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***ALOX5AP* and *LTA4H* polymorphisms modify augmentation of bronchodilator responsiveness by leukotriene modifiers in Latinos**

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Abstract

Background—Understanding the effects of interactions between multiple genes and asthma medications may aid in the understanding of the heterogeneous response to asthma therapies.

Objective—To identify modulating effects of *ALOX5AP* and *LTA4H* gene polymorphisms on the drug-drug interaction between leukotriene modifiers and albuterol in Mexicans and Puerto Ricans.

Methods—In a cross-sectional study of 293 Mexicans and 356 Puerto Ricans with asthma, *ALOX5AP* and *LTA4H* genes were sequenced, and interactions between gene polymorphisms and bronchodilator responsiveness to albuterol was analyzed between leukotriene modifier users and non-users.

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Clinical Implications: Identification of modulating effects of *LTA4H* and *ALOX5AP* gene polymorphisms on the augmentation of bronchodilator responsiveness by leukotriene modifiers highlights the complex interactions underlying the heterogeneous response to asthma medications.

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Results—In heterozygotes and homozygotes for the minor allele at *LTA4H* SNP rs2540491 and heterozygotes for the major allele at *LTA4H* SNP rs2540487, leukotriene modifier use was associated with a clinically significant increase in percent change in forced expiratory volume in 1 second (FEV₁) after albuterol administration of 7.10% (p=0.002), 10.06% (p=0.001), and 10.03% (p<0.001), respectively. Presence of the major allele at *ALOX5AP* SNP rs10507391 or the minor allele at *ALOX5AP* SNP rs9551963 augmented this response. When stratified by ethnicity, these findings held true for Puerto Ricans, but not Mexicans.

Conclusions—*LTA4H* and *ALOX5AP* gene polymorphisms modify the augmentation of bronchodilator responsiveness by leukotriene modifiers in Puerto Ricans but not Mexicans with asthma.

Keywords

asthma; leukotriene; leukotriene modifier; Latino; albuterol; drug responsiveness; association study; genetic polymorphism

Introduction

The identification of pharmacogenetic effects on an individual's responsiveness to asthma medications is important for the potential targeted use of specific medications in populations that are most likely to derive clinical benefit from their use. This is particularly important in ethnic minorities because they are underrepresented in clinical studies^{1,2} yet they disproportionately experience poor asthma outcomes.^{3,4}

Leukotrienes play an important role in the pathophysiology of asthma by mediating bronchoconstriction, mucus production, and airway edema.⁵ Leukotriene synthesis is initiated in airway leukocytes in response to a number of stimuli including allergens,⁶ and leukotriene synthesis begins with arachidonate 5-lipoxygenase (*ALOX5*) which catalyzes the synthesis of leukotriene A₄ from arachidonic acid, an activity facilitated by arachidonate 5-lipoxygenase-activating protein (*ALOX5AP*). This precursor to the biologically active leukotrienes is acted upon by leukotriene A₄ hydrolase (*LTA4H*) to form leukotriene B₄ (LTB₄) and leukotriene C₄ synthase (*LTC₄S*) to produce the cysteinyl leukotrienes. These leukotrienes then act upon their specific receptors on various target cells within the respiratory tract which propagate their biologic activity. The production and activity of these leukotrienes are modulated by 5-lipoxygenase inhibitors (zileuton) and cysteinyl leukotriene antagonists (montelukast, zafirlukast, pranlukast), respectively.

