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Pharmacogenetic Differences in Response to Albuterol between Puerto Rican and Mexican Asthmatics

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Abstract

Background: In the U.S., Puerto Ricans and Mexicans have the highest and lowest asthma prevalence, morbidity and mortality, respectively. Ethnic-specific differences in the response to drug treatment may contribute to differences in disease outcomes. Genetic variants at the beta$_2$ adrenergic receptor (β_2AR) may modify asthma severity and albuterol responsiveness. We tested the association of β_2AR genotypes with asthma severity and bronchodilator response to albuterol in Puerto Rican and Mexican asthmatics.

Methods: We used both family-based and cross-sectional tests of association with eight β_2AR SNPs in 684 Puerto Rican and Mexican families. Regression analyses were used to determine the interaction between genotype, asthma severity and bronchodilator drug responsiveness.

Results: Among asthmatic Puerto Ricans, the Arg16 allele was associated with greater bronchodilator response using both family-based and cross-sectional tests ($p = 0.01$-0.00001). We found a strong interaction of baseline Forced Expiratory Volume in one second or FEV_1 with the Arg16Gly polymorphism in predicting bronchodilator response. Among Puerto Rican asthmatics with baseline $FEV_1 < 80\%$ of predicted, but not in those with $FEV_1 > 80\%$, there was a very strong association between the Arg16 genotype and greater bronchodilator responsiveness. No association was observed between Arg16Gly genotypes and drug responsiveness among Mexican asthmatics.

Conclusions: Ethnic-specific pharmacogenetic differences exist between Arg16Gly genotypes, asthma severity and bronchodilator response in asthmatic Puerto Ricans and Mexicans. These findings underscore the need for additional research on racial/ethnic differences in asthma morbidity and drug responsiveness.

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Introduction (word count = 451)

Studies of racial/ethnic health disparities often define populations based on U.S. census definitions, which categorize Latinos as a single ethnic group. Latinos are the largest minority population in the U.S., and Latino children represent the largest demographic group of all U.S. children.[1] Although, the terms "Hispanic" or "Latino" American describe a common language and cultural heritage, these terms do not refer to race, uniform ethnicity or a common genetic background. The two largest Latino ethnic groups in the U.S., Mexicans (63%) and Puerto Ricans (11%),[1] are genetically complex and comprised of various proportions of Native American, African and European genetic origins.[2] The relative proportions of these ancestral populations to the contemporary Latino gene pool make each Latino ethnic group genetically unique.[2]

According to U.S. vital statistics, asthma prevalence, morbidity and mortality are the highest among Puerto Ricans and the lowest among Mexicans for a total of four-fold difference in asthma burden between these two ethnic groups.[3, 4] Although there are many potential explanations for this observation, including environmental and socioeconomic factors, one potential explanation is that the genetic predisposition to asthma or to greater asthma severity differs among subgroups within the Latino population. These subgroups may have genetic variants, which may explain the variation in asthma severity and response to drug therapy between Latino ethnic groups.

Albuterol, a short-acting beta$_2$ adrenergic receptor (β_2AR) agonist, is the most commonly prescribed treatment for asthma worldwide.[5] β_2AR mediates physiologic responses including bronchial smooth muscle relaxation (bronchodilation), vasodilatation and lipolysis.[6] Genetic variations at the β_2AR gene may play a role in the observed ethnic-specific differences in asthma severity and bronchodilator drug responsiveness.

Polymorphisms in the β_2AR gene have been inconsistently associated with varying degrees of bronchodilatory response to β-agonists[7-12] and with tachyphylaxis.[13, 14] In one study, specific haplotypes, as opposed to individual single nucleotide polymorphisms (SNPs), were significantly associated with drug responsiveness.[12] Consequently, there is no clear consensus regarding the extent to which genetic variants in the β_2AR gene may influence asthma severity or bronchodilator drug responsiveness.

We used a family-based genetic association study to determine whether polymorphisms (SNPs and haplotypes) in the β_2AR gene are associated with ethnic-specific differences in asthma severity and bronchodilator response in Puerto Rican and Mexican subjects with asthma participating in the Genetics of Asthma in Latino Americans (GALA) Study. We selected a family-based approach because it removes the effect of population stratification, a potentially important source of confounding.[15] Spurious associations between genetic variants and phenotypes may arise as a result of population stratification when non-family based approaches are used.[16] Moreover, racial and ethnic background can influence genetic variation and genotypic relative risk.[17] Some of the results of this study have been previously reported in the form of an abstract.[18]

Subjects and Methods (word count = 545

Study Participants

Recruitment and pulmonary function testing are described elsewhere and in detail in the online supplement.[19] Briefly, 684 asthmatic subjects (probands) and their biological parents were enrolled in the San Francisco Bay Area (SF), New York City (NY), Puerto Rico (PR), and Mexico City (MX). Clinical characteristics of all probands are shown in Table 1. Ethnicity was self-reported. Probands were enrolled only if both biological parents and all four biological grandparents were of Puerto Rican (for the NY and PR sites) or Mexican ethnicity (for the SF and MX sites).

Pulmonary Function Tests and IgE measurements

Spirometry was performed according to ATS standards.[20] Pulmonary function test results are shown in Table 1 and are expressed as a percentage of the predicted normal value using age-adjusted prediction equations from Hankinson.[21]

Asthmatic probands were classified as having "mild" or "moderate-severe" asthma based on their baseline Forced Expiratory Volume in one second (FEV_1) expressed as percent of predicted or Pre-FEV_1 levels. Patients with 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 responsiveness was calculated as Delta FEV_1, which is relative percent change in Pre-FEV_1 after albuterol administration. Total plasma IgE was measured in duplicate using Uni-Cap technology (Pharmacia, Kalamazoo, MI).

Genotyping

Genotyping details are described further in the online supplement. Eight β_2AR SNPs (-709, -654, -47, +46, +79, +252, +491, and +523) were genotyped and used to assign haplotypes.

Statistical Analysis

Statistical analyses are further described in the online supplement. Mendelian inconsistencies were identified using PEDCHECK.[22] Hardy-Weinberg Equilibrium was calculated by means of χ^2 goodness-of-fit tests. The degree of linkage disequilibrium was estimated by using the r^2 statistic.[23]

FBAT[24] and HaploFBAT[25] were used to assess the association between individual SNPs and haplotypes, respectively, with quantitative measures of asthma-related traits. Quantitative phenotypes included: asthma severity (defined by Pre-FEV_1), bronchodilator responsiveness (defined by Delta FEV_1, relative % of predicted) and IgE levels. Qualitative phenotypes included: Pre-FEV_1 greater or less than 80%, Delta FEV_1 greater or less than 12% and IgE levels greater or less than 100 IU/ml.

