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Admixture Mapping Identified Pharmacogenetic Gene-Gene Interaction for Asthma

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Admixture Mapping Identified Pharmacogenetic Gene-Gene Interaction for Asthma

by

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ABSTRACT

Rationale. A recent admixture mapping analysis identified interleukin 6 (IL-6) as a candidate gene for inflammatory diseases. The admixture signal was largely explained by a non-synonymous SNP Asp358Ala in the IL-6 receptor (*IL-6R)* gene, which affects serum levels of soluble IL-6R and IL-6. IL-6 signaling controls the balance between the production of pro- and anti-inflammatory factors in the airways during allergic inflammation. Moreover, anti-asthma medications such as albuterol have been shown to influence IL-6 production.

Objectives. To determine whether there are pharmacogenetic interactions between *IL-6* and *IL-6R* genes which are associated with variation in response to albuterol.

Methods. We analyzed 700 Mexican and Puerto Rican asthma families and 443 African American asthma cases and controls. We genotyped 4 SNPs in the *IL-6* gene [2 in the promoter (-174C/G and -572C/G) and 2 non-synonymous SNPs (Pro32Ser and Asp162Glu)] and the *IL-6R* Asp358Ala SNP. Family-based association tests and linear regression models were used to assess the association between individual SNPs and haplotypes with asthma, drug response, and asthma related phenotypes. Linear regression analyses were used to test for gene-gene interactions.

Measurements and Main Results. *IL-6* and *IL-6R* variants were significantly associated with asthma and bronchodilator drug response in Mexicans.

Although the frequency of the variants were low in Puerto Ricans and African Americans, we found similar trends for association. Single SNP associations differed among the three populations, yet we found a consistent gene-gene interaction which modified the bronchodilator drug response in Latinos and African Americans. We also demonstrated that the pharmacogenetic effect is modified by ancestral (racial) background.

Conclusions. Genetic variants in the *IL-6* and *IL-6R* genes act synergistically to modify bronchodilator drug responsiveness among ethnically diverse populations. Genotyping at these loci may be a useful approach for personalizing asthma bronchodilator therapy.

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Introduction

Race and Ethnic Background

There has been a recent debate about the use of racial classification in medicine and biomedical research[1, 2]. The argument behind this debate is whether race really does exist[3]. Some people propose that race is socially constructed where the formation of race was used to identify an inferior group of people who were conquered, colonized, and enslaved[3]. These conquered people were then identified by skin color, facial features, hair type, body type, and cultural differences. Unfortunately, this classification scheme was used to maintain a social order in which some groups dominated others[4]. Yet, other people propose that race is biologically constructed where geographic barriers along with natural factors have influenced the patterns of mating and reproduction of the human population to create genetically distinct groups of people, i.e. a group of people with homogeneous biological inheritance[3]. However, even with the view of race being socially constructed, race still serves as a meaningful categorization of humans since race serves as a surrogate for important environmental variations due to social stratification of race. This is further illustrated by the fact that the human population does not mate at random, but instead for several reasons, including geography, sociology, and culture, humans portray specific mating criteria and patterns[2]. Clearly, the categorization of humans by race is a blur between both social

and biological constructs, where the social labels placed on groups of people contributed to isolated patterns of mating amongst people with the same label.

Historically, within the United States and especially within minority populations, racial and ethnic background has been used as a cause for discrimination, prejudice, and marginalization[1]. The notorious racial segregation that affected every aspect of society from where one attended school to where one sat on the bus has remnants among U.S. citizens of today. Furthermore, this unjust use of race is highlighted by the higher U.S. poverty rate in certain racial groups as well as housing inequality along racial lines. Given this irrational use of race and current inequities based on perceived racial identities, many people strongly disagree with the use of race[2]. In clinical and epidemiological research, however, racial and ethnic categories are utilized for generating hypotheses about environmental and genetic risk factors in order to determine the interactions that effect disease outcome[1,2]. For example, to help untangle genetic and environmental factors, migrant studies are conducted on members of the same racial population, who are assumed to be genetically homogenous, that reside in different environments. In this scenario, if the migrants risk of disease remains the same, then researchers can conclude that there is a strong genetic factor behind the etiology of disease, however, if the migrants risk of disease becomes similar to the residents of the new environment, then researchers can conclude that there is a strong environmental factor predisposing to disease[2]. To support the genetic factor

underlying the etiology of disease, researchers can identify allele frequency differences between the migrant racial group and the residential racial group[2]; and to take this study a step further, researchers can establish if genetic-environmental interaction is taking place amongst either the migrant or residential racial populations. In addition, this strategy for categorizing humans is utilized to not only characterize the racial population risk of disease, but to also develop effective and efficient health prevention and treatment strategies that are tailored towards specific groups of people[2]. Risch et. al. has pointed out that if self-reported race/ethnicity is not taken into account for research studies, then, on average, any study in the U.S. will contain 75% Caucasians, 12% African Americans, 12% Latinos, 4% Asians, and few Pacific Islanders as well as Native Americans[2]. Clearly, this is troublesome since the majority of results will be derived and applicable to Caucasians, but not necessarily appropriate for minority racial populations.

Population genetics support the categorization of the world populations into five major groups – Africans, Caucasians, Pacific Islanders, East Asians, and Native Americans[2]. This categorization system depicts an evolutionary tree of human races with an "Out of Africa" theory, where populations outside of Africa are descendents of one or more migration events out of African within the last 100,000 years[2, 3]. Yet, despite the migration origination out of Africa, the greatest genetic diversity occurs within the African population[2]; correspondingly, genetic diversity between individuals is a function of the

degree of disconnection amongst ancestors[2]. Along similar lines, the greatest genetic diversity can be found between populations separated by continents[2]. Specifically, Africans contain their primary ancestry in sub-Saharan Africa, whereas Caucasians contain their primary ancestry in Europe, West Asia, Middle East, and North Africa. Furthermore, East Asians include ancestry from China, Indochina, Japan, Philippines, and Siberia[2]; on the other hand, Pacific Islanders include indigenous ancestry from Australia, Papua New Guinea, Melanesia, and Micronesia[2]. Nevertheless, Native Americans include indigenous ancestry in North and South America[2]. Ultimately, this ability to categorize people according to continental ancestry further underscores the validity of clinical and epidemiologic use of racial ancestry[4].

In the 2000 U.S. Census there were five major racial groups classified as Black or African American, White, Asian, Native Hawaiian or other Pacific Islander, and American Indian or Alaska Native. Interestingly, in this 2000 U.S. Census, 98% of the classified subjects reported themselves to be of a single race. Furthermore, 42% of respondents identified themselves as Latinos and thus checked the "other" box for race within the survey[2]. Having mentioned this, these racial constructs organize groups around geographic origin of a person's ancestry; however, this categorization does not take into consideration ethnic background. Furthermore, ethnic background casts a wider net when placing people into groups by taking into consideration cultural tradition, common history, religion, and often shared genetic heritage[1, 5].

Genetic Perspective of Race

From the prospective of genetics, gene structure is determined in the human population by patterns of mating and reproduction. Correspondingly, the greatest genetic differentiation in the human population occurs between continentally separated groups[2]. The scope of this genetic variation is great, with estimates of up to 15 million genetic polymorphisms[1]. A polymorphism is the existence of a gene with several different allelic forms. In further support of race as a biological entity, a study performed by Stephens et al. demonstrated that only 21% of single nucleotide polymorphism (SNPs) out of 3,899 present within 313 genes were found to be shared by four ancestral populations of African-American, Caucasian, Asian, and Latino[6]. These shared SNPs, or "Cosmopolitan SNPs" suggest that only a small proportion of genetic variation is shared across different ancestral populations. Yet, on the other hand, 25% of SNPs were identified as population specific alleles, thus indicating the occurrence of specific genetic variation within particular ancestral populations[6]. To highlight the potential magnitude of effect created by this percent difference of genetic variation, the human and chimpanzee share an identical 99% of DNA sequence[5], thus the 1% of difference in DNA sequence has allowed humans and chimpanzees to reside in drastically different habitats. In addition to different SNP frequencies across racial populations, studies have also identified ancestral haplotypes, or groups of SNPs strongly transmitted together, that are common in specific racial populations[3]. These racial populations that share similar SNPs and ancestral

haplotypes are believed to be due to the events of population bottlenecks and founder effects, of which both effect the distribution of genetic features in subsequent generations[3]. The importance behind this genetic variation is highlighted in such diseases as sickle cell disease or Alzheimer's disease where a change of only a single base pair is required to either cause or increase the risk of disease[1]. More importantly, these genetic variations that are associated with disease tend to occur more frequently in specific populations. For example, some diseases that have higher frequency in certain populations are sickle cell disease in the African-American population or Tay-Sachs disease in the Ashkenazi Jewish population[4]. The disease differences between racial groups can be used to identify disease-causing-mutations with high frequency in one particular racial population[3,4]. Subsequently, identification of common genetic variants within one particular racial/ethnic population allows more accurate clinical diagnosis, appropriately targeted health services, and adequately addressed public health education[3].

Complex Diseases and Race

Complex diseases are a result of multiple genetic and environmental factors. Moreover, complex diseases arise through various biological pathways and mechanisms[7]. Yet, aside from Mendelian diseases, where disease can be explained by a mutation in a single gene, the morbidity and mortality of complex diseases still segregates between stratified racial groups. For example, African Americans have an increased risk of developing and dying

from heart failure in comparison to Caucasians[8]. Another classic example is depicted with Alzheimer's disease and the APOE-e4 genetic variant which increases relative risk of Alzheimer's disease. In a study looking at three racial populations of Japanese, Caucasian, and African Americans, all of who have the APOE-e4 genetic variant, there is a racial difference in the risk of developing Alzheimer's disease. Specifically, Japanese who contain the genetic variant of APOE-e4 have an increased relative risk of 5 for developing Alzheimer's disease, Caucasians have an increased relative risk of 3, and African Americans have an increased relative risk of 1.1. Yet, the frequency of the genetic variants of APOE-e4 in these three racial populations displays substantial variation. In view of this, African Americans have the highest frequency of the APOE-e4 genetic variant, but the lowest relative risk of developing Alzheimer's disease, while Japanese have the lowest frequency of the APOE-e4 genetic variant, but the highest relative risk of developing Alzheimer's disease. From this study, the evidence suggests that there are unique genetic or environmental factors that modify the effects of the APOE-e4 genetic variant in each racial population.