Previous genetic association studies have evaluated the role of some leukotriene-related genes, such as *ALOX5* and *LTC₄S*,⁷ however there are few reports on the effects of the *ALOX5AP* and *LTA4H* genes. The activities of these gene products are necessary in the biological pathway leading to leukotriene production, and recent reports have identified these genes as important determinants of asthma susceptibility and the expression of asthma-related traits. In the Genetics of Asthma in Latino Americans (GALA) study, we recently reported that polymorphisms within both the *ALOX5AP* and *LTA4H* genes were protective for asthma in Latinos and associated with baseline lung function.⁸ Importantly, the effects of the polymorphisms were varied between the Puerto Rican and Mexican participants. Holloway JW, et al, previously reported similar associations for other polymorphisms with the same genes in Caucasians.⁹

In addition to the effects of the *ALOX5AP* and *LTA4H* genes on asthma outcomes, we also recently identified that leukotriene modifier use is associated with improved bronchodilator responsiveness to albuterol among Puerto Rican, but not Mexican American, children with asthma.¹⁰ These leukotriene modifiers are active in the biologic pathways leading to the

production and activity of leukotrienes, in which *ALOX5AP* and *LTA4H* are integral participants, and inhibition of leukotriene activity is known to lead to sustained bronchodilation.

Because Puerto Ricans and Mexicans in the GALA study experience differing clinical outcomes both from polymorphisms of the *ALOX5AP* and *LTA4H* genes and with the use of leukotriene modifiers that modulate the biologic pathways in which these genes are key players, we reasoned that distinct pharmacogenetic outcomes may exist in these two populations.

The present study aims to identify modulating effects of genetic variants in the *ALOX5AP* and *LTA4H* genes on the drug-drug interaction between leukotriene modifiers and albuterol in Latinos recruited in the GALA study. We hypothesized that polymorphisms within each of the genes would account for the augmentation of bronchodilator responsiveness by leukotriene modifiers which is present in Puerto Rican participants but absent in Mexican participants of the GALA study. Characterizing these complex interactions may help us to understand the heterogeneity in the response to leukotriene modifying medications within and between different ethnic groups.

Methods

Study Participants

Six hundred and forty-nine Latino individuals with asthma from the Genetics of Asthma in Latino Americans (GALA) Study were analyzed in the present study. This sample includes 293 individuals of Mexican origin, recruited in Mexico City and the San Francisco Bay Area, and 356 individuals of Puerto Rican origin, recruited in Puerto Rico and New York City. Pertinent clinical and demographic characteristics of these samples are shown in Table 1. Ethnicity was defined by all four grandparents being of Mexican or Puerto Rican origin. Further details on these samples have been previously published.⁶ Individuals were recruited if they had a diagnosis of asthma and were either taking a medication for asthma or had two or more asthma-related symptoms (wheezing, coughing, and/or dyspnea).¹¹ All subjects or legal guardians provided written informed consent, and local institutional review boards approved the study.

Clinical Data Collection

After obtaining age appropriate informed consent and assent, a trained interviewer administered the modified version of the 1978 American Thoracic Society-Division of Lung Diseases Epidemiology Questionnaire,¹² as previously described.¹¹ The medications: montelukast, zafirlukast, and zileuton, were all categorized as “leukotriene modifiers” and were not distinguished.

Pulmonary function was evaluated using spirometry performed to American Thoracic Society standards¹³ before and 15 minutes after the administration of albuterol, as previously described.¹¹ Briefly, albuterol was administered through a 5-cm plastic mouthpiece from a standard metered-dose inhaler at a dose of 2 puffs (180 µg) for participants aged <16 years and 4 puffs (360 µg) for participants aged ≥ 16 years. Baseline forced expiratory volume in one second (FEV₁) is reported as pre-FEV₁, and post-bronchodilator FEV₁ is reported as post-FEV₁. Bronchodilator responsiveness is reported as %ΔFEV₁, (post-FEV₁ in liters minus pre-FEV₁ in liters, times 100, divided by pre-FEV₁ in liters). Spirometric reference values from Hankinson et al. were used to determine percent predicted values.¹⁴ The reference values generated for Mexicans were also used for the Puerto Rican subjects since no reference values were available for this ethnic group. Subjects were classified as having either intermittent or persistent asthma based on data gathered from the 1978 American Thoracic Society-Division

of Lung Diseases Epidemiology Questionnaire and pulmonary function testing, as previously described.¹⁰

