease based on the “common disease–rare allele hypothesis” (Fig 1).^{14,24–27}

The analysis of a rapidly expanding catalog of rare variants remains a formidable challenge because of the low statistical power of conventional association studies and the potential for misclassification of the functional effect of individual variants. Analytic methods for rare variant analysis have evolved from simpler methods, which “collapsed” rare variants at the gene level, to more complex methods, which assign directionality of effect to individual rare variants and take advantage of predictive functional algorithms to optimize power while minimizing the potential for variant function misclassification.^{28–30} The role of variable genetic diversity and rare variants will be important to consider in future pharmacogenetic studies of subjects of African ancestry because rare variants appear to be more frequent in these ethnic or racial groups and thus might account for

interethnic differences in drug responses, especially for rare adverse events.¹⁰

GENETIC DIVERSITY FROM DIFFERENT ANCESTRIES IS A CRITICAL CONSIDERATION FOR GENETIC STUDIES IN ADMIXED POPULATIONS

Subjects from recently admixed ethnic groups have varying proportions of different ancestries represented within their genomes. The European colonization of the Americas and the African slave trade resulted in mixed genetic ancestries, which constitute the complex population structures identified in the genomes of African American, African Caribbean, Puerto Rican, and Mexican American subjects (Fig 1). The variable demographic histories of recently admixed populations with a common ancient African ancestral component has resulted in a population structure with greater genetic diversity compared with non-Hispanic subjects of European white descent. This increased genetic diversity has not been captured by most whole-genome genotyping platforms, which were designed for

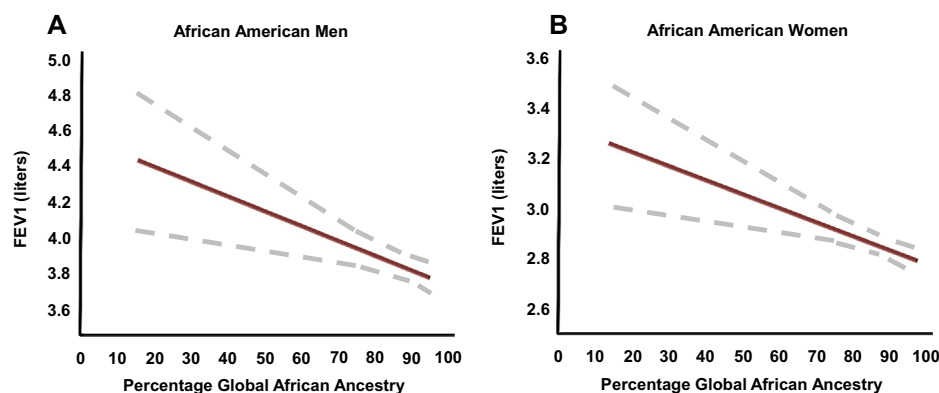


FIG 3. Relationship of global African ancestry with lung function in African American subjects. Kumar et al³³ demonstrated an inverse relationship between the percentage of global African ancestry and baseline FEV₁ (measured in liters) in self-identified African American men and women, as shown in **A** and **B**, respectively. Global African ancestry was estimated by using ancestry informative genetic markers. Reproduced from Kumar et al.³³

populations of a primarily European ancestry. Thus efforts are currently underway to develop more comprehensive whole-genome genotyping platforms to capture the genetic diversity of recently admixed populations, such as through the CAAPA Consortium.²³

In general, genetic clusters based on genome-wide genotype data have closely reflected self-reported ethnicity; however, subsequent genetic studies of recently admixed populations have demonstrated marked variability in genetic ancestry, particularly between different Hispanic subgroups.³¹⁻³³ For instance, African American subjects have estimated mean global African ancestry of 80% (20% European), whereas Hispanic ethnic subgroups, such as Puerto Rican and Mexican American subjects, have varying combinations of 3 different ancestries: African, European, and Amerindian or Native American (Fig 2).^{32,33} These percentages reflect the subgroup overall, but it is extremely important to remember that an individual patient might have a very different level of admixture.