Socioeconomic Status and Race

Socioeconomic status (SES) has also been correlated with race and ethnic background, as well as serving as a strong predictor of access to health care and quality of health care. In fact, the association of SES and disease is confounded by certain racial/ethnic groups, which are found within particular

levels of SES. This difference in disease prevalence is believed to stem from the unequal privilege and power between racially stratified socieities[4]. In this situation, SES may be serving as a complex multidimensional construct that captures many variables including unmeasured environmental factors[9]. Furthermore, socioeconomic factors can interact with other social experiences, gender, and race to produce different disease outcomes amongst different groups[9]. Take for example, African Americans with end-stage renal disease that are referred for renal transplant at lower rates than white Americans[1]. Previous studies have identified that higher rates of asthma exist in low socioeconomic populations, while lower rates of asthma exist in high socioeconomic populations[9]. Nevertheless, racial and ethnic differences in disease outcomes still exist after controlling for SES and health care access. For example, the rate of complications from type 2 diabetes mellitus varies according to racial or ethnic differences among members of the same health maintenance organization, despite uniform utilization of outpatient services, and after adjustment for levels of education, income, health behavior, and clinical characteristics[1].

Racial Drug Response

The categorization of humans by race is utilized to identify groups of individuals within the population who are more susceptible to particular diseases or more likely to respond to certain therapeutic treatments[2, 3]. Functional allelic variants in drug metabolizing enzymes have been identified

to demonstrate distinct differences in frequencies across different racial populations[2]; these differences of genetic variants lead to a differential drug response between different racial groups. For example, a study to determine the efficacy of angiotension-converting-enzyme (ACE) inhibitors in 1200 white and 800 black patients with congestive heart failure has revealed that the ACE inhibitor enalapril administered in the Black patients had no substantial effect in reducing hospitalization in comparison to placebo, whereas the Caucasian patients had significantly reduced hospitalization with enalapril[2,4]. From this study stems the notion of race-based-therapy that utilizes frequency differences in genetic variants that affect the efficacy of drug between different racial groups[4]. Further evidence is provided in a study performed by Choudhry et al. where they demonstrated that subgroups of Latinos with certain genetic variants exhibit variable bronchodilator drug response to Albuterol[10]. Specifically, they demonstrated that the Arg16allele of the β_2AR gene is correlated with enhanced bronchodilator drug responsiveness in Puerto Ricans, but not in Mexicans with asthma[10]. Even further evidence is provided by this same group by demonstrating that the Arg19Cys genetic variant of the β_2AR gene affects bronchodilator drug response in African American asthmatics only[10]. The patent for the first race-specific drug was BiDil, which was approved for therapeutic administration to African Americans with heart disease[8]. The ability to further grasp the genetic variability in drug response will aid physicians in optimally diagnosing and treating individual patients or particular groups of patients[11].

Racially Admixed Populations

Genetic admixture, which is the presence in a population of persons with multiple races or ethnic backgrounds has been documented to occur in the border regions of continents[1]. Recently, in the United States, mixture among different racial groups has occurred. In the 2000 U.S. Census, however, the majority of respondents still classified themselves as belonging to a single racial group. Genetic studies performed on African-Americans have revealed that a range of 7 to 20 percent white admixture exists within this community[1, 2]. Even though this white admixture exists within the community of African-Americans, they are still genetically similar to Africans. Another admixed population that exists within the United States is the Latino population, which appears to be the largest and fastest growing minority population in the United States. Correspondingly, this Latino population is an admixed group that includes White, African, and Native American ancestry. Within each of these populations, varying levels of admixture is present and is correlated with geographic region[1]. For example, Latinos in the Southwest of the United States, who are primarily Mexican-American, display admixture estimates of 39% Native American, 58% Caucasian, and 3% African[2]; on the other hand, Latinos in the East Coast of the United States, who are primarily from the Caribbean, display a larger amount of African admixture[2]. Choudhry et\ al. has shown that despite identifying both biological parents and all four grandparents as of either all Mexican or Puerto Rican ethnicity, there still was extreme variation of ancestry in both ethnic groups of asthmatic children[10].

Amongst the Puerto Rican asthmatics, some subjects displayed less than 10% African ancestry whereas other subjects had over 50% African ancestry; likewise, some subjects displayed less than 20% European ancestry whereas other subjects displayed over 80% European ancestry[11]. Amongst the Mexican asthmatics, some subjects displayed nearly 100% European ancestry whereas other subjects displayed nearly 100% Native American ancestry[11]. On average, Puerto Ricans have 66% European ancestry, 16% African ancestry, and 18% Native American ancestry; on the other hand, Mexicans have 45% European ancestry, 3% African ancestry, and 52% Native American ancestry[11]. Within the Mexican population, the European ancestry derives mainly from the Spanish regions of Castilla, Andalucia, and Extremadura; however, the Spanish population is compromised of people with background of Phoenician, Greek, Roman, Arabic, and Jew[12]. Moreover, the African component in the Mexican population derives from Africans on the West coast of Africa between the Senegal River and Portuguese Angola[12]. These varying levels of admixture present a unique opportunity for genetic-environmental studies. Based on this opportunity, Dr. Gonzalez-Burchard has launched the Genetics of Asthma in Latino Americans (GALA).

The Effects of Admixture in Populations

Ancestry informative markers have provided researchers with the ability to perform research on how different racial or ethnic backgrounds affect disease severity and disease progression within an admixed population[13]. These

ancestry informative markers (AIMS) have large differences in allele frequency between populations. Choudhry et al. found that among Puerto Ricans, asthma and ancestry is associated with SES[9]. Specifically, in African Americans with lower SES, European ancestry was associated with an increased risk of asthma while African ancestry was associated with a decreased risk of asthma[9]. Conversely, in African Americans with higher SES, African ancestry was associated with an increased risk of asthma while European ancestry was associated with a decreased risk of asthma[9]. Interestingly, healthy Puerto Ricans reporting upper SES had 9.1% lower African ancestry and 9.2% higher European ancestry than Puerto Ricans reporting moderate or middle SES. In addition, other studies have indicated that Puerto Ricans who self-identify as Black have lower mean household income and are more likely to live below the poverty level than those who self-identify as White. Along similar lines, Mexican Americans with dark skin/American Indian appearance are more likely to be discriminated against, receive less education, and hold occupations with lower prestige than other Mexicans with light skin/ European appearance[13]. The effects of admixture extend beyond SES and into the realm of health. Previous studies have demonstrated that among Mexican asthmatics, Native-American ancestry is associated with mild asthma whereas European ancestry is associated with severe asthma[14]. In fact, the effects of admixture can be seen even in previously believed homogenous ancestral populations. For example, within Caucasians, the hemochromatosis gene mutation C282Y has a frequency of

less than 1% in Armenians and Ashkenazi Jews, but 8% frequency in Norwegians[2]. A complex interaction between SES and ancestry has been demonstrated to effect disease severity. More specifically, Choudhry et al. identified that asthmatic patients of lower SES exhibited lower African ancestry and higher European ancestry compared to non-asthmatic lower SES controls, while asthmatic patients of higher SES had higher African ancestry and lower European ancestry in comparison with non-asthmatic high SES controls. These associations of SES and ancestry on disease severity are significant; however, the strongest evidence for a genetic contribution to group differences of disease would be the identification of a specific genetic variant associated with disease outcome that varies in frequency across racial/ethnic groups.

Population Stratification in Admixed Populations

In an admixed cohort, population stratification is the over- or underrepresentation of one particular ancestry in only cases or only controls[11]. Population stratification arises in admixed populations as a result of unequal distribution of risk of disease between different ancestral groups, thus causing an oversampling of subjects with an excess of ancestry with supposed high risk amongst the group of subjects with disease[11]. Choudhry et. al. have provided evidence of population stratification confounding genetic association studies of asthma in the Latino population[15]. Specifically, they observed a large degree of allelic association between pairs of ancestry informative markers on different chromosomes in Mexicans and Puerto Ricans, thus

contributing to evidence of recent admixture[15]. Moreover, in Puerto Ricans, they observed a significant difference in allele frequencies between asthmatic cases and controls; yet after adjustment of ancestry, only two alleles remained significantly associated with asthma; therefore, these results suggest that the effects of population stratification may influence the interpretation of results in case-control studies amongst admixed populations[15].

Determination of Ancestry Estimates

Two populations with no migration and who have separated eons ago will require less genetic markers to differentiate between the two groups as opposed to two populations who have recently separated and with recent migrations, which as a result, will require more genetic markers to genetically untangle the two groups[2]. In a study performed by Tsai et. al., the most influential factor in determining accuracy of admixture and in minimizing type I error is the number of markers that estimate admixture[16]. Specifically, they have identified that with a marker informativeness of 0.4, indicated by a mean delta value out of a range of 0.1 to 0.8, 100 markers would be required to receive admixture estimates that strongly correlate ($r>0.9$) with true admixture estimates[16]. In addition, they have discovered that the excess type I error rate is controlled at the 5% level when 100 admixture markers are used to estimate admixture[16].

Single Nucleotide Polymorphisms, Linkage Disequilibrium, And Haplotype Blocks

The largest amount of genetic variation within the Human Genome is captured in the form of SNPs[7], which are single base changes within the genetic code. In general, a polymorphism is a DNA sequence variant with an allele frequency of at least 1% in a population; however, SNPs with frequency of 5% or more are usually used for candidate gene studies[7]. Importantly, SNPs have been identified that account for susceptibility to certain diseases as well as specific patterns of drug metabolism and drug response[17]. In addition, SNPs have been identified to occur in discrete regions with high frequency and strong linkage disequilibrium separated by regions of low frequency and weak linkage disequilibrium. Correspondingly, these regions of high SNP frequency and strong linkage disequilibrium (LD) are known as haplotype blocks. The importance behind these haplotype blocks is stressed by the potential they offer to capture vast regions of genetic information, while reducing the workload of genotyping because a single or small number of SNPs within a haplotype block captures the same genetic information as genotyping every single SNP within the block. The exploited single SNP, or Tag SNP, represents the entire haplotype block based on high LD between the neighboring SNPs; if an individual contains one SNP within the haplotype block then they are also bound to contain every other SNP that resides within the same block. As a result of these Tag SNPs, genetic researchers can identify genetic regions, or even more specifically, single SNPs that are

associated with definite diseases[17]. SNPs can affect the gene in a variety of ways; nonsense SNPs cause premature termination of amino-acid sequence, non-synonymous SNPs cause amino acid change in protein, synonymous SNPS can alter splicing, insertion-deletion SNPs can cause an amino acid change or change the frame of the protein-coding region, promoter SNPs can affect level and location of gene expression, splice site SNPs change the splicing pattern, intronic SNPs might affect expression or mRNA stability, and intergenic SNPs might affect expression through enhancer or other mechanism[7]. In addition to addressing the function of the SNP, the frequency of the SNP in the population should be tested[7]. For example, SNPs with low allele frequencies need extremely large relative risk to be detected in candidate gene studies[7].

