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Pharmacogenetics of Asthma Controller Treatment

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Abstract

The interpatient variability in response to asthma controllers is significant and associates with pharmacogenomic variability. The goal of the present study was to identify novel variants that associate with response to common asthma controllers: fluticasone, combination of fluticasone + salmeterol and montelukast with single nucleotide polymorphisms (SNPs) in β2-adrenergic receptor, corticosteroid and leukotriene pathway candidate genes. Participants in a large clinical trial of step-down strategies volunteered for this pharmacogenetic study. 169 SNPs in 26 candidate genes were genotyped in 189 Caucasian participants with asthma who took either fluticasone (100 μg bid), fluticasone (100 μg) + salmeterol (50 μg) (FP/Salm) or montelukast (5 or 10 mg) each night for 16 weeks. Primary outcomes were the slopes of plots of Asthma Control Questionnaire (ACQ) scores vs. time following randomization; and the percent change in percent predicted FEV1 (ΔFEV1%pred) from enrollment to the end of the study. Associations between SNPs and outcomes were analyzed using general linear models. False Discovery Rate and Bonferroni corrections were

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used to correct for multiple comparisons. In all, 16 SNPs in seven genes were significantly associated with outcomes. For FP/Salm, 3 SNPs in *CHRM2* associated with ACQ slope ($p=2.8\times10^{-5}$), and rs1461496 in *HSPA8* associated with ΔFEV1%pred. For fluticasone, 5 SNPs in *CRHR1* ($p=1.9\times10^{-4}$), and 3 SNPs in *COL2A1* associated with ACQ slope and ΔFEV1%pred, respectively. For montelukast, 4 SNPs in *CHRM2* associated with ΔFEV1%pred and predicted an opposite effect compared to fluticasone ($p=9\times10^{-3}$). The present study indentified several novels SNPs that associate with response to common asthma controllers and support further pharmacogenomic study and the use of genetic variants to personalize asthma treatment.

Introduction

Asthma is a chronic complex disease characterized by variable and recurring symptoms, airflow obstruction, bronchial hyperresponsiveness and underlying inflammation. Asthma affects an estimated 20 million people in the US and 300 million people worldwide, and its prevalence is rising[1]. There are a number of different drugs and drug classes that can be categorized as either bronchodilators or as controllers, and are effective and generally safe in controlling asthma symptoms[2]. The three most common classes of asthma controllers include inhaled corticosteroids (lCS), ICS in combination with long acting beta agonists (LABA) (ICS + LABA), and leukotriene receptor antagonists (LTRA) either alone or in combination with lCS. Although effective, these drugs are associated with a significant degree of heterogeneity in patient response, which is due in large part to genetic variability[3,4]. This is known from evaluation of the repeatability of asthma treatment response[2,5,6], which is defined as the proportion of variance in a trait (FEV1, asthma exacerbations) that occurs between rather than within individuals.

Consistent with this understanding of the genetic basis for response heterogeneity, several polymorphisms have been associated with response to ICS[7,8], the combination of ICS and LABA[9,10], and LTRAs[11–13] in different studies. Knowledge of sequence variants that influence response to asthma controller drugs is important because it can lead to personalizing asthma therapy and the development of novel drugs. At present however, known sequence variants explain only a small fraction of the observed heterogeneity in response to asthma controller drugs. The goal of the present study was to identify novel variants that associate with response to common asthma controller drug therapy. A unique aspect of the present study is that we determine the pharmacogenetics of the 3 most common asthma controller drugs within the same study, providing a direct venue to compare pharmacogenetic response across therapies while increasing the specificity of our findings.

Methods

Study Design and Patients

The present pharmacogenetic study was ancillary to a large clinical trial entitled: The Leukotriene Modifier or Corticosteroid or Corticosteroid-Salmeterol Trial (LOCCS NCT00156819)[14]. Briefly, patients whose asthma was acceptably controlled with inhaled fluticasone proprionate (Flovent Diskus, Glaxo-SmithKline; 100 μg twice daily), were randomly assigned to receive double-blind treatment for 16 weeks with either continued