In addition to sharing common genetic ancestries, subjects from different ethnic groups also share common languages, cultural behaviors, religious affiliations, and often environmental exposures. These common characteristics might result in gene-environment interactions, which are implicit to individual ethnic groups and further confound genetic studies by making it difficult to dissect the effect of environment in determining differential drug responses between subjects from these various ethnic subpopulations.

ASSOCIATION BETWEEN GENETIC ANCESTRY AND ASTHMA-RELATED PHENOTYPES: A RATIONALE FOR PHARMACOGENETIC STUDIES IN ADMIXED POPULATIONS

Some admixed populations show measurable differences in asthma-related phenotypes not attributed to environmental factors. For instance, asthma-related morbidity and mortality are higher among Puerto Rican and African American subjects compared with non-Hispanic white and Mexican American subjects.³⁴⁻³⁶ These interethnic differences in disease severity might result from health care disparities but also might relate to genetic variants inherited from a specific ancestry associated

with disease severity or therapeutic response to different therapies. The effect of global genetic ancestry on asthma and related phenotypes has been evaluated by using SNPs with variable allele frequencies between different ancestral populations. These genetic markers are informative of genetic ancestry and thus are known as ancestry informative markers, which provide estimates of individual genetic ancestry. The majority of previous admixture-based genetic studies have been related to asthma susceptibility and severity, yet there are potential applications for determining the role of genetic ancestry on drug response.

Large cohorts of subjects with well-characterized asthma have consistently demonstrated the importance of lung function as a fundamental determinant of asthma disease severity.^{37,38} Admixture-based genetic studies in African American subjects from the general population have suggested that genetic variation related to African ancestry might affect both asthma susceptibility and severity based on lung function. The first of these studies was performed by Kumar et al,³³ which demonstrated that global estimates of percentages of African ancestry were inversely correlated with baseline lung function in 3 independent African American populations (Fig 3).

The observed inverse association between African ancestry and lung function was consistent with the trend in predicted baseline lung function described in the study by Hankinson et al,³⁹ in which self-designated African race predicted a lower lung function measure compared with that seen in self-designated white subjects of similar age, height, and sex. In addition, calculations of baseline lung function based on estimated ancestry by using genetic methods revealed that classic predictive equations misclassified asthma severity based on percent predicted FEV₁ in a subset of African American asthmatic patients. The potential for misclassification of disease severity in this African American cohort is because each asthmatic patient showed variable proportions of African versus European ancestries (ie, a continuous variable of percentage ancestry) not captured by using classic predictive methods based on self-reported race (ie, a dichotomous designation of self-reported race).³³ In 392 asthmatic patients African ancestry was associated with asthma exacerbations, suggesting that genetic variation leading to an increased risk for exacerbations is more common in African subjects, an important finding

requiring further replication and identification of genetic risk variants.⁴⁰

Admixture-based genetic studies in the Genetics of Asthma in Latino Americans (GALA) cohort have also evaluated the role for genetic ancestry in determining asthma susceptibility and severity in Puerto Rican and Mexican American subjects.³² Despite being considered from the same Hispanic or Latino ethnic or racial group, Puerto Rican and Mexican American subjects are ethnic subgroups that show remarkable differences in asthma disease severity, which might be related to genetic variation from their uniquely different ancestral origins. For instance, Puerto Rican subjects experience among the highest asthma-related morbidity and mortality when compared with all other ethnic groups in the United States, whereas Mexican American subjects experience the lowest asthma-related morbidity and mortality.⁴¹ Admixture-based studies from the GALA study group have demonstrated that Puerto Rican subjects have a higher degree of African and European Ancestry compared with Mexican American subjects, who have a higher degree of Amerindian or Native American ancestry (Fig 2).^{32,42}