Genetic Analysis

Genetic information on 6 single nucleotide polymorphisms (SNPs) was collected for all individuals. Four markers in the *LTA4H* gene and two in the *ALOX5AP* were selected to cover most of the gene regions while minimizing the number of comparisons. Markers with a minor allele frequency higher than 5%, low degree of linkage disequilibrium with other markers ($r^2 < 0.80$), and minor allele involved in haplotypes with a frequency higher than 10% were included in the analyses. Exact details on selection criteria and genotyping procedures have been previously described.⁸ Marker location and allelic and genotypic frequencies of the different SNPs are detailed in Table 2. All SNPs were in Hardy-Weinberg equilibrium after stratification by ethnicity ($p > 0.05$).

Statistical Analysis

Participants were stratified based on the use of leukotriene modifiers, then demographic and clinical characteristics were compared with the unpaired 2-tailed t test (continuous variables) or the chi-squared test (categorical variables).

The primary endpoint was the effect of *LTA4H* and *ALOX5AP* gene polymorphisms on the association between leukotriene modifier use and bronchodilator responsiveness to albuterol. The association between leukotriene modifier use and bronchodilator responsiveness was evaluated by comparing mean $\% \Delta FEV_1$ (as described in the “Clinical Data Collection” section) of participants using leukotriene modifiers with mean $\% \Delta FEV_1$ of those not using leukotriene modifiers ($\% \Delta FEV_1$ of leukotriene modifier users - $\% \Delta FEV_1$ of leukotriene modifier non-users), and this difference was described as the “difference in mean $\% \Delta FEV_1$.”

Analyses were performed with multiple linear regression with inclusion of variables specific to each analysis (relevant gene polymorphisms and an interaction term between leukotriene modifier use and relevant polymorphisms), variables previously found to have a significant effect on the model evaluating the effects of leukotriene modifiers on bronchodilator responsiveness (pre- FEV_1 and an interaction term between leukotriene modifier use and FEV_1),¹⁰ and variables included for face validity (age, sex, and genetic admixture). Individual genetic admixture estimates were obtained using a set of 106 ancestry informative markers (AIMs) as previously described¹⁵ and were included in the analyses to account for the potential presence of population stratification in these populations. The following variables were previously found not to have significant effects on the model evaluating the effects of leukotriene modifiers on bronchodilator responsiveness¹⁰ and were therefore not included in the analyses: asthma duration, plasma IgE level, allergic rhinitis, family history of atopy, tobacco smoke exposure, asthma medications other than leukotriene modifiers, and interaction terms between specific medications and pre- FEV_1 . All analyses were repeated with the inclusion of BMI as a predictor variable because there was a statistically significant difference in BMI between Mexican and Puerto Ricans, but this did not affect outcomes and was therefore not included in the final analyses. Each SNP was analyzed as a multilevel categorical predictor variable, therefore, Wald tests were performed for each regression analysis to assess for overall statistical significance of the effects of the polymorphisms on the model (i.e. to account for multiple pairwise calculations). In models identifying significant effects from *LTA4H* and *ALOX5AP* genes, the statistical analyses were repeated after stratifying by ethnicity.

As a secondary endpoint, gene-gene interactions were investigated between *LTA4H* and *ALOX5AP* by including 3-way interaction terms between relevant *LTA4H* polymorphisms, *ALOX5AP* polymorphisms, and leukotriene modifier use. Because of the multiple testing and

subsequent inflated risk of making a type 1 error, Holm correction was used to establish a more conservative $p \leq 0.008$ for the secondary endpoints. STATA V.9.1 software (STATA, College Station, Texas) was used for the statistical analyses.

Because this study involved secondary data analysis, sample size calculations were performed to ensure that the study was adequately powered to detect the primary endpoint – a clinically meaningful difference in mean $\% \Delta FEV_1$ between subjects with different alleles of each gene. With the sample sizes and 95% confidence intervals reported, the study was capable of identifying a 5% difference in mean $\% \Delta FEV_1$ with $\alpha=0.05$ and power=0.8.