To determine the magnitude of the interaction between genotype/haplotype, Pre-FEV_1, ethnicity, and drug responsiveness we used linear regression models (among asthmatics only) to test for an association between genotypes or haplotypes and bronchodilator responsiveness. In each model the most common homozygous genotype served as the reference group with heterozygotes and the less common homozygote treated as two

separate predictors. Other independent variables included in the model were Pre-FEV_1, age, birthplace, gender, regular or as needed use of $beta_2$–receptor agonist, use of long acting $beta_2$–receptor agonist (LABAs), use of steroids, albuterol dose used for spirometry, environmental tobacco smoke exposure and for selected analysis an interaction term between genotype and Pre-FEV_1 (genotype*Pre-FEV1). This interaction term was considered because the response to albuterol and effect of genotype on the response may be more substantial in patients with severe airway obstruction (low initial FEV_1) as opposed to patients with less airway obstruction at the time of pulmonary function testing. To correct for the effect of population stratification cross-sectional analyses were also adjusted for individual admixture using forty-four unlinked ancestry informative genetic markers (see on line supplement). All cross-sectional analyses were performed using STATA 8.0 S/E statistical software (College Station, TX).

Results

Allele Frequencies, Hardy-Weinberg Equilibrium and Linkage Disequilibrium

Of the 684 GALA trios, we obtained genotyping information for 667 trios, which were used for these analyses. The allele frequencies of 8 β_2AR SNPs in the combined (Puerto Rican and Mexican together), Puerto Rican and Mexican probands are listed in Table 2. All SNPs were found to be in conformance with Hardy-Weinberg equilibrium with the exception of SNP -47 in combined (p-value = 0.03) and SNPs +252 and +523 in Mexican parents (p-value 0.05 and 0.03, respectively). However, with 8 SNPs tested in combined and separate populations, these deviations could result from multiple testing. We did not find the rare allele of SNP –709 in any of our samples and therefore this SNP was

excluded from further analyses. For purposes of consistency, the haplotypes discussed below continue to list -709 position although it was invariant in our populations.

Pairwise linkage disequilbrium (LD) differed between Puerto Ricans and Mexicans among the seven SNPs, Figure 1. SNP pairs –654/+46, -47/+79 and +252/+523 were in very tight LD ($r^2 > 0.7$) in both populations. LD was stronger in the Mexican population (mean $r^2 = 0.5$) for SNPs –654, +46, +252 and +523 than in Puerto Rican population (mean $r^2 = 0.3$). In contrast, LD was stronger in the Puerto Rican population (mean $r^2 = 0.22$) for SNPs -47, +46 and +79 than in the Mexican population (mean $r^2 = 0.11$).

Association Analysis of β_2AR with Asthma Severity and Bronchodilator Response

Single SNP Analysis of Asthma Qualitative and Quantitative Traits: Association results differed when analyzed in the combined population (Table 3) versus separately in the Puerto Rican and Mexican populations (Table 4). Therefore, all subsequent analyses were performed in each ethnic group separately. Family-based analyses were used to test for associations between individual SNPs and Pre-FEV_1, Delta FEV_1 and log IgE. Among Puerto Ricans, significant associations ($p = 0.04$-0.002) were found for SNPs –654, -47, +46 and +79 and Pre-FEV_1 and Delta FEV_1 (Table 4). Among Mexicans, Gln27 was modestly associated ($p = 0.04$) with Delta FEV_1 (Table 4). There were no significant associations between individual SNPs and IgE levels in either Puerto Ricans or Mexicans.

Since family-based analyses suggested an association between SNPs and bronchodilator response, we tested the clinical magnitude of the effect of the Arg16Gly polymorphism

on bronchodilator response by cross-sectional analysis in Puerto Ricans and Mexicans separately. Regression analysis showed a highly significant association between number of Arg16 alleles and bronchodilator response in all Puerto Rican asthmatics ($p = 0.002$) and in those with Pre-FEV_1 less than 80% of predicted ($p = 0.0001$), Figure 2. This association was independent of age and albuterol dosage used for performing spirometry. Among all Puerto Rican subjects, the mean values of Delta FEV_1 stratified by codon 16 were as follows: individuals with Arg16/Arg16 (10.46% ± 2.44%), Arg16/Gly16 (6.13% ± 0.74%); and with Gly16/Gly16 (3.85% ± 0.99%). When the Puerto Rican probands were stratified based on Pre-FEV_1 less than or greater than 80%, Arg16 was significantly associated with higher levels of bronchodilator response among Puerto Rican asthmatics with Pre-FEV_1 less than 80%; Arg16/Arg16 (24.9% ± 6.86%) (n = 23), Arg16/Gly16 (8.8% ± 1.2%) (n = 70); and with Gly16/Gly16 (4.3% ± 2.2%) (n = 39). The 95% confidence interval for the difference of 20.6% ± 4.5% that we observed in bronchodilator drug responsiveness between Arg16/Arg16 and Gly16/Gly16 genotypes was 11.7% - 29.5%. Using an additive model, we found a mean increase of ~ 3% in Delta FEV_1 per each Arg16 allele ($p = 0.001$) in all Puerto Rican asthmatics and of ~ 8.2% in Puerto Ricans with Pre-FEV_1 less than 80% ($p = 0.00001$). In this model, the estimated variance in Delta FEV_1 explained by Pre-FEV_1 and Arg16Gly is 13.7% and 13.5%, respectively, among Puerto Rican asthmatics with Pre-FEV_1 less than 80%. However, when the interaction term (genotype for SNP+46*Pre-FEV_1) was added to the model, Pre-FEV_1 and Arg16Gly interacted to explain 44.4% of the variance in Delta FEV_1. This model suggested a substantially higher negative impact of Gly16 on Delta FEV_1 for patients with more severe asthma as defined by lower values of Pre-FEV_1. We did not see

any association between Arg16Gly genotypes and Delta FEV_1 among Puerto Rican asthmatics with Pre-FEV_1 greater than 80% (p = 0.87, Figure 2) and Mexican asthmatics with Pre-FEV_1 less than 80% (p = 0.72) or greater than 80% (p = 0.16). The results of these cross sectional analysis did not change when corrected for population stratification (see on line supplement for further details).

Haplotype-based Association Analysis

A total of seven, five and six different haplotypes (frequency of > 1%) were observed in the Puerto Rican, Mexican and both populations combined, respectively (Table 2). Haplotype associations differed between Puerto Ricans and Mexicans. However, haplotype results were consistent with single SNP results for Puerto Ricans and Mexicans, respectively, and analyses of the three most common haplotypes are presented in Table 5. Haplotype 1 (which carried the Arg16 allele) and haplotype 2 (which carried the Gly 16 allele) were over-transmitted in Puerto Rican patients with Delta FEV_1 > 12% and Delta FEV_1 < 12%, respectively. We did not find any association between the haplotypes and Delta FEV_1 among Mexicans (Table 5).