Admixture Mapping

Admixture mapping is a method used for localizing disease causing genetic variants that differ in frequency across populations [18-20]. This approach is most advantageous to apply to admixed populations, such as Latinos and African Americans, which descended from a recent mix of two or more ancestral groups that have been geographically isolated for many tens of thousands of years [15, 21-23]. The method assumes that near a disease causing gene there will be enhanced ancestry from the population that has greater risk of developing the disease. Thus, if one can calculate the ancestry along the genome for an admixed sample set, one could use this ancestry to identify

disease causing genetic variants. Evidence suggest that the admixture occurring in the Latino and African American population has occurred within the past 20 generations; this would mean that the genomic regions of LD have neither yet decayed nor does the LD extend too far to accurately identify a genomic region[11]. The advantage in using admixture mapping is the increased statistical power to identify genes of modest effect with different distribution across admixed populations[11]. This correlation to ancestry suggests that admixture mapping in ethnically diverse populations may be used to find genes underlying asthma.

The Latino Population

Within the United States, Latinos are the largest, youngest, and fastest growing minority population[11]. Excluding residents of Puerto Rico, there are currently 42.6 million Latinos residing in the United States[11]. In 2050, 25% of the U.S. population is predicted to be Latino[11]. A fact supporting this prediction is that the majority of immigrants to the United States come from Latin America[11]. The 10 main Latino sources of immigrants to the United States are Mexico, El Salvador, the Dominican Republic, Colombia, Guatemala, Peru, Cuba, Ecuador, Brazil, and Honduras[11]. Furthermore, in considering all children in the United States, Latinos represent the largest demographic group[11]. Yet, despite sharing similar cultural traditions and heritage, Latinos are not a homogenous population, but instead a heterogenous group, with dynamic variation in genetic, socioeconomic,

educational, and demographic factors[11]. Within the Latino population, asthma prevalence is highest in Puerto Ricans, intermediate in Dominicans and Cubans, and lowest in Mexicans and Central Americans[11]. Latinos are an admixed population composed of African, European, and Native American ancestry; as a result, they provide an optimal opportunity to unravel the clinical, social, genetic, and environmental factors underpinning the drastic differences in asthma morbidity and mortality amongst Latinos[11].

Asthma

Asthma is a common but complex respiratory ailment. In the U.S., asthma prevalence and severity are highest in Puerto Ricans, intermediate in African Americans, and lowest in Mexicans [24, 25]. Although there are many potential explanations for these observations, including socioeconomic and environmental factors, an additional contributor may be a genetic predisposition that varies among racial/ethnic groups. Latino and African American populations are admixed and share varying proportions of African, Native American and European ancestry [10, 22, 26]. The rich mixed ancestry of Latinos and African Americans provides the intrinsic variability needed to untangle complex gene-gene interactions that effect disease susceptibility, severity, and drug response.

The major characteristics of asthma are airflow obstruction, bronchial hyperresponsiveness, and airway inflammation[12]. These characteristics of

asthma manifest as symptoms of wheeze, dyspnea, and chest tightness[27]. The underlying mechanism of asthma involves not only inflammation, but also control of airway tone and activity[28]. Furthermore, asthma is a common and complex respiratory disease caused by an interaction of both genetic and environmental factors[9]. The key feature of asthma is the exaggerated bronchoconstriction after the inhalation of various stimuli[28]. Clinically, asthma is diagnosed based on the patient's medical history, physical examination, reversibility of airway obstruction, and exclusion of alternative diagnoses that resemble asthma[28]. Since asthma is a complex disease, it is not surprising that asthma prevalence and severity varies drastically between countries as well as between different populations with countries[9]. In fact, within the United States, asthma prevalence and mortality are the highest among Puerto Ricans and the lowest among Mexicans[9, 13]. Yet, for all U.S. children, the prevalence of asthma has increased from 3.6% in 1980 to 5.8% in 2003; the third leading cause of hospitalization among U.S. persons under 18 years of age is asthma[28]. Furthermore, the prevalence of asthma appears to be increasing world-wide [28]. Part of this increase in asthma prevalence is attributable to increased public awareness and changes in the diagnosis of this disease[28]. This sharp increase in the prevalence of asthma has occurred in such a short period of time, thus it is reasonable to hypothesize that this quick change in disease is not due to genetics, but rather mainly due to change in environmental exposure. Some environmental exposures that have been associated with asthma are tobacco smoke and air pollution. A meta-analysis

has concluded that parental smoking is causally related to acute lower respiratory tract illnesses in infancy and childhood asthma[28]. In addition, several studies have suggested that air pollution decreases lung function, triggers exacerbations of asthma, and increases rates of hospitalization for asthma[28]. Some studies even point out the evidence of association between obesity and asthma. Specifically, these studies have identified that weight gain can precede the development of asthma, whereas weight loss improves lung function; therefore, these studies suggest that recent changes in both diet and lifestyle are contributing to the increased prevalence of asthma[28]. Other studies have disproved the association of certain environmental exposures and asthma. For example, numerous birth-cohort studies have found no association between exposure to house-dust mites early in life and childhood asthma[28]. Further evidence suggests that exposure to environments rich in microbial substances, such as being raised in a farm, significantly decreases the risk of asthma[28]. Along similar lines, other studies suggest that exposure to infectious agents early in life protect against asthma[28]. All in all, these research results strongly support the interaction between environmental exposures and underlying genetic predispositions that result in the manifestation of asthma.

A characteristic of asthma inflammation is the increased numbers of lymphocytes, mast cells, and especially eosinophils that migrate into the airways of the lung[29]. Accordingly, this migration of inflammatory cells into the

airways is directed by activation of adhesion molecules on the endothelial cell surface[29]. Since eosinophils do not normally reside in the airways of non-asthmatics they serve as a specific hallmark of asthma[29]. With this hallmark, eosinophils are found in the airways of asthmatic patients; thus, as a result, treatment with drugs, such as inhaled corticosteroids, cause apoptosis of eosinophils and subsequent removal of eosinophils from the airways is associated with decreased symptoms in asthmatic patients[29]. Further research, pointing towards the culprit of asthma being eosinophils, has demonstrated that experimental treatments that prevent eosinophil migration in animal models inhibit bronchocontrictor response[29].

Heterogeneity of Asthma Therapy

There are three main modes of therapy for asthma – inhaled corticosteroids, β_2-agonists, and leukotriene inhibitors. Yet, since asthma is a complex disease that results from multiple genetic and environmental factors, there is drastic variation in drug response amongst asthmatics[27]. Even in patients with an identical clinical phenotype of asthma there still remains significant variation in response to asthma medications[27]. Current research supports variable drug response in asthmatics due to genetic factors[27]. The identified genetic factors behind the variability in drug response are polymorphisms that have been associated with corticosteroid resistance and polymorphisms within the genes encoding the drug target[27]. These pharmacogenetic loci have a strong association between a specific DNA sequence variant and clinical

response to a particular asthma medication[27]. In determining variable drug response, the repeatability versus heritability of the measured drug response needs to be established. With this approach, the within-individual variance in the treatment response, which is due to environmental variance and measurement error, is compared to the total population variance in the treatment response, which is due to genetic, environmental, and measurement error[27]. In a study performed by Drazen et. al., they estimated that the among-patient variability and within-population variability from the final percent of the predicted forced expiratory volume in one second (FEV_1) achieved after 6 weeks of treatment with inhaled triamcinolone acetonide to be 86.1%; therefore, these results suggest that a clinically significant proportion of the inhaled corticosteroid treatment response is heritable[27]. Likewise, the within-patient variability and within-population variability for β_2-agonists is 61.2%; therefore, these results also suggest that a clinically significant proportion of the β_2-agonists treatment response is heritable[27]. From these studies as well as others, Drazen et. al. conclude that at least half of the observed variation in treatment response to inhaled β_2-agonists, inhaled corticosteroids, and leukotriene inhibitors is due to genetic variation between patients[27].

A significant fraction of asthmatic patients have been identified as being resistant or non-responsive to inhaled corticosteroid therapy, so-called glucocorticoid-resistant asthmatics[27]. According to clinical definition, glucocorticoid-resistant asthmatics are asthmatic patients who fail to improve

their FEV_1 by 15% from a baseline of ≤75% predicted, yet they have a ≥15% reversibility to β_2-agonists[27]. The first description of glucocorticoid-resistant asthmatics was by Schwartz in 1967, and in 1981, Carmichael described 58 subjects with chronic asthma who were clinically resistant to inhaled corticosteroids[27]. Patients with glucocorticoid-resistant asthma are more likely to have a longer duration of asthma, a family history of asthma, poorer lung function, and a greater degree of bronchial reactivity[27]. These results indicate that both genetic factors, as suggested by family history, and environmental factors, as suggested by longer duration of asthma, play a role in the pathogenesis behind glucocorticoid-resistant asthma[27]. In searching for the mechanism behind this resistance, several studies have determined that glucocorticoid-resistant asthma is associated with both in vitro and in vivo impaired responsiveness of monocytes and T lymphocytes to the suppressive effects of glucocorticoids[27]. Furthermore, studies have compared the bioavailability of glucocorticoids in both glucocorticoid-sensitive and glucocorticoid-resistant asthmatics; in these studies, there was no difference in bioavailability between both groups, and both groups demonstrated similar, if not the same, plasma protein binding, distribution, and clearance of glucocorticoids[27]. To take this glucocorticoid resistance phenomenon a step further, studies have shown that the glucocorticoid-resistant asthmatics do not have an abnormality in glucocorticoid receptor nuclear translocation, density, or binding affinity[27].

In the normal steroid mechanism, glucocorticoids enter the cell by passive diffusion to bind to glucocorticoid receptors located in the cytoplasm[27]. Binding of glucocorticoids to the glucocorticoid receptors results in a conformational change resulting in dephosphorylation and dissociation into two 90 kDa associated heat shock proteins to form dimers that translocate into the nucleus[27]. In the nucleus, the glucocorticoid-formed dimers binds to specific sequences in the DNA, i.e., glucocorticoid response elements, in the promoter regions of the glucocorticoid responsive genes, thus resulting in either induction of inflammatory genes or suppression of anti-inflammatory genes[27]. Based on this sequence of steroid events, it is plausible to predict that polymorphisms within the glucocorticoid response element may effect the induction or suppression of inflammatory genes.