An inverse association between African ancestry and baseline lung function was observed in 943 Puerto Rican asthmatic cases and control subjects from the GALA cohort, a similar relationship to what had been previously observed in African American subjects.⁴³ An inverse relationship between European ancestry and baseline lung function measures was also observed in 181 Mexican American asthmatic patients from the GALA study.⁴² In contrast, Native American ancestry potentially exhibits a beneficial effect on lung function in Mexican American subjects and has been associated with a reduced risk of rapid lung function decrease and chronic obstructive pulmonary disease in a combined cohort of 305 Hispanic and 1,404 non-Hispanic white subjects from New Mexico.⁴⁴

Differences in genetic ancestry might also be important in the therapeutic efficacy of commonly used asthma therapies. The most relevant is related to a commonly used class of pharmacologic agents: β_2 -adrenergic receptor agonists (ie, β -agonists), which are divided into short-acting β -agonists (SABAs; fenoterol, isoproterenol, pirbuterol, levalbuterol, and albuterol) used for as-needed rescue therapy and long-acting β -agonists (LABAs; salmeterol and formoterol) used in combination with an inhaled corticosteroid (ICS) for chronic management. Recent surveillance studies and meta-analyses derived from these studies have suggested that LABAs increase the risk for life-threatening asthma exacerbations and asthma-related death when LABA therapy was administered without concomitant ICS therapy.^{10,45,46} The most cited of these studies is the Salmeterol Multicenter Asthma Research Trial (SMART) in which 26,355 subjects, including 4,685 African American subjects, were randomized to either salmeterol or placebo in addition to “usual therapy.”

The SMART trial was terminated early because of an increase in asthma or respiratory-related life-threatening exacerbations and death in the subgroup of African American subjects treated with salmeterol. This study was limited by its cursory surveillance design, which did not monitor compliance or concomitant medication use (eg, regular SABA use), and study participants were not routinely treated with an ICS. In addition, the African American subgroup consisted of asthmatic patients who were less frequently treated with an ICS and at least twice as likely to have experienced an asthma-related hospitalization or

life-threatening exacerbations before trial enrollment compared with their non-Hispanic white counterparts.¹⁰ Before and after the SMART trial, randomized controlled clinical trials and large meta-analyses have consistently demonstrated that the combination of a LABA and an ICS is safe and efficacious for asthma management.⁴⁷⁻⁵⁰ This LABA safety controversy has resulted in 2 advisory panel meetings by the US Food and Drug Administration, subsequent public health advisories, and a boxed warning for all LABA-containing inhalers.⁵¹ This controversy is currently being evaluated in an international study of more than 40,000 asthmatic patients mandated by the US Food and Drug Administration.^{51,52}

Data from different clinical trials suggest that African American subjects respond differently to combination therapies containing a LABA compared with non-Hispanic white subjects.^{8,9} The NIH/NHLBI Childhood Asthma Research and Education Network Best Add-on Therapy Giving Effective Response (BADGER) trial was a triple-crossover clinical trial consisting of a multiethnic cohort of 182 children with uncontrolled asthma who were randomized to a “step up” from low-dose ICS therapy to different combination therapies. These children were assessed for preferential therapeutic response based on the composite end point of lung function decrease, exacerbation, and a reduction of days with well-controlled symptoms. In the BADGER trial African American children did not show a preferential response to a step up from low-dose ICS therapy to a higher-dose ICS compared with a step up to LABA and ICS combination therapy, and thus the addition of a LABA to ICS therapy was no more efficacious than increasing the ICS dose. In contrast, Hispanic and non-Hispanic white subjects showed a preferential response to a step up from ICS therapy to LABA and ICS combination therapy compared with the other step-up combination therapies.⁸

In addition, a combined analysis of 795 non-Hispanic white and 233 African American adult asthmatic patients from 10 NIH/NHLBI Asthma Clinical Research Network Trials showed that African American subjects were more likely to experience treatment failures during randomized treatment with LABA therapy compared with non-Hispanic white subjects.⁹ African ancestry might also have a role in differential β -agonist response in other admixed ethnic groups. For instance, Puerto Rican subjects from the GALA cohort were less likely to show a significant increase in lung function in response to acute bronchodilation with a SABA.⁵³ These findings suggest that African ancestry might introduce genetic variation associated with a greater likelihood for alteration in therapeutic responses to inhaled β -agonists and thus provide the rationale for pharmacogenetic studies.