Results

Participant Characteristics

Participants using leukotriene modifiers and those not using leukotriene modifiers were similar for all characteristics except a higher percentage of leukotriene modifiers users had persistent asthma (Table 1). Mexicans and Puerto Ricans differed in that Mexicans were older, had a higher BMI, and a higher baseline FEV_1 than Puerto Ricans. The frequencies of the different alleles for each SNP are detailed in Table 2.

Leukotriene modifiers and bronchodilator responsiveness

The difference in mean (95% confidence intervals) $\% \Delta FEV_1$ between participants using leukotriene modifiers and those not using leukotriene modifiers was 5.04 (1.99 to 8.08) ($p=0.001$), indicating that subjects using leukotriene modifiers had a 5.04% greater improvement in FEV_1 after administration of a bronchodilator than did subjects not using this class of medication.

Effects of *ALOX5AP* and *LTA4H* polymorphisms

Neither of the two *ALOX5AP* SNPs had an effect on the association between leukotriene modifier use and bronchodilator responsiveness. The F-test statistic (numerator degrees of freedom, denominator degrees of freedom) was $F(2,568)=0.25$ ($p=0.776$) for the rs10507391 SNP and $F(2,568)=0.18$ ($p=0.835$) for the rs9551963 SNP.

Of the *LTA4H* SNPs, rs2540491 and rs2540487 had a significant effect on the association between leukotriene modifier use and bronchodilator responsiveness, with $F(2,570)=4.84$ ($p=0.008$) and $F(2,551)=3.43$ ($p=0.033$), respectively. The rs17525488 and rs2540493 SNPs did not have a significant effect on the model, with $F(2,545)=0.42$ ($p=0.655$) and $F(2,567)=1.28$ ($p=0.279$), respectively.

In heterozygotes and homozygotes for the minor allele at the rs2540491 SNP, the use of a leukotriene modifier was associated with an augmentation of bronchodilator responsiveness, with a difference in mean $\% \Delta FEV_1$ of 7.10 (2.61 to 11.59) ($p=0.002$) and 10.06 (4.29 to 15.82) ($p=0.001$), respectively. In homozygotes for the major allele, leukotriene modifier use had no effect on bronchodilator responsiveness, with a difference in mean $\% \Delta FEV_1$ of -0.31 (-4.91 to 4.29) ($p=0.896$). When stratified by ethnicity, Puerto Rican participants followed the same pattern in augmentation of bronchodilator responsiveness by leukotriene modifiers, but the Mexican participants did not, regardless of which allele was present at the rs2540491 SNP (Figure 1a).

At the rs2540487 SNP, heterozygotes demonstrated an augmentation of bronchodilator responsiveness with leukotriene modifier use (difference in mean $\% \Delta FEV_1$ of 10.03 (5.23 to 14.83) ($p<0.001$)) while homozygotes for either the major or minor alleles showed no association between leukotriene modifier use and bronchodilator responsiveness (difference

in mean % Δ FEV₁ of 2.52 (-1.16 to 6.20) (p=0.180) and 2.54 (-9.48 to 14.56) (p=0.679), respectively). When stratified by ethnicity, a similar pattern again held true for the Puerto Rican participants with no association evident in Mexican participants regardless of which allele was present (Figure 1b). Because the frequency of subjects homozygous for the minor allele of rs2540487 was low, further analysis was performed comparing carriers of the minor allele (Aa or aa) to homozygotes for the major allele (AA) to determine if carrying at least one copy of the minor allele was associated with augmented bronchodilator responsiveness in leukotriene modifier users. The use of a leukotriene modifier was associated with an augmentation of bronchodilator responsiveness in minor allele carriers in Puerto Rican participants, but not Mexican participants (difference in mean % Δ FEV₁ of 4.93 (0.11 to 9.74, p=0.045) and -3.77 (-10.03 to 2.48, p=0.236), respectively).