The most commonly prescribed asthma medication is albuterol, a B_2-agonist which is used as a rescue bronchodilator. Both short and long-acting B_2-agonists have shown protective effects against a variety of direct and indirect stimuli of bronchoconstriction[27]. In addition, clinical trials point towards an additive effect with joint administration of long-acting B_2-agonists and inhaled corticosteroids in controlling asthma[27]. There are numerous reports detailing differential response of B_2-agonists between different racial populations. For example, as previously mentioned, Choudhry et. al. has shown that Puerto Rican asthmatics have a poorer bronchodilator response to B_2-agonists in comparison to Mexican asthmatics[10]. An explanation behind this variable drug

response to B_2-agonists is genetic sequence variants within the B_2-adrenoceptor (B_2AR) gene. Accordingly, the B_2AR gene contains a single coding region with no introns and is located on the long arm of chromosome 5[10]. Within the single coding region 9 SNPs have been identified, four of which cause a single amino acid substitution (Ile-64, Met-34, Gly-16, and Glu-27)[27]. These four SNPs have functional consequences to the encoded protein; Ile-164 SNP results in a significant decrease in agonist binding affinity and coupling to adenylate cyclase, Gly-16 and Glu-27 significantly alter agonist-promoted receptor down-regulation and functional desensitization[27]. Accordingly, Gly-16 enhances down-regulation of the agonist-promoted receptor and Glu-27 protects against agonist-promoted down-regulation[27]. In epidemiologic studies, Gly-16 has been associated with nocturnal asthma and decreased bronchodilator response to B_2-agonists; Glu-27 has been associated with decreased airway reactivity in asthma and increased IgE levels and self-reported asthma in children[27]. In addition, SNPs at locus 16 of B_2AR have been shown to be significantly associated with tachyphylaxis to B_2-agonists salmeterol[27]. As a result, these results collectively suggest that variation in both asthma phenotype and bronchodilator drug response can be explained by variation in the genetic sequence of the B_2AR gene.

Some asthmatic patients fail to respond to leukotriene inhibitors[27]. A possible explanation behind this failure is genetic variation within genes of the leukotriene pathway. Accordingly, causal genetic variants have been found to

exist in the promoter of the ALOX5 gene and lead to decreased binding of the promoter and less gene transcription in comparison to wild-type ALOX5[27]. To determine if polymorphisms in the ALOX5 gene affected leukotriene treatments in asthmatics, Drazen et. al. performed a 12-week randomized, placebo-controlled and double-blind clinical trial of the effects of Zileuton inhibition conducted on patients stratified by genotype of the ALOX5 promoter locus[27]. In this clinical trial, patients who harbored the mutant ALOX5 promoter alleles had a decreased response or improvement in asthmatic symptoms as measured by change in FEV_1[27]. This study serves as an example of how genetic variants within drug-targeted genes can help predict whether an asthmatic patient will or will not response to the designated medication.

The IL-6 Pathway

The IL-6 pathway controls the balance between the productions of pro- and anti-inflammatory factors in the airways during allergic inflammation. IL-6, on chromosome 7p21, is activated via two different modes. Classically, IL-6 binds to a specific membrane-bound IL-6R (mIL-6R) and then the IL-6/IL-6R complex interacts with glycoprotein 130 (gp130) leading to signal initiation [32]. On the other hand, cells lacking mIL-6R can also respond to IL-6 via soluble IL-6 receptor (sIL-6R), since the IL-6/sIL-6R complex can activate target cells expressing gp130 in a process named IL-6 *trans*-signaling. The cells that are chiefly responsive to IL-6 in the presence of sIL-6R are embryonic stem cells,

early hematopoietic progenitor cells, T cells, neural cells, smooth muscle cells, mesothelial cells, and endothelial cells[33]. Accordingly, gp130 is expressed by most cells in the body while mIL-6R is expressed mainly by hepatocytes, neutrophils, monocytes/macrophages, and some lymphocytes[33]. The generation of sIL-6R is through both proteolytic cleavage of the membrane-bound IL-6R and alternative IL-6R mRNA splicing[33]. Although there are potentially two mechanisms of sIL-6R generation, the mechanism of generation depends on the individual's age, the inflammatory condition, and the stage of disease progression[33]. After generation, sIL-6R can be found in the plasma; therefore, sIL-6R has the ability to mediate both local and systemic IL-6 responses[34]. One of the systemic IL-6 responses is inflammation; this process is supported by clinical studies that have identified a positive correlation between the amount of leukocyte recruitment during inflammation and level of sIL-6R[34]. Specifically, sIL-6R has been demonstrated to differentially regulate the phenotypes of leukocytes that are recruited to inflammatory sites[34]. In this respect, IL-6 trans-signaling promotes transition from the acute innate immune response to the acquired immune response[33]. To support this claim, studies have suggested that IL-6 trans-signaling contributes to the retention of activated mononuclear cells within inflamed tissue.

In comparison to healthy controls, subjects with asthma have increased circulating levels of both IL-6 and sIL-6R which are further increased during an

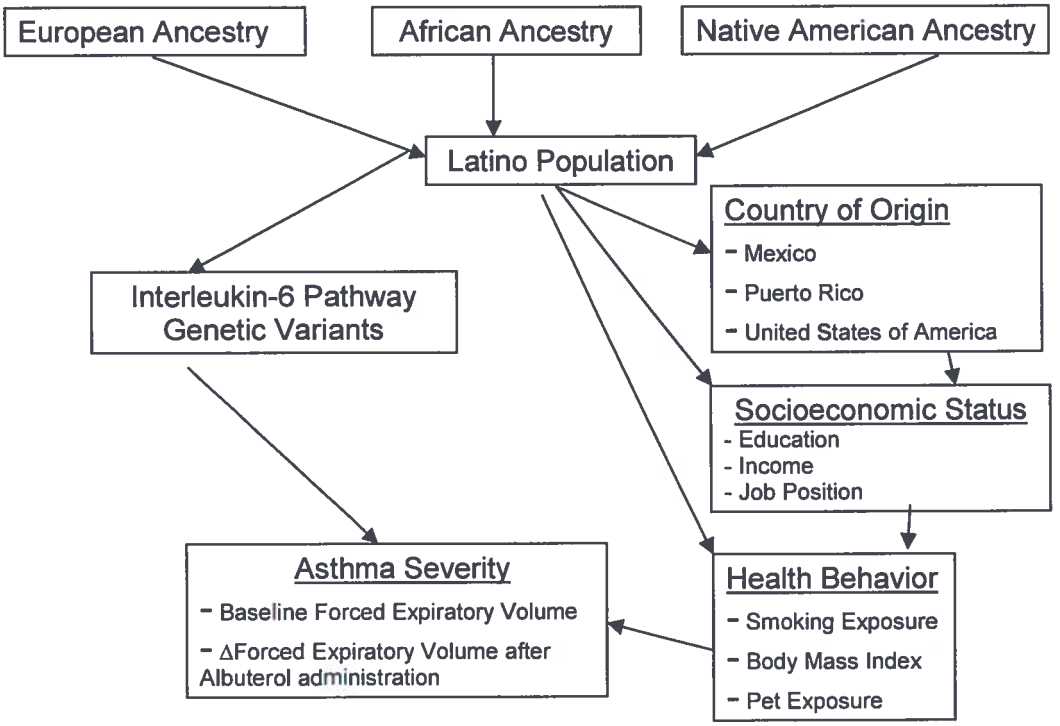
asthma attack or antigen inhalation [35, 36]. Genetic variants in the *IL-6* gene have been shown to affect IL-6 expression and IL-6 serum levels [33, 34, 37]. Furthermore, β-2 agonists have been shown to influence IL-6 production in vivo and in vitro [38, 39].

Interleukin-6 Receptor (IL-6R)

Circulating markers of inflammation are associated with risk of inflammatory diseases such as asthma [30]. A recent admixture mapping analysis of an African American cohort demonstrated an association between European ancestry on chromosome 1 and sIL-6R levels [31]. Fine mapping of this region determined that a single coding variant (Asp358Ala) of the *IL-6R* gene was strongly associated with sIL-6R and IL-6 serum levels in Blacks and Whites. Moreover, the frequency of this variant was highest in Europeans (35%) and lowest in West Africans (4%), which could be contributing tor differential risk of asthma across populations.

Based upon the biological, genetic and clinical evidence, we hypothesized that functional genetic variants in the IL-6 pathway interact to influence the drug response in Latino and African American asthmatics. To investigate whether *IL-6* and *IL-6R* variants alone or in combination modify bronchodilator drug response among subjects with asthma, we performed an analysis of subjects from the Genetics of Asthma in Latino Americans (GALA) Study and the Study of African Americans, Asthma, Genes, and Environments (SAGE) [14, 40].

Concept Map



Methods

Sampling and Recruitment

Subjects with asthma and their biological parents were enrolled over a 4-year period in the San Francisco Bay Area, California; New York City, New York: Puerto Rico: and Mexico City, Mexico. Subjects were recruited by investigators from community schools, clinics, and hospitals that served the Latino population. Medical records were reviewed in all health care centers in order to correctly identify patients with physician-diagnosed asthma, who then were contacted to participate in the study. Recruitment was standardized

across all clinical centers. Bilingual and bicultural physicians specialized in asthma were present at all interviews. In addition, all forms and questionnaires for subjects were available in English and Spanish. Although questionnaires at each recruitment site were identical, culturally and linguistically competent recruiters interviewed all subjects to account for differences in local Spanish dialects. Each participating subject had a blood sample collected, processed, and anonymously labeled and stored at the UCSF DNA bank.

Study Participants

In all, 700 Latinos consisting of 301 Mexicans and 399 Puerto Ricans with asthma (probands) and their biological parents were enrolled for the GALA study. African American asthmatics (n = 267) and healthy controls (n = 176) were enrolled for the SAGE study. In both GALA and SAGE studies, ethnicity was self-reported and subjects were enrolled only if both biological parents and all four biological grandparents were of the same ethnic (GALA) or racial (SAGE) background. Race and ethnicity were ascertained by standardized questions. Specifically, both biological parents and all biological grandparents had to idendity themselves as either of Puerto Rican ethnicity (for the NY and PR sites), Mexican ethnicity (for the SF and MX sites), or African American race (for the SF sites) in order for the subjects with asthma to participate in the study. Families with mixed ethnic or racial background were excluded from the study.

Asthma and Medical Questionnaire

Subjects were 8 to 40 years of age, had a current physician diagnosis of asthma, and had experienced the presence of asthma symptoms (wheezing, cough, or shortness of breath) over the last 2 years. A trained interviewer administered a modified version of the 1987 American Thoracic Society (ATS)–Division of Lung Disease Epidemiology Questionnaire [41], which was modified to assess the frequency and duration of asthma and allergy symptoms as well as environmental tobacco smoke exposure. Furthermore, questionnaires were available in Spanish and English. Local institutional review boards approved all studies, and all subjects provided written, age-appropriate informed consent.

Pulmonary Function Tests and IgE Measurements

Spirometry was performed according to ATS standards [42]. Specifically, subjects with asthma were instructed to withhold their bronchodilator medications for at least 8 hours before spirometry was performed. Spirometry was performed in the sitting position according to a protocol established by the Asthma Clinical Research Network. Then, albuterol was administered through a spacer device from a standard metered dose inhaler: Two puffs (180 μg) for subjects less than 16 years old, and four puffs (360 μg) for subjects 16 years old or older. Pulmonary function test results are expressed as a percentage of the predicted normal value using age-adjusted prediction

equations from Hankinson [43]. Baseline lung function was determined by forced expiratory volume in 1 second (FEV_1) expressed as percent of predicted (pre-FEV_1). Subjects with asthma were classified as having "mild" or "moderate-severe" asthma based on their pre-FEV_1 levels. Subjects with a pre-FEV_1 greater or less than 80% of predicted were categorized as having "mild" and "moderate-severe" asthma, respectively. A quantitative measure of bronchodilator drug responsiveness (ΔFEV_1) was calculated as the percent change in pre-FEV_1 after albuterol administration (post-FEV_1 in liters minus pre-FEV_1 in liters, times 100, divided by pre- FEV_1in liters). Total plasma IgE was measured in duplicate in all asthmatic subjects (Mexican, Puerto Rican, & African Americans) using Uni-Cap technology (Pharmacia, Kalamazoo, MI).