PHARMACOGENETIC STUDIES: A FOCUS ON β -AGONISTS

Pharmacogenetic studies in multiethnic populations have focused on the gene encoding for the β_2 -adrenergic receptor (*ADRB2*). *ADRB2* is located on chromosome 5q31 and encodes for a 7-transmembrane receptor that activates a G_s protein-coupled receptor pathway consisting of adenylyl cyclase to mediate smooth muscle relaxation.^{54,55} A common coding SNP, Gly¹⁶Arg, has been the most studied polymorphism; however, nearly 49 polymorphisms have also been identified through DNA sequencing in multiethnic asthma populations that include

TABLE I. Variant allele frequencies of asthma pharmacogenetic loci in different ancestral populations and US ethnic groups

Drug classes	Gene	Previously associated pharmacogenetic loci*	Alleles, major/minor	Minor allele frequency by racial group†						
				CEU	YRI	ASW	MEX	CHB	JPT	
Leukotriene receptor modifiers										
5-Lipoxygenase inhibitors (zileuton)	ALOX5	rs2029253 ⁷³	A/G	0.33	0.23	0.29	0.52	0.56	0.56	
		rs2115819 ⁷³	A/G	0.37	0.21	0.28	0.53	0.73	0.82	
Cysteinyl leukotriene antagonists (montelukast)	ALOX5	rs2115819 ^{71,72}	A/G	0.37	0.21	0.28	0.53	0.73	0.82	
	LTC4S	rs730012 ⁷¹	A/C	0.32	0.007	0.06	0.22	0.14	0.18	
	LTA4H	rs266845 ⁷¹	T/G	0.17	0.007	0.06	0.22	0.44	0.36	
Inhaled glucocorticoids (fluticasone, budesonide, flunisolide, triamcinolone)	STIP1	rs2236647 ⁷⁰	C/T	0.43	0.76	0.71	0.6	0.43	0.35	
	GLCCII	rs1011219 ⁷⁰	G/A	0.2	0.08	NA	NA	0.08	0.14	
		rs37972 ⁶⁰	C/T	0.45	0.14	0.29	0.46	0.43	0.38	
	T Gene	rs3127412 ⁶⁹	T/C	0.35	0.36	0.3	0.15	0.13	0.11	
		rs6456042 ⁶⁹	C/A	0.35	0.36	0.3	0.15	0.13	0.11	
		rs3099266 ⁶⁹	C/T	0.36	0.15	0.16	0.3	0.26	0.28	
		rs2305089 ⁶⁹	T/C	0.41	0.79	0.63	0.38	0.64	0.7	
	Inhaled β2-adrenergic receptor agonists, SABAs (albuterol)	ADCY9	rs2230739 (Met ⁷⁷² Ile) ⁶⁸	T/C	0.36	0.12	0.12	0.22	0.4	0.36
		ADRB2	rs1042713 (Gly ¹⁶ Arg) ^{56-58,65-67}	G/A	0.36	0.53	0.57	0.47	0.58	0.44
ARG1	rs2781659 ^{74,75}		A/G	0.34	0.73	NA	NA	0.28	0.4	
		rs2781667 ⁶²	C/T	0.31	0.79	0.72	0.36	0.32	0.37	
		rs7140310 ⁶²	T/G	0.11	0.24	0.21	0.12	0.25	0.26	
		rs10483801 ⁶²	C/A	0.16	0.42	0.36	0.29	0.4	0.42	
		SPATS2L	rs295137 ⁶¹	C/T	0.42	0.59	0.41	0.31	0.19	0.2
	LABAs (salmeterol and formoterol)	ADRB2	rs1042713 (Gly ¹⁶ Arg) ^{59,63,64,76}	G/A	0.36	0.53	0.57	0.47	0.58	0.44

ASW, African American subjects from the southwestern United States; CEU, Utah residents with ancestry from northern and western Europe; CHB, Han Chinese from Beijing, China; JPT, Japanese from Tokyo, Japan; MEX, Mexican American subjects from Los Angeles, California; YRI, subjects from Yoruba in Ibadan, Nigeria.