The three most common eight SNP haplotypes identified in the Puerto Rican and Mexican populations could be distinguished from each other using only 2 SNPs, (+46 and +79), which code for codon 16 and codon 27, respectively. Phase for the 2 SNP haplotypes were imputed for both Puerto Rican and Mexican probands using the Phamily Phase program.[26] The 2 SNP haplotypes were assembled as pairs and 6 common haplotype pairs with greater than 1% frequency were observed in Puerto Rican and

Mexican asthmatics. Among Puerto Rican probands, the mean Delta FEV_1 for the six common haplotype pairs is shown in Figure 3. Comparisons were made for haplotype pair Arg16Gln27 homozygote, which had the highest Delta FEV_1, and other haplotype pairs.

Among Puerto Rican asthmatics with Pre-FEV_1 < 80%, 2 SNP haplotype pairs were significantly associated with Delta FEV_1 (p= 0.009). Subjects homozygous for Arg16/Gln27 had highest Delta FEV_1 (24.4% ± 6.9%) and those with Gly16Gln27 homozygotes had lowest (3.9% ± 6.5%) (Figure3). No association was observed between 2 SNP haplotypes pairs among Puerto Rican subjects with Pre-FEV_1 greater than 80% (p = 0.55) and Mexican subjects with Pre-FEV_1 either less than 80% (p = 0.21) or greater than 80% (p = 0.39).

Discussion

Although most studies of racial/ethnic health disparities often categorize Latinos as a single ethnic group this classification masks important ethnic-specific differences in health outcomes and therapeutic effectiveness. Despite this classification, it is well accepted that in all regions of the United States, Puerto Ricans have higher asthma morbidity and mortality rates than other Latino ethnic groups. This observation has not so far been satisfactorily explained by environmental or socioeconomic factors. Our results demonstrate that Puerto Rican asthmatics have an ethnic-specific genetic predisposition to more severe asthma. Specifically, our study of β_2-adrenergic receptor polymorphisms shows that the Arg16 allele is significantly associated with asthma severity (Pre-FEV_1)

and bronchodilator responsiveness (Delta FEV_1) in Puerto Rican but not in Mexican asthmatics. The large size and clinical diversity of these two study populations have allowed us to address many outstanding questions relating to the clinical relevance of β_2-adrenergic receptor polymorphisms in terms of asthma severity and bronchodilator drug responsiveness. Most importantly, however, this comprehensive family-based study of β_2-adrenergic receptor polymorphisms demonstrates striking ethnic-specific pharmacogenetic differences between Puerto Rican and Mexican asthmatic subjects. Despite the fact that Puerto Ricans and Mexicans are classified as the same populations "Hispanic or Latino", our results demonstrate that there are different patterns of linkage disequilibrium, haplotypes and genetic associations between these two ethnic groups. To our knowledge, this is the first report of an ethnic-specific pharmacogenetic association for asthmatic subjects.

Martinez and colleagues previously demonstrated that when compared to Gly16 homozygotes, Arg16 homozygotes were 5.3 times and Arg16Gly heterozygotes were 2.3 times more likely to respond to albuterol, respectively.[8] Our results demonstrate that Arg16Gly is an important modifier of asthma severity and drug responsiveness among Puerto Ricans. These results corroborate and extend previous reports of associations between β_2-adrenergic receptor polymorphisms and asthma.[8, 9, 27, 28] Among Puerto Rican asthmatics with low baseline FEV_1, for whom the β_2–agonist response is probably most crucial, there was approximately a 20% difference in Delta FEV_1 between the Arg16 homozygotes and the Gly16 homozygotes, a highly significant and clinically important difference. The intermediate value of heterozygotes is consistent with an additive model,

which corroborates a dose-response effect of this allele in Puerto Ricans.

We did not see a convincing association between β_2AR genotype and drug responsiveness among Mexican asthmatics. The lack of a clear association may be due to several factors including differences in linkage disequilibrium (LD) patterns between Puerto Ricans and Mexicans. For example, the Arg16Gly allele may be in LD with another important risk allele in Puerto Ricans, but not in LD with this risk allele in Mexicans. There may also be ethnic-specific genetic and/or environmental modifiers that attenuate the effect of the Arg16Gly allele in Mexicans. By design, neither environmental nor cultural differences were a primary focus of the GALA study, and therefore, could be confounders.

Drysdale *et. al.* demonstrated that haplotypes, but not individual SNPs were associated with bronchodilator responsiveness.[12] Our results differ in that both individual SNPs and haplotypes modulate asthma severity and bronchodilator drug responsiveness. Specifically, Drysdale *et. al.* demonstrated that Drysdale haplotype 6 (corresponding to GALA haplotype 2) was associated with increased measures of bronchodilator drug responsiveness whereas Drysdale haplotype 4 (corresponding to GALA haplotype 1) was associated with lower measures of bronchodilator drug responsiveness. In contrast, our results demonstrate that GALA haplotype 1 (Drysdale haplotype 4) was associated with increased measures of bronchodilator responsiveness whereas GALA haplotype 2 (Drysdale haplotype 6) was associated with lower levels of bronchodilator responsiveness. There are several important differences between our study and the study

of Drysdale *et. al.*, however, which might help to explain differences in our respective results. Drysdale *et. al.* studied 121 unrelated Caucasian asthmatic subjects, who all had a Delta FEV_1 greater than 12% from baseline. Our study was a family-based study and included asthmatic probands from two populations, Puerto Ricans (n = 393) and Mexicans (n = 274). Unlike case-control and case-only genetic association studies, family-based studies are robust to the effects of population stratification.[16] It is unlikely that inadequate sample size precluded the replication of the previously reported association. We analyzed data from 667 family trios for a total of 2001 individuals, which exceeds the sample size (n = 121) that Drysdale *et. al.* used for their study. Although it is possible that the limited sample size precluded Drysdale *et. al.* from finding associations between individual SNPs and asthma related phenotypes, there might be other potential reasons why our results differed from those reported previously. One important difference is that we studied different populations and racial/ethnic-specific differences in environmental or genetic risk factors may account for the observed differences.[17] Drysdale *et. al.* performed their study exclusively in Caucasians from the U.S., while we studied Latinos recruited from Mexico City, Puerto Rico, and the continental U.S..