Socioeconomic Status

The Federal Financial Institutions Examination Council's (FFIEC) Geocoding/Mapping System (http://www.ffiec.gov/geocode/default.htm) was used as a proxy for SES. Accordingly, the FFIEC classifies subjects into 1 of 4 U.S. Census 2000 tract income levels: Low, Moderate, Middle, and Upper. The classification is based on the census tract's median family income (MFI) divided by the median family income of the metropolitan area (MA MFI) that the tract is located, or MFI%. If the MFI% is less than 50%, then the income level is classified as "Low"; if the MFI% is ≥50% and <80%, then the income level is classified as "Moderate"; if the MFI% is ≥80% and <120%, then the income level is classified as "Middle"; and if the MFI% is ≥120%, then the

income level is classified as "Upper". In assigning SES, the individual's clinic recruitment site address and the individual's home address were used.

Individual Ancestry Estimation

To determine individual ancestry estimates, we genotyped 104 ancestry informative markers (AIMs) in all Mexican, Puerto Rican, and African American asthmatics. The 104 AIMs were selected to be distributed across the genome and identified as encompassing large allele frequency differences between Native American, West African, and European ancestral populations. Accordingly, the ancestral allele frequencies have been characterized extensively by genotyping individuals of the following populations: Irish, English, German, and Spanish (European, n=243); Nigerian, Central African Republic, Sierra Leone (African, n=481); Mayan, Pima, Cheyenne, and Pueblo (Native American, n=148). Detailed information with regard to polymorphic site, flanking sequence and other relevant information for all the 104 AIMs is available in dbSNP (http://www.ncbi.nlm.nih.gov/SNP/). Genotyping data of these AIMs were imputed into the program STRUCTURE 2.1 in order to determine individual ancestral estimates in each asthmatic subject. To correct for population stratification, individual ancestry estimates were included in the regression models.

Selection of Single Nucleotide Polymorphisms for IL-6 & IL-6R

Based upon our biological hypothesis, we chose to evaluate functional *IL-6* and *IL-6R* SNPs. Two promoter *IL-6* SNPs -174C/G (rs1800795) and -572C/G (rs1800796) have been extensively studied and have been shown to affect IL-6 expression and serum levels [33, 34, 37]. Two *IL-6* coding variants, Pro32Ser (rs2069830) and Asp162Glu (rs13306435), have been recently described to affect IL-6 binding to gp130 [44-46]. The genetic variant *IL-6R* Asp358Ala (rs8192284), which was identified through admixture mapping, corresponds to the proteolytic cleavage site of the IL-6R and affects serum levels of sIL-6R and IL-6 [31, 47].

We determined the frequencies of the selected SNPs by genotyping them in our SNP Discovery Panel, which includes 72 unrelated asthmatics of each ethnic group: 24 Mexicans, 24 Puerto Ricans and 24 African Americans. The genetic variants with a minor allele frequency greater than 5% were further selected to be genotyped in the following populations: *IL-6R* Asp358Ala and *IL-6* -572C/G were genotyped in all three populations; *IL-6* -174C/G in both Puerto Ricans and Mexicans; *IL-6* Pro32Ser in both Puerto Ricans and African Americans; and *IL-6* Asp162Glu in Mexicans only.

Genotyping

The 104 AIMs were genotyped using the AcycloPrime-FP™ (PerkinElmer) method. PCR conditions were 6 µl volume with Platinum Taq PCR buffer, 2.5

mM $MgCl_2$, 2.4-4.0 ng genomic DNA, 50 µM dNTPs, 0.1-0.2 µM primers, 0.1-0.2 units Platinum Taq (Invitrogen) plus 1 µl extra water to counteract evaporation. Cycling conditions were: 95◦C for 2 minutes, 35 cycles of 95◦C for 10 seconds, 55◦C for 20 seconds, 68◦C for 30 seconds, and final extension at 68◦C for 10 minutes. Enzymatic cleanup and single base extension genotyping were performed with AcycloPrime-FP kits. Plates were read on an En Vision fluorescence polarization plate reader (PerkinElmer). PCR and genotyping primer.

Statistical Analyses

In the GALA trios, Mendelian inconsistencies were identified using PEDCHECK [48]. Families with Mendelian inconsistencies were excluded from further analyses. Hardy-Weinberg equilibrium (HWE) was calculated by means of x^2 goodness-of-fit tests for each ethnic group. Pair-wise linkage disequilibrium (LD) was estimated by using the program LD Plotter, which uses an iterative expectation-maximization (EM) algorithm to calculate r^2 values [49].

Single SNP associations: Family-based association tests (FBAT) [50] and family-based tests for associating haplotypes (HBAT) [51] were used to assess the association between individual SNPs and haplotypes with asthma and asthma related traits in Latino asthmatics. Based on the *ALOX5AP*, *LTA4H*, and *IL-6* genotypes, haplotypes were determined using HBAT, which

constructs possible phases and conditional probabilities for each family to determine excess transmission of a particular haplotype from parents to asthmatic probands. Multiple linear regression models were used to assess the associations between individual SNPs with asthma and asthma-related traits in African American asthmatics. Asthma-related traits were categorized as quantitative and qualitative measures. Quantitative phenotypes included: asthma severity (defined by pre-FEV_1), bronchodilator responsiveness (defined by ΔFEV_1), and IgE levels (IgE values were $\log_{10}$-transformed to achieve normal distribution). Qualitative phenotypes included: pre-FEV_1 greater or less than 80% of predicted, ΔFEV_1 greater or less than 12%, and IgE levels greater or less than 100 IU/ml. In addition, analyses were also performed separately for patients with pre-FEV_1 greater or less than 80% (mild versus severe-moderate asthmatics). Both additive and dominant SNP models were used for FBAT analysis.

Gene-gene interactions: The effects of gene-gene interaction on asthma severity (baseline FEV_1) and drug response (ΔFEV_1) were determined by using multiple linear regression models. Specifically, SNPs of the *ALOX5AP* gene and *LTA4H* gene were paired and SNPs of the *IL-6* gene were paired with the *IL-6R* SNP to model the effect of gene-gene interaction on baseline FEV_1 and on ΔFEV_1. The following variables were tested for their inclusion in the regression model based on their potential for confounding: age, gender, ancestry, asthma duration, and use of short-acting β_2-agonists, long-acting β_2-

agonists, inhaled corticosteroids, oral corticosteroids, leukotrienes, theophylline, and cromolyn. An F-test determined which of these variables significantly affected the gene-gene-interaction model. All regression analyses were performed using the statistical software package STATA/SE 9.0 (StataCorp, College Station, TX). Analyses were not adjusted for multiple testing because tested phenotypes are strongly associated with each other in these populations and we were attempting to replicate described preliminary results of associations between SNPs in these genes and inflammatory phenotypes such as asthma.

Pharmacogenetic effect modification by ancestry: Based on the effects of bronchodilator drug response for IL-6 and IL-6R interaction in Latino and African American asthmatics, i.e., higher drug response in Latinos and lower drug response in African Americans, three-way gene-gene-ancestry interaction terms were generated to assess the effect of gene-gene-ancestry interaction on ΔFEV_1 in a multiple linear regression model. For this analysis, we focused on SNPs genotyped in all three populations, IL-6 -572C/G and IL-6R Asp358Ala. To further investigate possibleeffect modification by ancestry on IL-6 (-572 C/G)-IL-6R (Asp358Ala) gene-gene interaction, mean ΔFEV_1 for this gene-gene interaction was obtained for every quartile % individual European and Native American ancestry (0-24, 25-49, 50-74, 75-100).

RESULTS

Characteristics of the subjects

Demographic and clinical characteristics of the 700 probands with asthma (301 Mexicans and 399 Puerto Ricans) and 267 African Americans with asthma are shown in **Table 1**. The median age of the Mexican, Puerto Rican and African American asthmatics was 16.5, 14 and 19 years, respectively. Both the Mexican and Puerto Rican asthmatics were approximately 50% male whereas the African American asthmatics were 39% male. The median pre-FEV_1 was 88.9%, 83.7% and 91.5% in Mexican, Puerto Rican and African American asthmatics, respectively. This baseline lung function measurement demonstrates that African American asthmatics had less severe asthma in comparison to both Mexican and Puerto Rican probands. However, as previously described, the bronchodilator responsiveness measured by the ΔFEV_1 was significantly greater for Mexican asthmatics than for Puerto Rican or African American asthmatics ($p<0.0001$) [14].

Allele frequencies, Hardy-Weinberg equilibrium and linkage disequilibrium

The minor allele frequencies of *IL-6R* and *IL-6* SNPs for Mexicans, Puerto Ricans and African Americans are listed in **Table 2**. The observed distribution of genotypes within each ethnic group was in Hardy-Weinberg equilibrium and the frequencies did not differ from those reported in public databases (**table 2**

lists the frequencies available in public databases in different ethnic groups for comparison). Pair-wise linkage disequilibrium (LD) was computed between the *IL-6* SNPs using the r^2 statistic in Mexicans, Puerto Ricans and African Americans. No significant linkage disequilibrium was observed between the *IL-6* SNPs (r^2<0.07, *see* **Table 3** in the online supplemental data for details).

Association analysis of *IL-6R* variant with asthma and asthma-related phenotypes

Among Mexicans, single-locus family-based analyses using an additive model, demonstrated that the minor allele coding for Alanine of the *IL-6R* Asp358Ala SNP was associated with a decreased bronchodilator drug response, defined by ΔFEV_1 (p = 0.03, **Table 4**). Furthermore, in Mexican subjects with moderate-severe asthma, this allele was significantly associated with asthma (OR=0.67, 95% C.I.=0.40-1.02, p = 0.02), asthma severity (defined by pre-FEV_1, p = 0.01), bronchodilator drug response (ΔFEV_1, p = 0.003), and with asthma in asthmatics with higher drug response, defined by a ΔFEV_1 greater than 12% (OR=0.39, 95% C.I.=0.20-0.76, p =0.005, **Table 4**). In Puerto Ricans, *IL-6R* Asp358Ala was also associated with asthma in the asthmatics with higher drug response, defined by a ΔFEV_1 greater than 12% (OR=1.77, 95% C.I.=1.10-2.85, p = 0.03, **Table 4**). In African Americans, linear regression modeling demonstrated no significant associations of the *IL-6R* Asp358Ala SNP with asthma or asthma-related phenotypes (**Table 7**); however, the SNP frequency was much lower compared to Latinos (0.13 in

African Americans vs. 0.54 and 0.41 in Mexicans and Puerto Ricans, respectively, **Table 2**). There were no significant associations with IgE levels in Mexicans, Puerto Ricans, or African Americans (**Table 4 & Table 7**).