*Pharmacogenetic loci classified by specific drug class where associations with therapeutic responses were observed.⁵⁶⁻⁷⁶

†Minor or less common variant allele frequencies are based on data from the International HapMap Project Genome Browser release 28 accessed on August 27, 2013 (http://hapmap.ncbi.nlm.nih.gov/cgi-perl/gbrowse/hapmap28_B36/).

polynucleotide insertions-deletions and rare functional variants. The minor allele of this SNP has variable allele frequencies between different ancestral populations but is more frequent in African and Asian populations (Table I).⁵⁴⁻⁷⁶ *In vitro* β -agonist stimulation results in enhanced downregulation of receptors expressing the Gly¹⁶ variant compared with Arg¹⁶.^{77,78} Additional *ADRY2* polymorphisms identified in most ethnic groups include additional coding variants (Cys^{19[BUP]}Arg, Gln²⁷Glu, Thr¹⁶⁴Ile), with varying allele frequencies resulting in complex haplotypes, some of which are specific to certain ethnic groups.^{54,55,79}

Pharmacogenetic studies have evaluated the genotypic effects of the Gly¹⁶Arg locus on the response to chronic regular exposure to β -agonist therapies, revealing genetic effects that might affect populations with a significant African ancestry. Two early studies were genetic association studies of prior clinical trials that randomized asthmatic patients to regular chronic albuterol exposure. These studies consistently demonstrated that asthmatic patients homozygous for the Arg¹⁶ allele were more likely to experience adverse effects on peak flow rate during regular SABA treatment compared with Gly¹⁶ homozygotes.^{56,57} These pharmacogenetic findings were confirmed in a prospective, genotype-stratified, crossover trial performed by the NHLBI's Asthma Clinical Research Network investigators Beta Agonist Response by Genotype (BARGE) trial. In the BARGE trial 37 Arg¹⁶ homozygotes and 41 Gly¹⁶ homozygotes were prospectively randomized to regular albuterol or placebo for 16 weeks, with ipratropium provided as the rescue inhaler. In this study Arg¹⁶ homozygotes did not experience a change in peak expiratory flow rate (PEFR) and a deterioration in symptom

control during albuterol treatment. In contrast, Gly¹⁶ homozygotes demonstrated improvements in PEFR and symptom control during albuterol treatment.⁵⁸

In the BARGE trial 22% of Arg¹⁶ homozygotic subjects were African American, whereas 17% were Gly¹⁶ homozygotes; however, the allele frequency of the Arg¹⁶ allele is higher among African American subjects (allele frequency = 0.53 in an African population, Table I) compared with non-European white subjects (allele frequency = 0.36, Table I) in the general population.⁵⁸ This difference in allele frequency is likely because Gly¹⁶ is the ancestral allele at the Gly¹⁶Arg locus; thus chromosomes from an ancient African ancestry have had more time to distribute the more recent Arg¹⁶ variant compared with chromosomes from a European ancestry.⁸⁰ This difference in allele frequency at the Gly¹⁶Arg locus has the potential to contribute to a disparity in adverse responses between ethnic groups during chronic β -agonist exposure and has been further evaluated in subsequent, prospective, genotype-stratified trials for LABA therapy in asthmatic patients (Table I).^{59,76}