In contrast to results of Drysdale *et. al.*, our results corroborate recently published results from the Childhood Asthma Management Program (CAMP), a large family-based study primarily consisting of subjects with mild asthma.[10] Similar to GALA, the CAMP Study was a family-based study and included genotype data of eight β_2AR SNPs in 700 complete trios (mother, father and asthmatic child); 516 Caucasian, 70 African American, 56 Hispanic and 56 listed as "other". An important difference between the CAMP and

GALA Studies is that we directly compared two Latino ethnic groups consisting of subjects with mild and moderate-severe asthma. Results from GALA and CAMP differ in that CAMP demonstrated association of Pre-FEV_1 of less than 80% with SNPs –654/-47/+46/+79 and association of bronchodilator responsiveness only with the SNP +523. The most significant individual haplotype results were for GALA haplotype 2 (corresponding to CAMP haplotype 3) which had lower bronchodilator response measures in both CAMP and GALA Mexicans.

We have previously demonstrated that asthmatic Puerto Ricans have significantly more airway obstruction, more health care utilization and lower bronchodilator response to albuterol than asthmatic Mexicans.[19] Although our results do not entirely explain the significant disparities in asthma prevalence, morbidity and mortality among Latino ethnic groups, our study raises several provocative issues regarding ethnic-specific pharmacogenetic differences between these two populations. This large family-based study consists of two well-characterized, ethnically diverse populations of Puerto Rican and Mexican asthmatics from four geographically diverse locations. Studies performed in these populations may have important public health implications since Latino children represent the largest demographic group of all U.S. children[1] and since asthma morbidity and mortality are highest amongst Puerto Ricans and the lowest amongst Mexicans for a four-fold difference in asthma burden between these two ethnic groups.[3, 4] The fact that Puerto Ricans and Mexicans have the highest and lowest asthma prevalence, morbidity and mortality, respectively, underscores the clinical significance and public health implications of these findings.

Our study demonstrates that the two largest Latino ethnic groups in the U.S. have different patterns of linkage disequilibrium, haplotypes and pharmacogenetic associations, and therefore should be considered as separate groups in future drug trials and pharmacogenetic studies of asthma. Ethnic-specific pharmacogenetic differences among asthmatics have not been previously reported and thus merit further investigation.

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Figure Legends:

Figure 1. Linkage disequilibrium between β_2AR SNPs in Puerto Rican and Mexican asthmatic subjects. The degree of linkage disequilibrium was estimated by using the r^2 statistic. The results are color coded as shown by the color bar below.

Figure 2. Responsiveness to β_2AR agonist expressed as Delta FEV_1 (relative % pred) in different codon 16 genotypes among all Puerto Rican asthmatics (colored bars), Puerto Rican asthmatics with baseline Pre-FEV_1 < 80% of predicted (open bars) and those with baseline Pre-FEV_1 > 80% of predicted (hatched bars).

Figure 3. Responsiveness to β_2AR agonist expressed as Delta FEV_1 (relative % of predicted) in different (codon 16-codon 27) haplotype pairs among all Puerto Rican asthmatics (colored bars), Puerto Rican asthmatics with baseline Pre-FEV_1 < 80% (open bars) and those with baseline Pre-FEV_1 > 80% (hatched bars).

Table 1. Characteristics and pulmonary function results of GALA asthmatic probands included in the β_2AR analysis.

	Comparison by Ethnicity		
	Puerto Ricans (n = 393)	Mexicans (n = 274)	p-value
Characteristic:			
Age (years)	12.0 (10:15)	13.1 (11:19)	< 0.001
Gender (% male)	55.9%	54.1%	0.66
BMI (kg/m^2)	21.2 (17: 26)	23.8 (20: 28)	< 0.000
Serum IgE (IU/ml)	258.5 (92: 628)	270.0 (99: 615)	0.92
Moderate-Severe Asthma (%)	30.8%	40.5%	< 0.02
Baseline Spirometry:			
Pre-FEV_1	83 (74: 93)	90 (77: 100)	< 0.0001
Pre-FEV_1 < 80%	33.9%	28.8%	0.17
Bronchodilator Responsiveness:			
Delta FEV_1 (relative % pred)	5.0 (0.6: 10)	7.4 (4: 12)	< 0.0001
IgE Levels (IU/ml)	258.5 (92: 628)	270.0 (99: 615)	0.91

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

Table 2. Allele frequencies for β_2AR SNPs and haplotypes (> 1% frequency) in Combined (PR + Mex), Puerto Rican (PR) and Mexican (Mex) probands. The allele frequencies from the CAMP study and Drysdale *et. al.* are listed for comparison.

SNP Position	Alleles/ Haplotype	Amino Acid Change & Codon	Frequency (%)							
			Allele/ Haplotype	PR + Mex Probands	PR Probands	Mex Probands	CAMP* Parents	Drysdale Caucasians	Drysdale African-Americans	Drysdale Hispanic-Latinos
-709	C/A	-	C	100	100	100	98.0			
-654	A/G	-	A	37.9	37.0	39.1	36.0			
-47	C/T	Arg19/Cys19	C	23.2	27.4	17.6	37.0			
+46	A/G	Arg16/Gly16	A	43.9	45.2	42.1	40.0			
+79	G/C	Glu27/Gln27	G	22.9	26.5	17.8	37.0			
+252	A/G	-	A	32.0	26.2	40.6	23.0			
+491	C/T	Thr164/Ile164	C	98.5	98.0	99.2	99.0			
+523	C/A	-	C	70.0	75.5	62.3	80.0			
Haplotype 1	C A T A C G C C	-	1	36.8	34.9	39.7	35.4	33.0	29.7	40.0
Haplotype 2	C G T G C A C A	-	2	26.8	21.9	34.2	18.2	13.2	31.3	13.3
Haplotype 3	C G C G G G C C	-	3	22.7	26.8	16.5	36.3	48.3	6.3	26.7
Haplotype 4	C G T A C G C C	-	4	7.1	8.9	4.2		0.7	29.7	10.0
Haplotype 5	C G T G C A C C	-	5	1.7	1.8	1.6		0.7	0.0	3.3
Haplotype 6	C G T G C A T A	-	6	1.3	1.6	N.P.		1.0	1.6	3.3
Haplotype 7	C G C G C G C C	-	7	N.P.	1.3	N.P.		N.P.	N.P.	N.P.

*Include combined allele frequency of Caucasian, African-American, Hispanic and Others.
N.P. = Not Present

Table 3. Family-based association analysis of qualitative and quantitative asthma traits with β_2AR SNPs in the combined population.