Association analysis of *IL-6* variants with asthma and asthma related phenotypes

Among Mexicans, the *IL-6* -572 C/G SNP was significantly associated with asthma (risk allele: C, OR=1.26, 95% C.I.=0.95-1.67, p = 0.03, **Table 5**). Moreover, *IL-6* -572 C/G and *IL-6* Asp162Glu SNPs were both associated with asthma in mild asthmatic subjects, as defined by a pre-FEV_1 greater than 80% (OR=1.27, 95% C.I.=0.90-1.81, p = 0.04 and OR=0.5, 95% C.I.=0.29-0.85, 0.005, respectively), and in lower drug response subjects, as defined by a □FEV_1 less than 12% (OR=1.40, 95% C.I.=1.00-1.96, p = 0.02 and OR=0.57, 95% C.I.=0.36-0.90, p=0.002, respectively, **Table 5**). In contrast, no significant association with asthma or asthma-related phenotypes was found for *IL-6* -572 C/G SNP in Puerto Ricans or African Americans (**Table 5 & Table 7**), although the frequency of the SNP was lower (0.14 in Puerto Ricans and 0.12 in African Americans vs. 0.34 in Mexicans; **Table 2**). Furthermore, both *IL-6* -572 C/G SNP in Mexicans and *IL-6* Pro32Ser in Puerto Ricans were associated with asthma in atopic asthmatics, defined by IgE levels greater than 100 IU/ml (OR=1.45, 95% C.I.=1.04-2.02, p=0.01 and OR=3, 95% C.I.=0.97-9.3, p =0.04, respectively, **Table 5**).

Association analysis of *IL-6* haplotypes with asthma and asthma related phenotypes

A total of four different haplotypes (frequency of > 1%) were observed in Mexican and Puerto Ricans asthmatics. The haplotype association results were consistent with those of the individual SNPs (**Table 8**). Among Mexicans, haplotype 4 was protective; it carries the protective alleles of both *IL-6* -572C/G and Asp162Ala SNPs. Furthermore, haplotype 4 was undertransmitted to the Mexican probands ($p = 0.05$ for all, $p = 0.003$ in mild asthmatics) and was also associated with traits related to mild asthma and bronchodilator responsiveness (**Table 8**). For Puerto Ricans, haplotype analyses revealed an association between the haplotype 4 (carrying the risk alleles T of *IL-6* Pro32Ser) and IgE levels greater than 100 IU/ml ($p=0.04$, **Table 8**). In African Americans, linear regression analyses revealed no significant associations of *IL-6* haplotypes with asthma or asthma-related phenotypes.

***IL-6* and *IL-6R* gene-gene interaction analyses**

Given the biologic properties of the polymorphisms studied, we tested for the effects of gene–gene interaction between *IL-6R* and *IL-6* genes on asthma severity (pre-FEV_1) and drug response (ΔFEV_1) in Mexican, Puerto Rican, and African American asthmatics. All of the SNPs genotyped, which were categorized as a dominant model, were used to analyze the *IL-6R/IL-6* gene–gene interaction (i.e., *IL-6R* Asp358Ala modeled with *IL-6* -174C/G, -572C/G

and Asp162Glu among Mexicans; *IL-6R* Asp358Ala modeled with *IL-6* -174C/G, -572C/G and Pro32Ser among Puerto Ricans; and *IL-6R* Asp358Ala modeled with *IL-6* -572C/G and Pro32Ser among African Americans). The gene-gene interaction was found to significantly modify the bronchodilator drug response but not asthma severity. Results from the gene-gene interaction analysis on □FEV_1 are shown in **Table 9**.

In Mexicans asthmatics, *IL-6R* Asp358Ala significantly interacted with the three *IL-6* SNPs (-174C/G, -572C/G and Asp162Glu) to increase bronchodilator drug responsiveness (p= 0.005, 0.009 and 0.005, respectively). In Puerto Ricans, the interaction of *IL-6R* Asp358Ala and two of the three SNPs of *IL-6* (-572C/G and Pro32Ser) had marginal association with increased bronchodilator drug response (p = 0.08 and 0.06, respectively). On the other hand, in African Americans, the interaction of *IL-6R* Asp358Ala with *IL-6* Pro32Ser significantly decreased bronchodilator drug response (p = 0.04).

The *IL-6* and *IL-6R* interaction analysis demonstrates differential drug response between Latinos and African American asthmatics; Latinos display an increased bronchodilator drug response while African Americans display a decreased bronchodilator drug response. We have previously shown that pharmacogenetic associations for asthma differ among racial/ethnic groups [1, 52]. These results suggest that there may be racially specific factors (genetic or environmental) which may attenuate or accentuate the effects of this gene-

gene interaction in each racial group. One possible explanation behind this differential drug response is that the IL-6 and IL-6R interaction has effect modification by ancestry. To explore this possibility we conducted three way gene-gene-ancestry interaction analysis in a multiple regression model; we focused on SNPs genotyped in all three populations, IL-6 (-572 C/G) and IL-6R (Asp358Ala). In this analysis, we found marginally significant effect modification with European ancestry and significant effect modification with Native American ancestry within Mexican asthmatics. Specifically, in Mexican asthmatics, IL-6 (-572C/G)-IL-6R (Asp358Ala) interaction with effect modification by European ancestry resulted in marginally significant decreased bronchodilator drug response ($p=0.09$), while IL-6 (-572C/G)-IL-6R (Asp358Ala) interaction with effect modification by Native American ancestry resulted in significantly increased bronchodilator drug response ($p=0.03$). Although IL-6 (-572C/G) and IL-6R (Asp358Ala) interaction with effect modification by ancestry did not reach statistical significance in Puerto Rican or African American asthmatics, a similar trend was observed; IL-6 (-572C/G)-IL-6R (Asp358Ala) interaction with effect modification by European ancestry resulted in a lower bronchodilator drug response in both Puerto Rican and African American asthmatics, while this same gene-gene interaction with effect modification by Native American ancestry resulted in a higher bronchodilator drug response in Puerto Rican asthmatics. Based on this similar trend of effect modification by ancestry with both Mexican and Puerto Rican asthmatics, we combined these two populations together to illustrate the mean

bronchodilator drug response (ΔFEV) for IL-6 (-572 C/G)-IL-6R (Asp358Ala) gene-gene interaction with effect modification by ancestry (**Figure 1**). Mean ΔFEV for IL-6 (-572 C/G)-IL-6R (Asp358Ala) interaction decreased with increasing amounts of European ancestry; on the other hand, mean ΔFEV for IL-6 (-572 C/G)-IL-6R (Asp358Ala) interaction increased with increasing amounts of Native American ancestry

DISCUSSION

In this study, we identified genetic variants of the *IL-6 receptor* and *IL-6* genes that jointly modify bronchodilator drug responsiveness in Latino and African American asthmatics. Although genetic associations between individual *IL-6* and *IL-6R* SNPs and asthma differed between Mexicans, Puerto Ricans and African Americans on the gene level, we have found a consistent gene-gene interaction. Among admixed populations, complex genetic associations may be more informative on the gene level rather than on the level of individual SNPs (Jorgenson et. al. 2006). Moreover, cross-ethnic validation provides strong evidence for true genetic effects on disease (Mountain et al. 2004). These findings are further supported by the fact that both IL-6 and IL-6R have been implicated in the pathogenesis of asthma. As a whole, these results suggest that there is a genetic interaction between *IL-6* and *IL-6R* genes and asthma. To our knowledge, this is the first study of asthma to analyze loci identified by admixture mapping. Furthermore, this is the first study to test a

pharmacogenetic gene-gene interaction between *IL-6* and *IL-6R* genes in Latino and African American asthmatics.

Results of an admixture mapping analysis for inflammatory markers led us to explore the IL-6 pathway as a potential modifier of asthma and bronchodilator drug response [31]. In the analysis of inflammatory markers, Reich *et al.* demonstrated that a missense mutation, SNP *IL-6R* Asp358Ala, fully accounts for an admixture peak. Furthermore, this genetic variant influences circulating markers of inflammation [31]. The *IL-6R* Asp358Ala SNP corresponds to the proteolytic cleavage site of membrane-bound IL-6R (mIL-6R) that has been shown to affect sIL-6 and IL-6 serum levels [31, 47, 53]. In asthma, sIL-6R serum and airway levels are increased and the blockade of sIL-6R in mice suppresses airway inflammation [54, 55]. Based on these results, we considered *IL-6R* Asp358Ala as a possible risk factor for asthma. Indeed, our results demonstrate an association of *IL-6R* Asp358 allele with drug response in Mexican and Puerto Rican asthmatics but not in African American asthmatics. Yet, given the lower prevalence of the SNP in African Americans compared to Latinos, such results were not expected to achieve statistical significance in the African American cohort. In fact, the *IL-6R* Ala358 allele is over-represented in Native Americans in comparison to other ethnic groups (SNP500Cancer Database). Among our study participants, Native American ancestry, on average, is highest in Mexicans (52%), intermediate in Puerto Rican (18%) and lowest in African Americans (< 1%) [11]. Similarly, our results

are in parallel with the pattern of Native American ancestry in that Mexicans displayed the highest frequency of the IL-6R Ala358 (0.54) allele, while Puerto Ricans displayed an intermediate frequency (0.41), and African Americans displayed the lowest frequency (0.13).

To further explore the IL-6 pathway, we selected functional *IL-6* gene variants to investigate the interaction with the *IL-6R* gene. Although IL-6 plays a major role in the inflammation process, balancing the production of pro- and anti-inflammatory markers, the exact pathogenetic mechanism of IL-6 in asthma remains unclear. The proliferation of the mucosal Th_2 cells, thought to play a major role in asthma, depends on IL-6 *trans*-signaling via the sIL-6R. In contrast, suppression of regulatory T cells and differentiation of $CD4^+$ cells into Th_2 within the lung depends on gp130 signaling mediated by the mIL-6R [54]. Moreover, in comparison to wild type mice, *IL-6*-deficient mice manifested exaggerated inflammation whereas mice over-expressing IL-6 manifested diminished inflammation in the bronchial airways [55]. These studies suggest that abnormalities in the production and/or effector functions of IL-6 can contribute to the generation, severity, and chronicity of asthma. Based on these findings, we studied *IL-6* functional variants that affect IL-6 expression, production or binding to gp130, as a potential risk factor for asthma. Our results revealed that individual genetic variants in the *IL-6* gene, as well as *IL-6* haplotypes, affect asthma susceptibility and drug response in Mexicans but not in Puerto Ricans and African Americans. Similar to the results observed for

the *IL-6R* variant, these discrepancies may be explained by lower frequencies of *IL-6* genetic variants in Puerto Ricans and African Americans with asthma.