In contrast to the BARGE trial, the NHLBI's Asthma Clinical Research Network Long-Acting Beta Agonist Response by Genotype (LARGE) trial did not identify differential therapeutic LABA responses related to Gly¹⁶Arg genotype in 42 asthmatic Arg¹⁶ homozygotes and 45 Gly¹⁶ homozygotes randomized to salmeterol or placebo in combination with ICS therapy by using a similar crossover, genotype-stratified study design. Despite the fact that both genotypes (Arg¹⁶ or Gly¹⁶ homozygotes) experienced equal improvements in PEFR during either treatment, Gly¹⁶ homozygotes experienced a bronchoprotective effect or an decrease in methacholine bronchial reactivity, which

was not observed in Arg¹⁶ homozygotes.⁷⁶ In the subgroup of African American subjects from the LARGE trial (8 Gly¹⁶ homozygotes and 9 Arg¹⁶ homozygotes), Gly¹⁶ homozygotes experienced significantly greater improvements in morning and evening peak flow rates with salmeterol compared with placebo, whereas no such effect was observed among Arg¹⁶ homozygotes.⁸¹ A second genotype-stratified trial of Gly¹⁶Arg genotypes by Bleeker et al⁵⁹ randomized 183 Gly¹⁶ homozygotes, 182 Gly¹⁶ Arg heterozygotes, and 179 Arg¹⁶ homozygotes to salmeterol monotherapy or salmeterol with an ICS for 16 weeks without a crossover design. Similar improvements in PEFR were observed in all subjects and all ethnic subgroups during LABA therapy with or without ICS therapy independent of Gly¹⁶Arg genotype.

LESSONS LEARNED FROM PHARMACOGENETIC STUDIES RELATED TO ADMIXED ETHNIC POPULATIONS

There has been conflicting pharmacogenetic evidence for prospective pharmacogenetic studies of *ADRB2* gene variation, which highlights the challenges of performing these studies in different ethnic groups. First, admixed ethnic groups represent the minority of subjects enrolled in most pharmacogenetic studies, thus resulting in sample sizes that might be underpowered to detect genetic associations.

Second, there are often environmental factors related to cultural or habitual exposures, such as access to health care, compliance with medications, and different environmental exposures, such as cigarette smoke.

Third, the study of admixed populations is further complicated by the increased genetic diversity at the individual level. This increased genetic diversity complicates genetic association studies because of altered allele frequencies and potentially altered gene-gene interactions between subjects who are self-reporting the same ethnicities. For instance, an SNP in *GSNOR* (rs11547772) was shown to interact with Gly¹⁶Arg in *ADRB2* to determine albuterol bronchodilator response in 336 Puerto Rican asthmatic families, a pharmacogenetic interaction not observed among Mexican American subjects.⁸² In addition, 426 SNPs from 254 plausible candidate genes were evaluated by using Bayesian networks to identify predictive gene pathways for acute albuterol bronchodilator response in 308 non-Hispanic white subjects from the Childhood Asthma Management Program. This systems biology approach used genetic data to generate a network of 15 SNPs within 15 genes, which predicted SABA response with greater accuracy than each locus.⁸³ Systems biology approaches will be particularly important for pharmacogenomic studies in admixed populations in which variable allele frequencies alter complex gene-gene interactions and potentially contribute to variable therapeutic responses.⁸⁴

A final consideration is that there is an increased frequency of rare variants in populations with African ancestry compared with non-Hispanic white populations.^{22,54} Life-threatening adverse responses to LABAs are uncommon events but represent a strong effect that might be determined by rare genetic variants, particularly among subjects of African descent in whom rare variants have been most frequently identified. Rare genetic variants have a greater potential for stronger effects on phenotype compared with common variants, such as Gly¹⁶Arg (Fig 1).