Because participants were recruited from different sites in the United States or their country of origin and different environmental exposures in the various locations could account for some of the observed findings, the regression analyses were repeated with inclusion of variables for birth location and recruitment site. Adjustment of the analyses for these variables had no effect on the reported differences in mean % Δ FEV₁, with the largest measured effect on any of the above reported significant outcomes being a change in the magnitude of the difference in mean % Δ FEV₁ of 0.17. Likewise, the outcomes were not significantly affected in the analyses stratified by ethnicity.

Interactions between *ALOX5AP* and *LTA4H*

Because *ALOX5AP* and *LTA4H* are in same enzymatic pathway for the production of the proinflammatory leukotriene B₄, gene-gene interactions were sought that altered the effects of *LTA4H* SNPs on the augmentation of bronchodilator responsiveness by leukotriene modifiers. Only those *LTA4H* SNPs found to have a significant effect in the primary analysis were analyzed for the presence of interactions with *ALOX5AP*. The number of participants homozygous for the minor alleles at the *LTA4H* SNPs were small, therefore, homozygotes for the minor alleles and heterozygotes were combined to identify a dominant effect of the minor allele.

For *LTA4H* SNPs rs2540491 and rs2540487, only participants with genotypes containing the minor allele demonstrated augmentation of bronchodilator responsiveness by leukotriene modifiers, and both *ALOX5AP* SNPs interacted with the *LTA4H* SNP's effects (Table 3). For the *ALOX5AP* rs10507391 SNP, only participants with genotypes containing the major allele demonstrated augmentation of bronchodilator responsiveness by leukotriene modifiers, suggesting a dominant effect of the major allele. For the *ALOX5AP* rs9551963 SNP, only participants with genotypes containing the minor allele demonstrated augmentation of bronchodilator responsiveness by leukotriene modifiers, suggesting a dominant effect of the minor allele. When stratified by ethnicity, these findings held true for Puerto Ricans but not for Mexicans.

Discussion

This study identified interactions between gene polymorphisms in leukotriene production enzymes and the effects of leukotriene modifiers on albuterol responsiveness in Latinos with asthma. Furthermore, in subgroup analyses, these interactions were found to be present in Puerto Rican, but not Mexican, participants. This highlights the complex interactions underlying the heterogeneous response to asthma medications amongst individuals and emphasizes the importance of seeking ethnic-specific differences in gene-drug interactions.

LTB₄ and the cysteinyl leukotrienes (leukotrienes C₄, D₄, and E₄) exert their proinflammatory effects through interactions with their specific receptors on the surface of inflammatory cells.

LTA4H is involved in the enzymatic pathway for the production of LTB₄, and *ALOX5AP* is in the common pathway for the production of both LTB₄ and the cysteinyl leukotrienes.⁵ Most leukotriene modifiers block cysteinyl leukotriene receptors but still allow LTB₄ to act through its receptor. The regulatory role that polymorphisms in the *ALOX5AP* and *LTA4H* genes play on the effect of leukotriene modifiers may be explained by the alteration of both LTB₄ and cysteinyl leukotriene production. Genetic variants in the *ALOX5AP* and *LTA4H* genes would modify both the effects of leukotriene modifiers at the cysteinyl leukotriene receptor and the effects of LTB₄ at its receptor, thereby affecting these medications' ability to augment bronchodilator responsiveness.

The identified polymorphisms in the *LTA4H* gene that are associated with a greater augmentation of bronchodilator responsiveness in leukotriene modifier users do not exactly match those that were previously associated with asthma-related traits.⁸ However, the present study shows clear evidence of the involvement of *LTA4H* together with *ALOX5AP* in the effect of leukotriene modifiers on bronchodilator responsiveness. The studied polymorphisms were selected to capture most of the genetic diversity in these genes and, as previously supported, gene-based approaches are more informative than approaches at a SNP-based level.¹⁶

Our results are tempered by the limitations of cross-sectional study design, such as the inability to adjust for all potential confounders. Furthermore, the analysis of multiple gene and drug interactions limits the ability to identify small effects of each of the predictor variables; nonetheless, Wald testing confirmed the presence (and absence) of statistically significant interactions between individual gene polymorphisms and the augmentation of bronchodilator responsiveness by leukotriene modifiers in the primary analysis.