Finally, we identified a pharmacogenetic gene-gene interaction between variants of the *IL-6* and *IL-6R* genes, which results in differential drug responsiveness to albuterol. The interaction between these two variants is most likely attributable to IL-6 cytokine binding to the IL-6 receptor that in turn results in changes in the inflammatory process. This observed pharmacogenetic gene-gene interaction, however, is contextual and dependent upon the background individual ancestry. In view of this effect, previous studies have demonstrated that differences between two populations in environmental conditions or in genetic background can result in different impacts of the genetic variant [59].The interaction of genes and ancestry has been observed in both murine models and humans. In murine models, strain background has been identified to modify the effect of loci associated with airway hyperresponsiveness[60]. Furthermore, in humans, ancestral background has been recognized to modify the genetic effects of risk on inflammatory diseases [61]. Our results revealed that the IL-6 and IL-6R gene-gene interactions resulted in higher drug response in Latinos, but lower drug response in African Americans. To explain this discrepancy in drug response, our analysis suggests that ancestral background may modify the effects of the gene-gene interaction on ancestry; In Latino asthmatics, increased European ancestry modified the effects of IL-6-IL-6R gene-gene interaction resulting in

decreased drug response while increased Native American ancestry modified the effects of IL-6-IL-6R gene-gene interaction resulting in increased drug response. Although statistical significance for the effect modification by ancestry was achieved only in Mexican asthmatics, parallel trends were observed for effect modification by ancestry in also Puerto Rican asthmatics. Similar to previous results, this lack of significance in Puerto Rican asthmatics may be explained by lower frequencies of the gene-gene interaction in each ancestral background. Taken together, our results coupled with previous results suggest higher level gene-gene interactions. Yet, in addition to ancestral factors, there may also be different environmental factors between Latinos and African Americans that modify the effects of the gene-gene interaction. In either way, further functional studies will be needed to evaluate the biological interaction between *IL-6* and *IL-6R* genetic variants and to elucidate the casual mechanism behind differential bronchodilator drug responsiveness.

In summary, this study provides evidence of significant gene-gene pharmacogenetic interaction between *IL-6* and *IL-6R* that modulates bronchodilator drug response in ethnically diverse asthmatic populations. Although no other studies report any *IL-6R/IL-6* gene interaction in asthma, a significant *IL-13/IL-4R* gene interaction has been shown in African Americans, Caucasians and Chinese with asthma [56-58]. Interestingly, these genes interacted to modify lung function; however, the genetic variants that

interacted varied among ethnic groups. Nevertheless, given the extent of these results, Latino or African American asthmatics with the two-locus genotype may benefit from tailored asthma therapy that would support a favorable drug response. This potentially targeted therapeutic intervention will require prospective clinical studies that evaluate the bronchodilator drug response to albuterol in Latino and African American asthamatics who contain the combined *IL-6/IL-6R* genetic variants.

Table 1: Demographic and clinical characteristics of the Mexican and the Puerto Rican subjects with asthma from the GALA study and the African American subjects with asthma from the SAGE study

	Mexicans (n = 301)	**Puerto Ricans** (n = 399)	**African Americans** (n = 267)
Characteristics:			
Age (years)	16.5 (11:22)	14.0 (9:19)	19.3 (13:26)
Gender (% male)	53.8%	55.9%	39.3%
BMI (kg/m²)	24.5 (20:29)	22.3 (18:27)	26.9 (22:32)
Plasma IgE (IU/ml)	470.4 (50:891)	487.9 (58:918)	292.4 (0:590)
Baseline Spirometry:			
Pre-FEV_1 (% pred)	88.9 (76:101)	83.7 (72:95)	91.5 (79:103)
Pre-FEV_1 (% pred) < 80%	30.7%	39.9%	30.7%
Bronchodilator Responsiveness:			
ΔFEV_1 (relative % change)	10.1 (2:19)	6.2 (0:15)	9.8 (3:17)

Definition of abbreviations: BMI = body mass index, Pre-FEV_1 = baseline FEV_1 expressed as percentage of predicted, ΔFEV_1 = relative percent change in Pre-FEV_1 after albuterol administration.

Values are expressed as median (25^{th}: 75^{th} percentile) and were missing for some subjects. Analyses were done by Mann Whitney rank test.

Table 2. Minor allele frequencies and p-value for Hardy-Weinberg equilibrium (HWE) analysis for *IL-6R* and *IL-6* polymorphisms in Mexican and Puerto Rican GALA probands and parents and African American SAGE cases and controls.

	Mexicans		Puerto Ricans		African Americans		HapMap**		SNP500 Cancer Database**
	Probands	Parents	Probands	Parents	Cases	Controls	Caucasians	Africans	Hispanics
***IL-6R* Asp358Ala**									
Ala	0.54	0.55	0.41	0.41	0.13	0.12	0.35	0.04	0.48
HWE (p-value)	0.41	0.31	0.95	0.06	0.49	0.07			
***IL-6* -174C/G**									
C	0.13	0.14	0.23	0.24	NA*	NA*	0.53	0	0.22
HWE (p-value)	0.28	0.46	0.34	0.84					
***IL-6* -572C/G**									
C	0.34	0.31	0.13	0.14	0.10	0.12	0.04	0.09	0.28
HWE (p-value)	0.34	0.70	0.33	0.52	0.74	0.82			
***IL-6* Asp162Glu**									
A	0.11	0.13	NA*	NA*	NA*	NA*	0.0	0.03	0.02
HWE (p-value)	0.68	0.06							
***IL-6* Pro32Ser**									
T	NA*	NA*	0.03	0.02	0.08	0.10	0.0	0.09	
HWE (p-value)			0.62	0.52	0.25	0.22			

*NA: Not Applicable, due to a low minor allele frequency (<5%) in the populations.
**Allele frequencies from public database are listed for comparison.

Table 3. Pairwise linkage disequilibrium (r^2) between the *IL-6* SNPs in Mexicans, Puerto Ricans and African Americans.

Mexicans			
SNP	**-174C/G**	**-572C/G**	**Asp162Glu**
-174C/G	-		
-572C/G	0.067	-	
Asp162Glu	0.022	0.063	-

Puerto Ricans			
SNP	**-174C/G**	**-572C/G**	**Pro32Ser**
-174C/G	-		
-572C/G	0.045	-	
Pro32Ser	0.008	0.004	-

African Americans		
SNP	**-572C/G**	**Pro32Ser**
-572C/G	-	
Pro32Ser	0.011	-

Table 4. Family-based association analysis of *IL-6R* Asp358Ala and asthma, asthma severity, bronchodilator response and IgE levels among Mexican and Puerto-Rican trios.

IL-6R Asp358Ala	Mexican trios		Puerto-Rican trios	
Ala allele	All (n=301)	Moderate-severe (n=82)	All (n=399)	Moderate-severe (n=134)
Asthma	(-) 0.35	**(-) 0.02***	(+) 0.24	(+) 0.28
Quantitative traits:				
Pre-FEV_1 (% pred)	(+) 0.12	**(+) 0.01***	(-) 0.63	(-) 0.13
ΔFEV_1 (relative % change)	**(-) 0.03***	**(-) 0.003***	(+) 0.51	(+) 0.50
$Log_{10}IgE$	(+) 0.67	(+) 0.9	(+) 0.89	(+) 0.45
Qualitative traits:				
Pre-FEV_1 (% pred) < 80%	(-) 0.07	**(-) 0.02***	(+) 0.24	(+) 0.28
Pre-FEV_1 (% pred) > 80%	(+) 0.82	NA	(+) 0.24	NA
ΔFEV_1 (relative % change) >12	(-) 0.17	**(-) 0.005***	**(+) 0.03***	(-) 0.19
ΔFEV_1 (relative % change) <12	(-) 1.00	(+) 0.71	(+) 0.52	(+) 0.78
IgE level > 100 IU/ml	(-) 0.45	(-) 0.07	(+) 0.56	(+) 0.45
IgE level < 100 IU/ml	(-) 0.58	(+) 0.59	(+) 0.25	(+) 0.80

n refers to the number of trios analyzed. Analyses were performed using an additive model in all the trios and in the moderate severe asthma trios defined by a Pre-FEV_1 <80% for the proband.
(+) and (-) indicate the direction of the association. For quantitative traits, (+) means that the Ala allele is associated with a higher value of the trait and (-) indicates a lower value of the trait. For qualitative traits, (+) means that the Ala allele is overtransmitted to probands with asthma and (-) indicates undertransmitted to the probands with asthma.
*p-values < 0.05
For definition of abbreviations, see table 1.

Table 5. Family-based association analysis of *IL-6* variants and asthma, asthma severity, bronchodilator response and IgE levels among Mexican and Puerto Rican trios.

IL-6 Genotypes	Mexican trios (n=301)				Puerto Rican trios (n=399)		
	-174 C/G CC+GC	-572 C/G CC+CG	Asp162Glu AA+AT		-174 C/G CC+CG	-572 C/G CC+CG	Pro32Ser TT+CT
Asthma	(-) 0.92	**(+) 0.03***	(-) 0.09		(-) 0.67	(-) 0.49	(+) 0.51
Quantitative traits:							
Pre-FEV_1 (% pred)	(+) 0.19	(+) 0.59	(-) 0.06		(-) 0.73	(+) 0.15	(+) 0.16
ΔFEV_1 (relative % change)	(-) 0.43	(-) 0.22	(+) 0.58		(-) 0.65	(-) 0.24	(-) 0.72
$Log_{10}IgE$	(+) 0.68	(-) 0.86	(+) 0.65		(-) 0.63	(+) 0.94	(+) 0.82
Qualitative traits:							
Pre-FEV_1 (% pred) < 80%	(-) 0.93	(+) 0.58	(+) 0.46		(-) 0.94	(-) 0.18	NA
Pre-FEV_1 (% pred) > 80%	(+) 0.94	**(+) 0.04***	**(-) 0.005***		(-) 0.74	(-) 0.66	(+) 0.81
ΔFEV_1 (relative % change) >12	(-) 0.18	(+) 0.93	(+) 0.12		(+) 0.56	(-) 0.28	NA
ΔFEV_1 (relative % change) <12	(+) 0.25	**(+) 0.02***	**(-) 0.002***		(-) 0.33	(-) 0.96	(+) 0.16
IgE level > 100 IU/ml	(-) 0.26	**(+) 0.01***	(-) 0.18		(-) 0.69	(-) 0.48	**(+) 0.04***
IgE level < 100 IU/ml	(+) 0.40	(+) 0.28	(-) 0.55		(-) 0.33	(-) 0.37	NA

n refers to the number of trios analyzed. Analyses were performed using a dominant model.
(+) and (-) indicate the direction of the association. For quantitative traits, (+) represents genotypes associated with higher value of the trait and (-) represents genotypes associated with lower value of the trait. For qualitative traits, (+) represents genotypes overtransmitted to probands with asthma and (-) represents genotypes undertransmitted to the probands with asthma.
*p-value<0.05
For definition of abbreviations, see table 1.