SNPs	-654 (A/G)	-47 (C/T)	+46 (A/G)	+79 (G/C)	+252 (A/G)	+491 (C/T)	+523 (C/A)
Amino Acid and Codon		Arg19Cys	Arg16Gly	Glu27Gln			
				p-value			
Quantitative Traits							
Pre-FEV_1			0.024 (G)	0.045 (G)			
Delta FEV_1 (relative % pred)	0.024 (A)		0.011 (A)				
Log $_{10}$ IgE level							0.047 (C)
Qualitative Traits							
Pre-FEV_1 < 80% predicted							
Pre-FEV_1 > 80% predicted							
Delta FEV_1 (relative % pred) > 12%	0.018 (A)						
Delta FEV_1 (relative % pred) < 12%					0.038 (A)		
IgE level > 100 IU/ml							
IgE level < 100 IU/ml							

For quantitative traits, the allele in the parenthesis was associated with higher value of the trait. For qualitative traits, the allele in the parenthesis was over-transmitted to asthmatic probands. All other p-values are not shown because they were not significant.

Table 4. Family-based association analysis of qualitative and quantitative asthma traits with β_2AR SNPs in Puerto Ricans* and Mexicans†. Only significant associations (p-value < 0.05) are shown.

SNPs	-654 (A/G)	-47 (C/T)	+46 (A/G)	+79 (G/C)	+252 (A/G)	+491 (C/T)	+523 (C/A)
Amino Acid and Codon		Arg19Cys	Arg16Gly	Glu27Gln			
	p-value						
Quantitative Traits							
Pre-FEV_1	0.035 (G)*		0.048 (G)*				
Delta FEV_1 (relative % pred)	0.003 (A)*		0.006 (A)*	0.043 (C) †			
Log_{10} IgE level							
Qualitative Traits							
Pre-FEV_1 < 80% predicted							
Pre-FEV_1 > 80% predicted							
Delta FEV_1 (relative % pred) > 12%	0.006 (A)*		0.044 (A)*				
Delta FEV_1 (relative % pred) < 12%							
IgE level > 100 IU/ml							
IgE level < 100 IU/ml							

For quantitative traits, the allele in the parenthesis was associated with higher value of the trait. For qualitative traits, the allele in the parenthesis was over-transmitted to asthmatic probands. All other p-values for both ethnic groups are not shown because they were not significant.

Table 5. Family-based association analysis of qualitative and quantitative asthma traits with the three most common β_2AR haplotypes in the combined (PR + Mex), Puerto Rican (PR) and Mexican (Mex) populations. Only significant associations (p-value < 0.05) are shown.

	Haplotype 1 C A T A C G C C			Haplotype 2 C G T G C A C A			Haplotype 3 C G C G G G C C		
	p-value			p-value			p-value		
	PR + Mex	PR	Mex	PR + Mex	PR	Mex	PR + Mex	PR	Mex
Quantitative Traits									
Pre-FEV_1									
Delta FEV_1 (relative % pred)									
Log $_{10}$ IgE level									
Qualitative Traits									
Pre-FEV_1 < 80% predicted		**0.029**							
Pre-FEV_1 > 80% predicted									
Delta FEV_1 (relative % pred) > 12%		**0.003**							
Delta FEV_1 (relative % pred) < 12%					**0.023**				
IgE level > 100 IU/ml								0.038	
IgE level < 100 IU/ml				**0.015**	**0.028**				

For quantitative traits, the p-values in bold represent haplotypes over-transmitted to asthmatic probands. All other p-values are not shown because they were not significant.

Figure 1

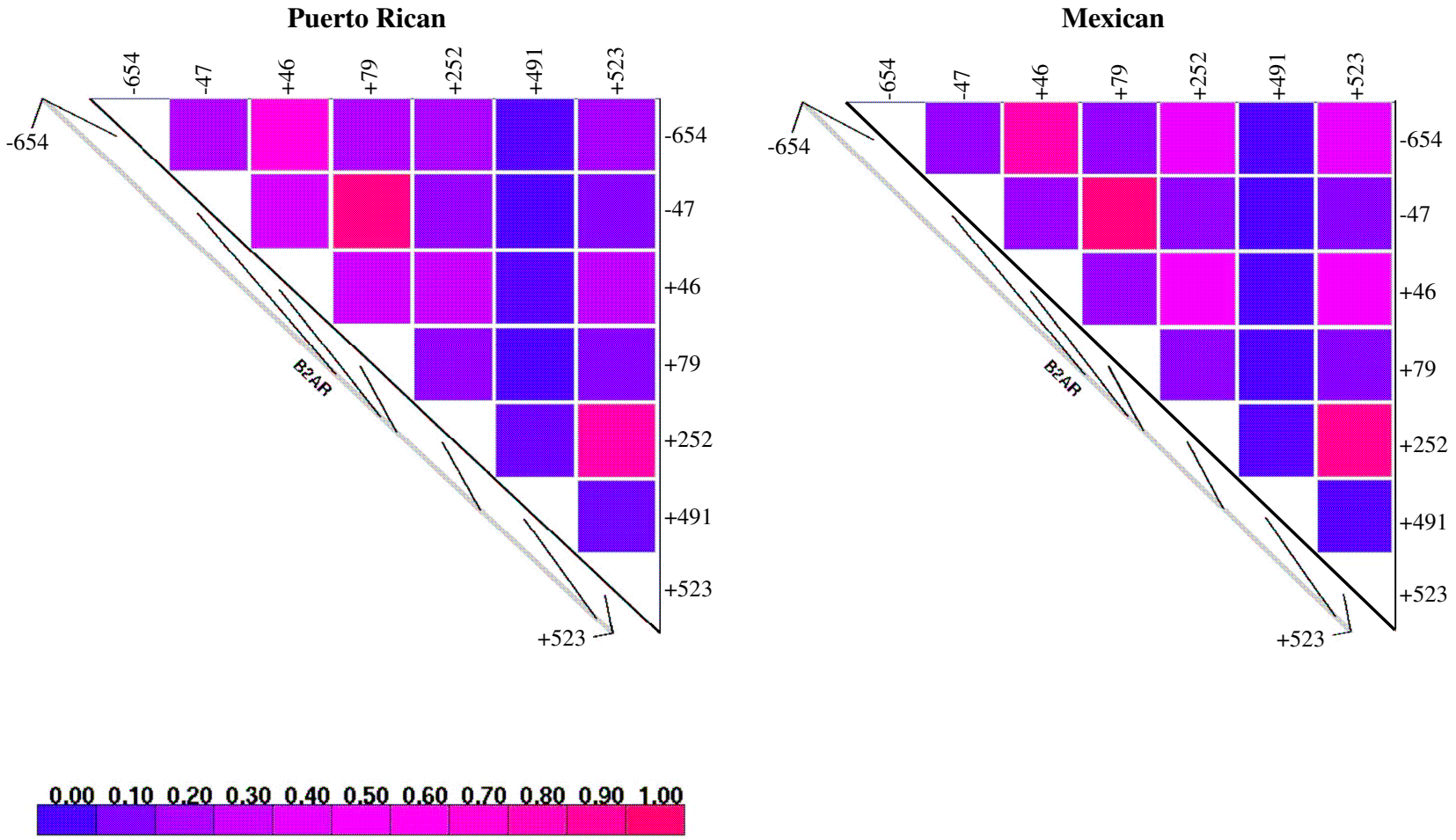

Pairwise LD (r^2)
Puerto Rican
Mexican
-654
-47
+46
+79
+252
+491
+523
B2AR
0.00 0.10 0.20 0.30 0.40 0.50 0.60 0.70 0.80 0.90 1.00