A limitation in our finding that *LTA4H* and *ALOX5AP* polymorphisms affect Puerto Rican and Mexicans differently is that there were differences in clinical characteristics between the two ethnic groups (i.e., age, BMI, baseline FEV₁). To address these potential confounding factors, age and baseline FEV₁ were included in the statistical models, and a difference was still identified between the two ethnic groups. Furthermore, all the analyses were repeated with the inclusion of BMI as a predictor variable, and this had no effect on the outcomes. Additionally, participants were collected in their regions of origin (Mexico and Puerto Rico) and in mainland US metropolitan regions (San Francisco Bay Area and New York City) and may have been born in a separate location than that from which they were recruited, therefore, environmental differences could explain the observed differences between populations. Although we cannot fully discard the possibility of systematic environmental differences between Mexican and Puerto Rican participants, adjustment of the statistical analyses for birth location and recruitment center had no effect on the outcomes. Furthermore, within an ethnic origin, there were no major differences between recruitment sites in the associations found. Altogether, this indicates that our conclusion that the described gene polymorphisms are associated with augmentation of bronchodilator responsiveness by leukotriene modifiers in Puerto Ricans, but not Mexicans, is both statistically and clinically significant.

Although pharmacogenetic studies evaluating the effects of specific medications on asthma outcomes have previously been reported,¹⁷⁻¹⁹ studies such as this one analyzing interactions between two genes linked in a common pathway and its effects on drug-drug interactions are only recently being investigated.²⁰ Such investigations of multiple gene-drug interactions will allow for a more detailed understanding of the heterogeneous response to asthma medications and can potentially aid in the development of optimal therapeutic regimens for different individuals.

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Abbreviations

% Δ FEV ₁	percent change in forced expiratory volume in one second
<i>ALOX5AP</i>	arachidonate 5-lipoxygenase-activating protein
FEV ₁	forced expiratory volume in one second
GALA	Genetics of Asthma in Latino Americans
<i>LTA4H</i>	leukotriene A ₄ hydrolase
LTB ₄	Leukotriene B ₄
SNP	single nucleotide polymorphism

Figure 1. Difference in mean %ΔFEV₁ between leukotriene modifier users and non-users stratified by LTA4H genotype and ethnicity. Results for markers rs2540491 (a) and rs2540487 (b) are shown. ALL, all participants; PR, Puerto Ricans; MX, Mexicans.

Table 1

Clinical and demographic characteristics of participants, stratified by leukotriene modifier use and ethnicity.

	All	Non-LT modifier users	LT modifier users	p-value (non-LT vs. LT)	MX	PR	p-value (MX vs. PR)
N	649	546	103	-	293	356	-
Age, y	12.3 (9.9-16.7)	12.4 (10.0-16.8)	12.1 (9.5-16.3)	0.3629	13.2 (10.6-19.7)	11.9 (9.5-14.9)	0.0001
Male sex, %	54.9	53.7	61.2	0.1661	53.6	55.9	0.649
BMI	22.7 (18.6-27.6)	22.9 (18.7-27.6)	21.9 (17.9-25.9)	0.3915	23.7 (19.8-28.2)	21.2 (16.7-25.9)	0.0002
Serum IgE, IU/mL	254 (93.5-615)	261 (96.6-602.0)	226 (84.9-632.0)	0.2273	251 (95.7-600.0)	258 (92.4-627.0)	0.7577
Persistent asthma, %	67.2	64.1	83.5	0.0001	66.9	67.4	0.969
FEV ₁ baseline							
liters/sec	2.21 (1.76-2.84)	2.24 (1.76-2.85)	2.11 (1.76-2.75)	0.3971	2.34 (1.89-3.01)	2.12 (1.67-2.73)	0.0028
% predicted	86.9 (75.3-97.5)	87.2 (75.9-97.5)	84.1 (73.0-97.6)	0.2700	89.7 (77.8-100.3)	83.1 (73.9-93.4)	0.0002

LT: leukotriene modifier, MX: Mexicans, PR: Puerto Ricans.