inhaled fluticasone (100 μg twice daily), FP/Salm (100 μg fluticasone propionate + 50 μg salmeterol, Advair Diskus, Glaxo-SmithKline, once daily), or montelukast (Singulair, Merck and Co.; 6–14 years old, 5 mg chewable tablet; ≥15 years old, 10 mg capsule once daily). Acceptable control was defined as all of the following during the last two-weeks of the fluticasone treatment run-in period: pre-bronchodilator FEV1 > 80% predicted; Asthma Control Questionnaire (ACQ) score less than 1.5; rescue β_2-agonist use less than 16 puffs per week (excluding use as a premedication for exercise, one nebulizer use was considered equivalent to 2 puffs of β_2-agonist); no hospitalization or unscheduled medical care visit for asthma; no oral corticosteroid use; no need for any additional asthma medication for asthma symptoms. All centers that participated in this ancillary study received approval from the relevant institutional review boards and all participants gave written informed consent prior to participation[14]. Treatments are subsequently referred to as fluticasone, FP/Salm, or montelukast, respectively. In the present study we report data from Caucasians only (64 FP/Salm, 65 fluticasone, and 60 montelukast). Details of patient characteristics at enrollment and randomization from the parent trial can be found in the main manuscript[14]. In the parent trial, 64.2% of participants who received study treatment were Caucasian and 93.7% of those were non-Hispanic. Ethnicity of the participants in the pharmacogenomic study paralleled the parent trial with 68.4% being Causacian and of those 93.1% were non-Hispanic. Race or ethnic group was self-reported. A replicate cohort for the montelukast arm was obtained from a previous ALA-ACRC study[15].

Outcomes

Two outcomes were considered: the slope of the least squares regression line fit to a plot of scores from the Asthma Control Questionnaire[16] (ACQ) versus time at weeks 0, 2, 4, 8, 12, and 16 after randomization, and percent change in percent predicted FEV1 (pre-bronchodilator) from enrollment to the end of the study (ΔFEV1%pred), defined as (%predicted FEV1 (week 16) - %predicted FEV1 (enrollment) / %predicted FEV1 (enrollment)) × 100. Higher ACQ scores are indicative of asthma worsening. Predicted values for pulmonary function were calculated as described by Hankinson et al.[17]. Prior to pulmonary function testing, all participants were instructed to withhold short- and long-acting β_2-agonist drugs for 4 and 12 hours, respectively. Recorded maneuvers had to meet criteria for acceptability (sharp start of flow volume curve; no cough within the first second; expiratory effort for at least 6 seconds) and reproducibility (the second largest FVC should be within 0.2 L of the largest acceptable FVC and the second largest FEV1 should be within 0.2 L of the largest acceptable FEV1). At least 3 acceptable and 2 reproducible efforts had to be obtained (up to a maximum of 8 attempts) and the largest FEV1 and FVC were recorded.

Genotyping

SNPs were genotyped by micro-sequencing of limited primer extension products as previously described[8]. P-values for deviation from Hardy-Weinberg equilibrium (HWE) between observed and expected genotype distributions were calculated using an exact test as described by Guo and Thompson[18] (Table S2). For SNPs located on the X chromosome (*CYSLTR1*), HWE was determined in females. Linkage disequilibrium (LD) was assessed and displayed for the CEU population using Haploview[19] (http://www.broadinstitute.org/

haploview) and SNP Annotation and Proxy Search Version 2.2[20] (http://www.broadinstitute.org/mpg/snap/index.php).

Statistical Analyses

PASW Statistics release 17.0.3 (Aug 22, 2009) was used to perform basic statistical analyses. Homogeneity of baseline groups was assessed with Pearson's X^2 goodness-of-fit test[21] for categorical variables, or with analysis of variance[21] (ANOVA) for continuous variables (Table 1).

Association Analyses

We tested associations between individual SNPs and outcome phenotypes stratified by treatment using general linear models as previously described[8]. The effect size was then calculated as the slope of the line between the adjusted means of the response variable for each genotype. Each model was adjusted for age, gender, height, and height^2. All analyses were performed in SAS V.9 (SAS Institute, Cary, NC).

To compensate for the effects of multiple comparisons, we used the False Discovery Rate (FDR) method of Storey and Tibshirani[22,23] as implemented in the R Statistical Package[24] computer program QVALUE[23]. We chose to limit the number of estimated false positives to <1 within the results that are called significant. Additionally, we calculated the threshold P-value for association significance using the Bonferroni correction[21], which was 3.01×10^{-4}.

Results

Baseline Characteristics of Participants

Baseline characteristics of the Caucasian patients who participated in this study (189 total: 64 FP/Salm, 65 fluticasone, and 60 montelukast) were not significantly different between the treatment groups (Table 1). The one notable exception was the frequency of smoking, with significantly fewer individuals in the fluticasone treatment group having ever smoked, although total pack years was consistent among smokers of all treatment groups. This pattern was also evident in the clinical trial[14].

Marker Associations with Pharmacodynamic Outcomes

In total, 169 markers were genotyped in 26 candidate genes in the β_2-adrenergic, corticosteroid and leukotriene pathways (Table E1 & Table E2). Three of the markers were monomorphic in our population and were not analyzed further. Genotype frequencies for 5 markers were not in HWE ($p<0.05$) including rs706765 in *CPAMD8*, rs1876831 in *CRHR1*, rs12319274 in *HAL*, rs2540483 in *LTA4H*, and rs7941773 in *STIP*. Of these only rs1876831 was found to be significantly associated with outcomes. The significant associations (16 markers in 7 genes) are summarized in Table 2.