Figure 2

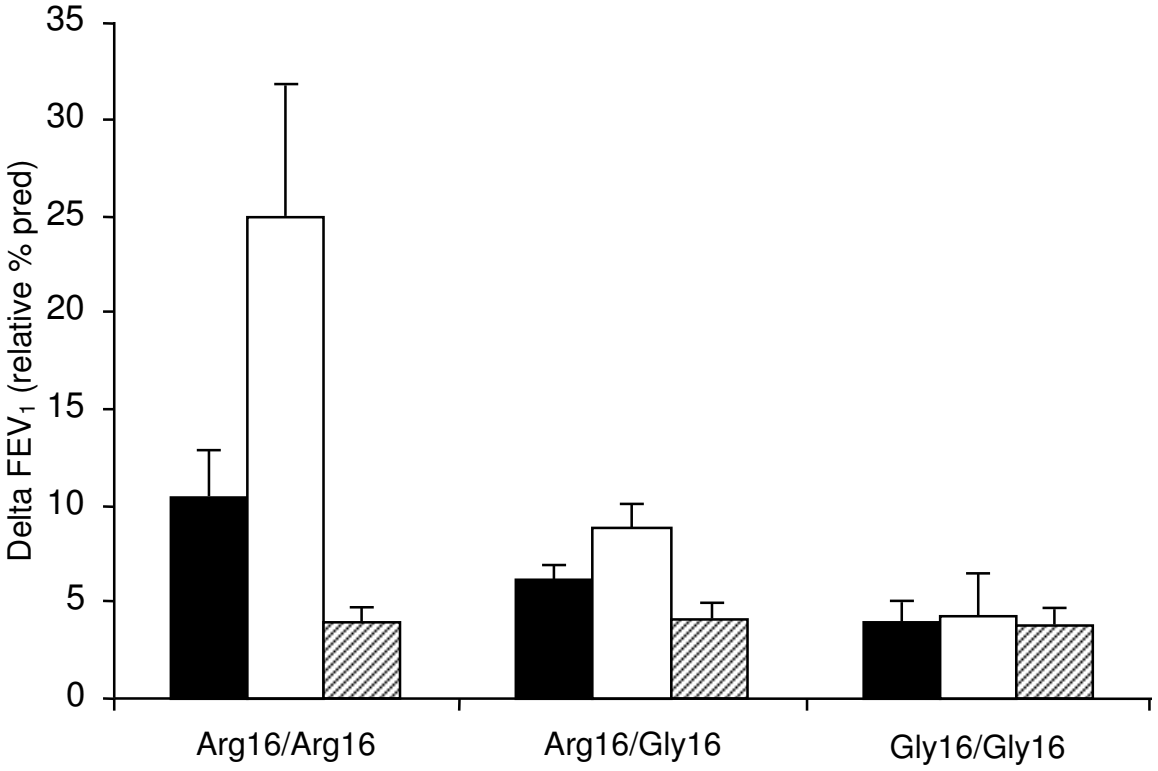
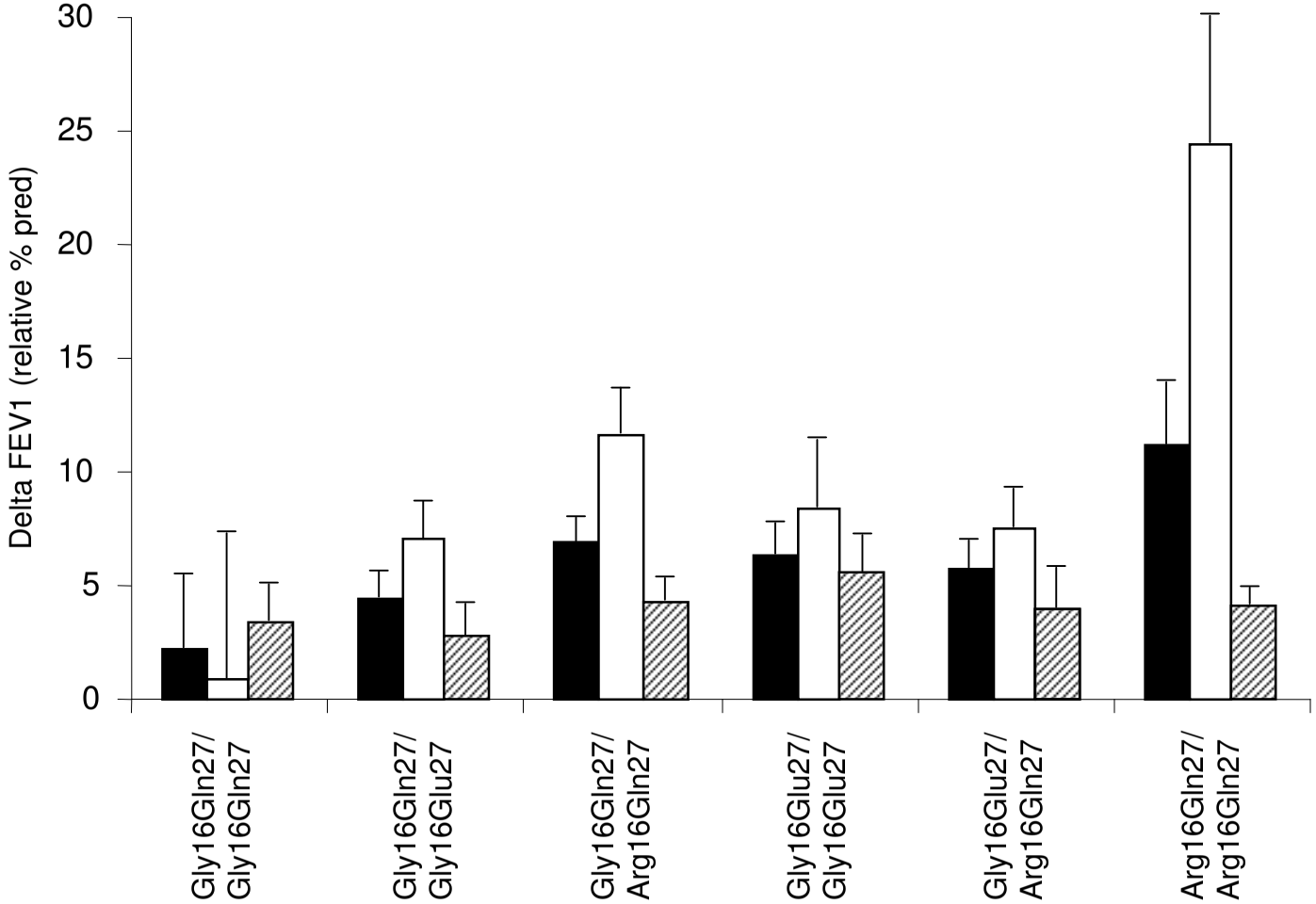