Data are given as median (25th-75th percentiles) unless otherwise indicated.

Table 2

Genotype and allele frequencies of analyzed SNPs in the LTA4H and ALOX5AP genes.

Gene	SNP	Location	N	Genotype frequencies				MAF		
				AA (%)	Aa (%)	aa (%)		GALA	MX	PR
LTA4H	rs2540487	5'UTR	599	388 (64.8)	179 (29.9)	32 (5.3)		20.3	15.8	24.0
	rs17525488	5'UTR	594	502 (84.5)	89 (15.0)	3 (5.1)		8.0	8.3	7.7
	rs2540491	intron 3	618	260 (42.1)	259 (41.9)	99 (16.0)		37.0	28.0	44.5
	rs2540493	intron 5	619	458 (74.0)	151 (24.4)	10 (1.6)		13.8	10.2	16.9
ALOX5AP	rs10507391	intron 1	615	185 (30.1)	314 (51.1)	116 (18.9)		44.3	43.8	44.9
	rs9551963	intron 4	614	173 (28.2)	301 (49.0)	140 (22.8)		47.3	42.4	51.5

A: common allele; a: minor allele; MAF: minor allele frequency; GALA: all subjects in GALA study; MX: Mexicans; PR: Puerto Ricans

Table 3

Interactions between LTA4H and ALOX5AP SNPs and their effect on the augmentation of bronchodilator responsiveness by leukotriene modifiers.

	LTA4H rs2540491		LTA4H rs2540487	
	AA	Aa / aa	AA	Aa / aa
ALOX5AP rs10507391	AA 0.91 (-7.05-8.87) P=0.82 N=84	9.12 (2.43-15.81) P=0.008 N=94	0.20 (-6.11-6.51) P=0.95 N=119	15.41 (6.70-24.12) P=0.001 N=57
	Aa -1.63 (-8.38-5.13) P=0.64 N=117	8.79 (3.96-13.62) P<0.001 N=191	3.38 (-1.39-8.15) P=0.16 N=191	10.28 (3.98-16.58) P=0.001 N=104
	aa 0.76 (-8.84-10.37) P=0.88 N=55	4.45 (-4.20-13.11) P=0.31 N=57	3.89 (-4.16-11.93) P=0.34 N=70	1.09 (-8.82-11.00) P=0.83 N=42
ALOX5AP rs9551963	AA -0.68 (-12.72-11.35) P=0.91 N=71	5.07 (-1.26-11.41) P=0.12 N=97	5.40 (-0.94-11.75) P=0.10 N=116	-0.13 (-9.97-9.71) P=0.98 N=50
	Aa 0.23 (-6.15-6.60) P=0.94 N=125	8.31 (2.87-13.75) P=0.003 N=167	1.40 (-3.66-6.45) P=0.59 N=184	11.16 (4.32-18.01) P=0.001 N=101
	aa -0.66 (-8.73-7.42) P=0.87 N=55	10.20 (3.75-16.65) P=0.002 N=84	0.30 (-6.89-7.49) P=0.93 N=79	10.28 (3.03-17.52) P=0.006 N=53

Data is presented as the difference in mean %AFEV₁ (95% confidence intervals) of subjects using leukotriene modifiers compared to those not using leukotriene modifiers with each combination of LTA4H and ALOX5AP SNP genotype. A: common allele; a: minor allele.