Table 6. Family-based association analysis of *IL-6* -174C/G, -572C/G and Asp162Glu (T/A) polymorphisms and asthma, asthma severity, bronchodilator response and IgE levels among Mild asthmatic Mexican trios.

IL-6 Genotypes	Mild asthmatic Mexican trios (n=201)		
	-174 C/G CC+CG	-572 C/G CC+CG	Asp162Glu AA+AT
Asthma	(+) 0.95	**(+) 0.04***	**(-) 0.005***
Asthma severity:			
Pre-FEV_1 (% predicted)	**(+) 0.05***	**(+) 0.40**	(-) 0.20
Pre-FEV_1 (% predicted) < 80%			
Pre-FEV_1 (% predicted) > 80%	**(+) 0.95**	**(+) 0.04***	(-) 0.005*
Bronchodilator Responsiveness:			
ΔFEV_1 (relative % change)	(-) 0.19	(-) 0.30	**(+) 0.31**
ΔFEV_1 (relative % change) >12	(-) 0.09	(-) 0.86	
ΔFEV_1 (relative % change) <12	**(+) 0.19**	**(+) 0.03***	(-) 0.001*
Plasma IgE (Log of IU/ml):			
Log_{10}IgE	(-) 0.61	**(+) 0.31**	**(+) 0.76**
IgE level > 100 IU/ml	(-) 0.48	**(+) 0.006***	(-) 0.01*
IgE level < 100 IU/ml	**(+) 0.27**	(-) 0.74	(-) 0.28

n refers to the number of trios analyzed. Analyses were performed using a dominant model in the mild asthmatic Mexican trios as defined by a Pre-FEV_1 > 80% for the proband.
(+) and (-) indicate the direction of the association. For quantitative traits, (+) represents genotypes associated with higher value of the trait and (-) represents genotypes associated with lower value of the trait. For qualitative traits, (+) represents genotypes overtransmitted to probands with asthma and (-) represents genotypes undertransmitted to the probands with asthma.
*p-value<0.05
For definition of abbreviations, *see* table 1.

Table 7. Linear regression analysis of *IL-6R* and *IL-6* variants with asthma, asthma severity, bronchodilator response and IgE levels among African American cases and controls.

African American (n=267 cases and n=176 controls)	*IL-6R Genotypes*	*IL-6 Genotypes*	
	Asp358Ala CC+AC	-572 C/G CC+CG	Pro32Ser TT+CT
Asthma	(+) 0.75	(+) 0.60	(+) 0.56
Quantitative traits:			
Pre-FEV_1 (% pred)	(-) 0.90	(+) 0.66	(-) 0.79
ΔFEV_1 (relative % change)	(+) 0.68	(-) 0.10	(-) 0.23
$Log_{10}IgE$	(+) 0.13	(+) 0.52	(+) 0.68
Qualitative traits:			
Pre-FEV_1 (% pred) < 80%	NA	NA	NA
Pre-FEV_1 (% pred) > 80%	(-) 0.63	(-) 0.29	(+) 0.87
ΔFEV_1 (relative % change) >12	(-) 0.46	(-) 0.85	(-) 0.32
ΔFEV_1 (relative % change) <12	NA	NA	NA
IgE level > 100 IU/ml	(-) 0.87	(-) 0.47	(+) 0.65
IgE level < 100 IU/ml	NA	NA	NA

n refers to the number of cases or controls analyzed. Analyses were performed using a dominant model.
(+) and (-) indicate the direction of the association: (+) represents genotypes associated with higher value of the trait and (-) represents genotypes associated with lower value of the trait.
For definition of abbreviations, *see* table 1.

Table 8. Family-based association analysis of *IL-6* -174C/G, -572C/G and Asp162Glu (T/A) haplotypes and asthma, asthma severity, bronchodilator response and IgE levels among Mexican trios.

IL-6 haplotypes	All Mexican trios (n=301)					Mild asthmatic Mexican trios (n=201)			
	GGT 0.40%	GCT 0.30%	CGT 0.13%	GGA 0.13%		GGT 0.43%	GCT 0.30%	CGT 0.13%	GGA 0.13%
Asthma		**(+) 0.09**		(-) 0.05*					(-) 0.003*
Asthma severity:									
Pre-FEV_1 (% predicted)				(-) 0.09				**(+) 0.09**	
Pre-FEV_1 (% predicted) < 80%									
Pre-FEV_1 (% predicted) > 80%				(-) 0.002*					(-) 0.003*
Bronchodilator Responsiveness:									
ΔFEV_1 (relative % change)									
ΔFEV_1 (relative % change) >12									
ΔFEV_1 (relative % change) <12		**(+) 0.05***		(-) 0.002*					(-) 0.0006*
Plasma IgE (Log of IU/ml):									
$Log_{10}IgE$						(-) 0.07	**(+) 0.09**		
IgE level > 100 IU/ml		**(+) 0.02***					(-) 0.009*		(-) 0.009*
IgE level < 100 IU/ml						(-) 0.09			

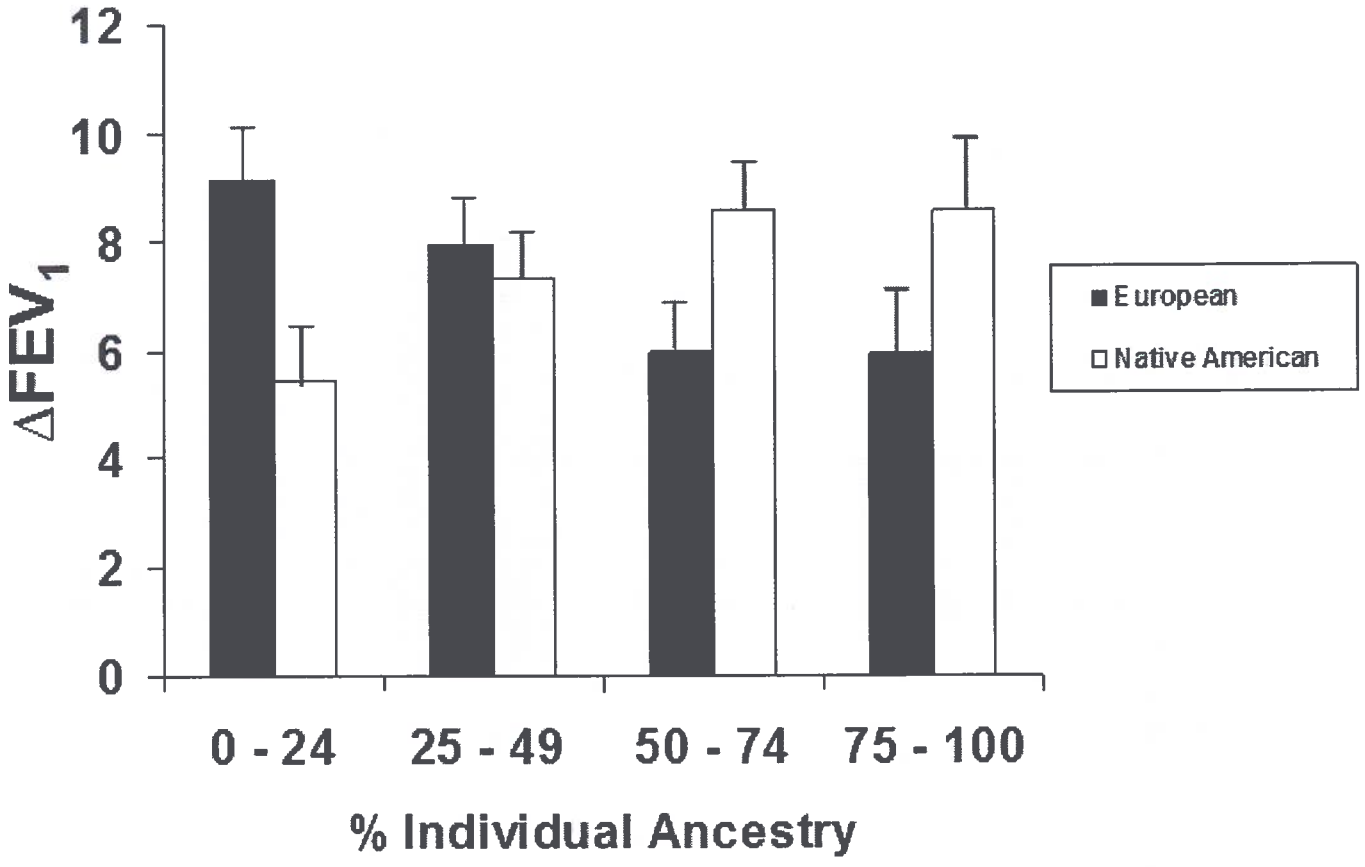
n refers to the number of trios analyzed. P-values > 0.1 are not shown in the table. Analyses were performed in all Mexicans and the mild Mexican asthmatic trios as defined by a Pre-FEV_1 > 80% for the proband.
(+) and (-) indicate the direction of the association. For quantitative traits, (+) represents haplotypes associated with higher value of the trait and (-) represents haplotypes associated with lower value of the trait. For qualitative traits, (+) represents haplotypes overtransmitted to probands with asthma and (-) represents haplotypes undertransmitted to the probands with asthma.
*p-value<0.05
For definition of abbreviations, *see* table 3.

Table 9. P-values for gene-gene interaction on drug responsiveness (ΔFEV_1) among Mexican, Puerto Rican and African American subjects with asthma

IL-6	*IL-6R*	Gene-Gene Interaction		
		Mexicans	Puerto Ricans	African Americans
-174C/G	Asp358Ala	(+) 0.005*	(+) 0.22	NA
-572C/G	Asp358Ala	(+) 0.009*	(+) 0.08	(-) 0.28
Asp162Glu	Asp358Ala	(+) 0.005*	NA	NA
Pro32Ser	Asp358Ala	NA	(+) 0.06	(-) 0.04*

Multiple linear regression analysis was performed with genetic variants categorized in a dominant model. For ΔFEV_1, positive (+) direction of association indicates that the gene-gene interaction is associated with an increased drug response and negative (-) direction of association indicates that the gene-gene interaction is associated with a decreased drug response.
*p-values < 0.05
For definition of abbreviations, *see* table 1.

Figure 1. Mean ΔFEV_1 for IL-6 (-572 C/G) – IL-6R (Asp358Ala) gene-gene interaction among both Mexican and Puerto Rican asthmatics with effect modification by ancestry



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