35
30
25
20
15
10
5
0
Delta FEV1 (relative % pred)
Arg16/Arg16
Arg16/Gly16
Gly16/Gly16

Figure 3


Delta FEV1 (relative % pred)
30
25
20
15
10
5
0
Gly16Gln27/ Gly16Gln27
Gly16Gln27/ Gly16Glu27
Gly16Gln27/ Arg16Gln27
Gly16Glu27/ Gly16Glu27
Gly16Glu27/ Arg16Gln27
Arg16Gln27/ Arg16Gln27

ONLINE DATA SUPPLEMENT

Pharmacogenetic Differences in Response to Albuterol between Puerto Rican and Mexican Asthmatics

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Subjects and Methods

Study Participants

Recruitment and patient characteristics are described in detail elsewhere.[E1] Briefly, 684 asthmatic subjects (probands) and their biological parents were enrolled in the San Francisco Bay Area (SF), New York City (NY), Puerto Rico (PR), and Mexico City (MX). Clinical characteristics of all probands are shown in Table 1. For this analysis, all asthmatic probands had a physician diagnosis of asthma and were currently taking asthma medications or had two or more asthma symptoms (wheezing, coughing, and shortness of breath) in the previous two years. 659 subjects were classified as having either mild or moderate-severe disease according to the study protocol. [E1]

Asthmatic probands were assessed using a modified version of the 1978 ATS-Division of Lung Disease Epidemiology Questionnaire.[E2] Ethnicity was self-reported. Probands were enrolled only if both biological parents and all biological grandparents were of Puerto Rican (for the NY and PR sites) or Mexican ethnicity (for the SF and MX sites). Local Institutional Review Boards (IRBs) approved the study.

Pulmonary Function Tests and IgE measurements

Asthmatic subjects were instructed to withhold their bronchodilator medications for at least eight hours before spirometry. Spirometry was performed according to ATS standards.[E3] Pulmonary function test results are shown in Table 1 and are expressed as a percentage of the predicted normal value using age-adjusted prediction equations from Hankinson.[E4] Baseline Forced

Expiratory Volume in one second (FEV_1) percent of predicted is described herein as Pre-FEV_1. Albuterol was administered using an extension tube connected to a standard metered dose inhaler (180µg or 2 puffs for subjects < 16 years old and 360µg or 4 puffs for subjects ≥ 16 years old), and FEV_1 measured again at least 15 minutes later (Post-FEV_1, also as % of predicted). Quantitative measure of bronchodilator responsiveness was calculated as change from baseline Pre-FEV_1: Delta FEV_1 (relative % of predicted): Relative percent change in FEV_1 percent of predicted after albuterol administration = [(Post-FEV_1-Pre-FEV_1) x 100/Pre-FEV_1] all expressed as percent of predicted FEV_1. The qualitative measure of bronchodilator responsiveness included Delta FEV_1 (relative % of predicted) greater or less than 12%.

Asthmatic probands were classified as having "mild" or "moderate-severe" asthma based on four "yes/no" questions related to medication use, asthma symptoms, nocturnal awakenings and Pre-FEV_1.[E1] According to this definition, 67.1% and 66.7% of the Puerto Rican and Mexican probands had moderate-severe asthma respectively. However, since this definition of asthma severity may be susceptible to reporting bias, we classified asthma severity based on Pre-FEV_1 levels. Patients with Pre-FEV_1 greater or less than 80% of predicted were categorized as having "mild" and "moderate-severe" asthma, respectively. Total plasma IgE was measured in duplicate using Uni-Cap technology (Pharmacia, Kalamazoo, MI). An IgE level above 100 IU/ml was used to define atopy as a qualitative measure.

Genotyping

This set of SNPs included 2 promoter SNPs (-709 and –654), a nonsynonymous SNP at position (-47[Arg19/Cys19]) in the B upstream peptide (BUP), three nonsynonymous SNPs within the β_2AR coding region; (+46 [Arg/Gly 16], +79 [Gln/Glu 27], and +491 [Thr/Ile 164]), and two synonymous SNPs within the β_2AR coding region (+252, +523). Genotyping was performed with Sequenom MassEXTEND system as previously reported.[E5]

Statistical Analysis

Mendelian inconsistencies were identified using PEDCHECK.[E6] Families with Mendelian inconsistencies were excluded from further analysis including Hardy-Weinberg Equilibrium and family-based association tests. Hardy-Weinberg Equilibrium was calculated by means of χ^2 goodness-of-fit tests. The degree of linkage disequilibrium was estimated by using the r^2 statistic.[E7] An r^2 statistic of 1 implies complete linkage disequilibrium and an r^2 statistic of 0 implies no linkage disequilibrium.

FBAT [E8] and HaploFBAT [E9] were used to assess association between individual SNPs and haplotypes, respectively, with quantitative measures of asthma severity as defined by Pre-FEV_1, bronchodilator responsiveness and IgE levels. Quantitative phenotypes selected for the association studies included Pre-FEV_1, Delta FEV_1 and log IgE. Qualitative phenotypes selected for association analysis included Pre-FEV_1 greater or less than 80%, Delta FEV_1 greater or less than 12% and IgE levels greater or less than 100 IU/ml.

Adjusting Cross-sectional Analyses for Population Stratification

Forty-four unlinked autosomal ancestry informative markers (AIMs) (see Table 1S) were genotyped in a subset of Mexican and Puerto Rican subjects. In addition, we genotyped our panel of 44 AIMs in individuals of African (n = 481), European (n = 243) and Native American (n = 148) ancestry to estimate their allele frequencies in the ancestral populations. The forty-four AIMs were genotyped using the AcycloPrime-FP^{TM} (PerkinElmer) method.[E10, E11] 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 92°C for 10 seconds, 58°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 reactions were performed with AcycloPrime-FP kits. Plates were read on an EnVision fluorescence polarization plate reader (PerkinElmer). Individual admixture estimates were derived using a Bayesian approach, as implemented in the program STRUCTURE.[E12] All cross-sectional analyses were adjusted for population stratification by including individual admixture estimates in the logistic regression model as a covariate.