For instance, the rare Thr¹⁶⁴Ile variant in *ADRB2* is located in the fourth transmembrane domain and has been associated with receptor ligand binding and decreased receptor coupling to G_s protein in response to different SABAs.^{85,86} Similar *in vitro* effects for this rare variant have also been observed in response to LABAs, resulting in impaired binding of salmeterol to its receptor “*exosite*.”⁸⁶ The rare Ile¹⁶⁴ allele was also associated with reduced baseline lung function and an increased susceptibility to airflow obstruction in 62,748 subjects from the Copenhagen General Population Study and the Copenhagen City Heart Study, which included a subset with self-reported asthma (n = 1,300); however, the effect of this rare variant on therapeutic responses to β -agonist remains to be studied in an asthma trial cohort.⁸⁷ A polycytosine repeat variant in the 3' untranslated region of *ADRB2* was not associated with therapeutic responses to LABA and ICS combination therapy in 2,192 asthmatic patients; however, nearly half of the polycytosine allele lengths had allele frequencies of less than 5%, which is consistent with a rare variation that remains to be studied.⁸⁸ Sequencing of *ADRB2* has also identified additional potentially functional rare variants at an increased frequency among African American compared with non-Hispanic white asthmatic patients.^{22,54} Of these rare functional variants, a novel rare coding variant, Ser²²⁰Cys, and a rare promoter 25-bp insertion at nucleotide position -376 were only identified in African American subjects, whereas a rare coding SNP, Thr¹⁶⁴Ile, was identified at a much greater frequency in non-Hispanic white subjects.⁵⁴

PHARMACOGENETIC GENOME-WIDE ASSOCIATION STUDIES AND FUTURE STUDIES OF MULTIETHNIC POPULATIONS

Although there have been no pharmacogenetic genome-wide association studies (GWASs) related to asthma therapies in African American or Hispanic ethnic groups, recent GWASs in non-Hispanic white asthmatic populations have provided a sound rationale for gene variants with potential pharmacogenetic effects. A GWAS of ICS response in 118 probands from the Childhood Asthma Management Program and 935 asthmatic patients from 4 replication cohorts consisting primarily of non-Hispanic white subjects revealed an SNP in the promoter region of the glucocorticoid-induced transcript 1 gene (*GLCCI1*), rs37972, to be associated with lung function response to ICSs.⁶⁰ More recently, a GWAS of albuterol bronchodilator response in 1,644 non-Hispanic white asthmatic patients from 6 clinical trials identified SNPs adjacent to a gene that determines β_2 -adrenergic receptor expression, rs295137 and rs295114 in the spermatogenesis-associated, serine-rich 2-like (*SPATS2L*) gene, which is associated with the acute bronchodilator response to SABAs.⁶¹

The less common or minor allele of rs37972 in *GLCCI1* was associated with a diminished response to ICSs and is less common in populations of African descent compared with a European population (Table I).^{60,89,90} In contrast, the minor allele of rs295137 in *SPATS2L* was associated with a greater acute SABA bronchodilator response and is more common in an African population compared with populations of European descent (Table I).^{61,89,90} Thus the difference in allele frequencies at these pharmacogenetic loci potentially contributes to a greater frequency of treatment failures or adverse responses to LABA

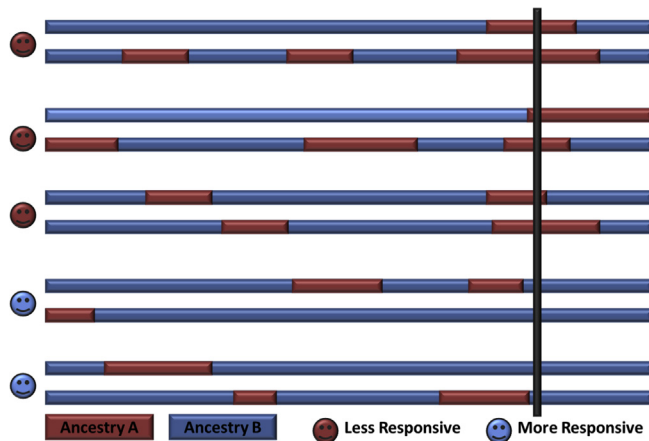


FIG 4. Illustration of admixture mapping. The hypothesis behind mapping by admixture linkage disequilibrium or admixture mapping is that chromosomes from an admixed population (shown with red and blue genetic regions from a specific ancestry) contain a susceptibility allele for reduced therapeutic responsiveness, which is more frequent in the red (ancestry A) ancestral region versus the blue (ancestry B) ancestral region. A hypothetical mapping by admixture linkage disequilibrium for a pharmacogenetic study would identify an increased proportion of ancestry A at a susceptibility locus in subjects who are less likely to respond to a pharmacologic therapy (region intersected by thick black line). Reproduced from Montana and Pritchard.⁹⁴

treatment and a relative preference for ICS therapy among African American when compared with non-Hispanic white subjects.⁸⁻¹⁰