Since cross-sectional analysis of unrelated individuals may be susceptible to genetic confounding due to population stratification, especially in recently admixed populations such as Puerto Rican and Mexican populations, we estimated individual admixture proportions in a subset of our Puerto Rican (n = 181) and Mexican (n = 268) asthmatic subjects using the program STRUCTURE. We performed cross-sectional analyses of bronchodilator responsiveness,

Arg16Gly and baseline Pre-FEV_1 with and without adjusting for individual admixture estimates to test for potential spurious associations. Although, the sample size (n = 449) used for this analysis has been reduced from what was originally used for the analysis (n = 667), the association among Puerto Rican asthmatics with Pre-FEV_1 less than 80% remained significant before and after genomic adjustment (0.015 and 0.016 respectively) and non-significant among Mexicans (0.7 and 0.75 respectively).

Table E1. Forty-four ancestry informative markers with their NCBI reference numbers, chromosomal locations, allele frequencies and differences in allele frequencies (δ) between African and European (Afr/Eur), African and Native American (Afr/NA), and European and Native American (Eur/NA) populations are shown.

Marker Name	dbSNP Accession	Location	African	European	Native American	Afr/Eur δ	Afr/NA δ	Eur/NA δ
MID-575	rs140864	1p34.3	0.11	0.01	0.55	0.10	**0.45**	**0.55**
MID-187	rs3188519	1p34.1	0.76	0.37	0.32	**0.39**	**0.44**	0.05
FY-null	rs2814778	1q23.2	0.00	0.99	0.99	**0.99**	**0.99**	0.00
F13B	rs6003	1q31.3	0.70	0.08	0.03	**0.62**	**0.67**	0.05
TSC1102055	rs2065160	1q32.1	0.50	0.92	0.17	**0.42**	**0.32**	**0.75**
TSC0053441	rs723632	1q32.3	0.10	0.92	0.67	**0.82**	**0.57**	0.25
WI-16857	rs3287	2p16.1	0.73	0.20	0.21	**0.53**	**0.52**	0.01
WI-11153	rs17203	3p12.3	0.81	0.15	0.76	**0.66**	0.05	**0.61**
TSC1157118	rs584059	3q22.3	0.49	0.14	0.47	**0.35**	0.03	**0.33**
GC1	rs7041	4q13.3	0.93	0.41	0.45	**0.52**	**0.48**	0.04
TSC0255473	rs930072	5p13.2	0.96	0.10	0.45	**0.86**	**0.51**	**0.35**
SGC30610	rs3309	5q11.2	0.40	0.28	0.69	0.12	0.29	**0.41**
SGC30055	rs3317	5q23.1	0.05	0.59	0.73	**0.53**	**0.68**	0.15
WI-17163	rs3340	5q33.2	0.06	0.19	0.65	0.13	**0.59**	**0.47**
WI-9231	rs2763	7p22.3	0.86	0.84	0.48	0.03	**0.38**	**0.36**
TSC0003523	rs1985080	7p14.3	0.10	0.64	0.97	**0.54**	**0.87**	**0.32**
WI-4019	rs2161	7q22.1	0.44	0.30	0.62	0.14	0.18	**0.32**
LPL	rs285	8p21.3	0.97	0.52	0.45	**0.45**	**0.52**	0.07
WI-11909	rs2695	9q21.31	0.81	0.86	0.22	0.05	**0.59**	**0.64**
TSC0879894	rs518116	9q33.3	0.13	0.67	0.58	**0.54**	**0.45**	0.09
TSC0000409	rs7349	10p11.22	0.04	0.87	0.96	**0.83**	**0.92**	0.08
TSC0316844	rs994174	10q23.1	0.76	0.25	0.26	**0.51**	**0.49**	0.02
D11S429	rs594689	11q11	0.09	0.47	0.13	**0.37**	0.04	**0.34**
TYR-192	rs1042602	11q21	0.00	0.47	0.05	**0.46**	0.05	**0.41**
DRD2-BclI	rs1079598	11q23.1	0.06	0.14	0.63	0.08	**0.57**	**0.49**
DRD2-Taq	rs1800498	11q23.1	0.14	0.65	0.09	**0.51**	0.05	**0.56**
TSC0322307	rs326946	11q23.1	0.61	0.17	0.07	**0.44**	**0.54**	0.10
TSC0072803	rs146026	13q13.1	0.26	0.92	0.83	**0.66**	**0.57**	0.09
TSC0030203	rs736394	14q32.12	0.52	0.74	0.99	0.22	**0.48**	0.26
OCA2	rs1800404	15q13.1	0.14	0.72	0.48	**0.58**	**0.34**	0.23
WI-14319	rs2862	15q14	0.38	0.17	0.69	0.21	**0.30**	**0.51**
CYP19	rs4646	15q21.2	0.32	0.29	0.72	0.03	**0.40**	**0.43**
rs223830	rs223830	16q13	0.03	0.19	0.64	0.16	**0.62**	**0.45**
MC1R-314	rs2228478	16q24.3	0.51	0.14	0.04	**0.37**	**0.47**	0.09
WI-14867	rs2891	17p13.2	0.02	0.51	0.43	**0.49**	**0.40**	0.08
WI-7423	rs2816	17p12	0.00	0.49	0.08	**0.49**	0.07	**0.42**
TSC0745571	rs203096	17q21.33	0.65	0.72	0.28	0.07	**0.37**	**0.44**
TSC0042312	rs2077863	18p11	0.51	0.93	0.93	**0.41**	**0.42**	0.00
CKM	rs4884	19q13.32	0.15	0.29	0.86	0.13	**0.71**	**0.57**
TSC0000580	rs1989486	19q13.42	0.04	0.58	0.40	**0.53**	**0.36**	0.17
MID-154	rs3188520	20q11.22	0.83	0.35	0.44	**0.48**	**0.39**	0.09
TSC0050288	rs722098	21q21.1	0.90	0.18	0.72	**0.73**	0.19	**0.54**
TSC1023401	rs235936	21q21.3	0.18	0.49	0.37	**0.31**	0.20	0.11
MID-93	rs16383	22q13.2	0.27	0.80	0.11	**0.52**	0.17	**0.69**
					average	0.42	0.42	0.30

δ >0.3 for African/European, African/Native American and Native American/European are shown in bold.

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