ADMIXTURE MAPPING AS A MORE POWERFUL APPROACH TO GENOME-WIDE STUDIES

The greatest challenge in performing GWASs in admixed populations is the ability to enroll a sufficient sample size of these populations that have historically been underrepresented in clinical trial cohorts. The recent mixing of ancestries in these populations provides for an alternative method for genome-wide studies, which takes advantage of interindividual differences in genetic ancestry in admixed populations: mapping by means of admixture linkage or admixture mapping. Admixture mapping is a genome-wide approach that uses the variable allele frequencies of multiple SNPs between different ancestral populations (traditionally ancestry informative markers, but higher-resolution methods have also used arbitrary SNPs) to estimate genetic ancestry throughout the genome.⁹¹⁻⁹⁴

Admixture mapping uses estimates of ancestry at each SNP to test for associations with a phenotype in contrast to GWASs, which compare allele frequencies with phenotype (Fig 4).⁹⁴ Admixture mapping has greater statistical power than GWASs because it requires a smaller number of genetic markers while providing coverage for genomic regions that contain rare variants.⁹¹⁻⁹⁴ Admixture mapping is best applied in recently admixed populations in which a marked racial disparity exists in disease phenotype, which is not clearly attributed to environmental factors and has successfully identified novel asthma susceptibility loci in African American, Puerto Rican, and Mexican subjects.^{93,95-100} Admixture mapping estimates ancestry at genetic markers, which represent the surrounding genomic region (ie, region of admixture linkage disequilibrium; Fig 4), and therefore does not have the resolution of a GWAS or

whole-genome sequencing. Despite this limitation, admixture mapping is still a powerful method with the potential to identify regions containing pharmacogenetic loci unique to a particular ancestry within admixture mapping association peaks. Thus the optimal setting for admixture-based approaches in future pharmacogenetic studies will be in combination with whole-genome genotyping or sequencing to potentially identify genetic variation, which influences differential responses to asthma therapies observed between different ethnic or racial groups (Fig 4).⁸⁻¹⁰

NEXT-GENERATION SEQUENCING AND THE FUTURE PATH TO PERSONALIZED MEDICINE IN ADMIXED POPULATIONS

Most pharmacogenetic studies of admixed ethnic groups with asthma have been small candidate gene association studies that have identified “tagging” SNPs that are potentially co-inherited with another causative variant. Large consortium-based sequencing studies, such as the NIH/HLBI GO Exome Sequencing Project, CAAPA, and the 1000 Genomes Project are currently using next-generation whole-exome and whole-genome sequencing studies to provide an increasingly diverse genetic map of genomes from different admixed populations.^{22,23} These large-scale sequencing projects have revealed that admixed ethnic groups demonstrate a remarkable degree of genetic diversity related to an ancient African ancestry. Such genetic diversity has resulted in shorter regions of shared chromosomal segments (ie, linkage disequilibrium) and a greater frequency of rare variants in ethnic groups with an African ancestry compared with European ancestral populations.²²

Future pharmacogenetic approaches will need to enroll a larger number of subjects from underrepresented ethnic groups into clinical trials for GWASs, admixture mapping, and genotype-stratified trials based on comprehensive whole-genome genotyping platforms, which account for the genetic diversity of different ancestral backgrounds. Eventually, our understanding of the complex genetic factors influencing treatment response will improve, and the costs of genotyping and DNA sequencing and the issues of data storage will progress to the point that we will be able to develop genetic biomarker panels that will predict the most efficacious therapies at the least risk for an individual asthmatic patient. The path to personalized medicine for all ethnic groups will require that we improve our ability to decipher the expansive volumes of genotype and sequence data using different analytic methods that account for ancestral genetic structure, complex haplotypes, gene-gene interactions, and rare variants to detect and replicate novel pharmacogenetic loci.

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