FP/Salm Therapy—Three markers in *CHRM2* (rs8191992, rs6962027, and rs6967953) were associated with ACQ slope for FP/Salm therapy and are in linkage disequilibrium (Table 2 and Figure E1, $r^2 = 0.710 – 0.839$). Patients homozygous for the major allele improved by −0.019±0.001 ACQ points per week, while patients who were homozygous for

the minor allele got worse by 0.0056±0.001 ACQ points per week ($p=2.81\times10^{-5}$; Figure 1A and Table 2). As a consequence, by week 16, patients who were homozygous for the major allele had an ACQ score of 0.57±0.19 points compared to minor allele homozygotes who scored 0.93±0.14 points (p=0.12, Figure 1B).

An association between rs1461496 in *HSPA8* and ΔFEV1%pred was observed for participants receiving FP/Salm therapy (Figure 2). Participants homozygous for the minor allele had a 17% increase in adjusted mean ΔFEV1%pred compared to homozygous major allele participants ($p=1.28\times10^{-3}$, Table 2). No associations with *CHRM2* or *HSPA8* were observed for either fluticasone or montelukast therapies (data not shown).

Fluticasone Therapy—Five markers in *CRHR1* (rs242941, rs739645, rs1876831, rs1876829, and rs1876828) were significantly associated with ΔFEV1%pred for participants receiving fluticasone therapy (Table 2). Four of the markers in *CRHR1* were in linkage disequilibrium (rs739645, rs1876831, rs1876829, and rs1876828), as previously reported[25]. Patients who were homozygous for the minor alleles showed an improvement of 24.2±9.10% in adjusted mean ΔFEV1%pred between enrollment and week 16 of treatment compared to 10.1±2.67% for heterozygotes and 2.98±1.88% for major allele homozygotes ($p=1.89\text{x}10^{-4}$; Figure 3 and Table 2). Conversely, *CRHR1* rs242941 was associated with decreased ΔFEV1%pred. By week 16, patients homozygous for rs242941 scored 18.3±5.59% lower than patients who were homozygous for the major allele ($p=2.07\times10^{-3}$; Figure 3 and Table 2) suggesting that ΔFEV1%pred in these patients was not protected by fluticasone therapy and in fact deteriorated over the course of the trial to finish at a value lower than that recorded at enrollment.

The recessive model identified a novel association between three markers in *COL2A1* (rs2276458, rs2276455, and rs2276454) and ΔFEV1%pred for fluticasone therapy. Markers rs2276458, rs2276455, and rs2276454 in *COL2A1* are in linkage disequilibrium (Figure S2, $r^2= 0.870 – 1.00$). Patients who were homozygous for the minor allele did not show an improvement in FEP while on fluticasone, averaging an adjusted mean ΔFEV1%pred of −1.96±1.52% and resulting in a differential of 14.5±2.37% between homozygous major and homozygous minor allele patients ($p=4.25\text{x}10^{-3}$; Figure 4 and Table 2).

Montelukast Therapy—The linked markers in *CRHR1*: rs739645, rs1876831, rs1876829, and rs1876828 were associated with ΔFEV1%pred for montelukast therapy, however the effect observed was opposite in direction to that seen for fluticasone therapy (adjusted mean ΔFEV1%pred effect size for fluticasone vs. montelukast therapy =10.4±1.40% vs. −6.26±1.19, Table 2 and Figures 3 and 5). Patients homozygous for the major allele improved by 4.6±1.8% while patients who were homozygous for the minor allele showed no improvement over that recorded at enrollment ($p=8.6\times10^{-3}$; Figure 5 and Table 2).

CRHR1 marker rs242950, which is not in LD with rs739645, rs1876831, rs1876829, and rs1876828, associated with ACQ slope for participants receiving montelukast therapy. In rs242950 heterozygotes, ACQ slope was negative indicating reduced asthma symptoms while ACQ slope for major allele homozygotes was positive indicating increased asthma

symptoms ($p=3.57x10^{-3}$; Figure 6 and Table 2). Mean ACQ scores (±SE) in homozygotes and heterozygotes at week 16 were 0.963±0.109 and 0.543±0.087, respectively (p=0.075).

In an attempt to evaluate the validity of the associations between response to montelukast and genotype at rs739645, rs1876831, rs1876829, and rs1876828 in *CRHR1*, we determined associations between rs1876829 and response (ΔFEV1%pred, ACQ scores) in a replicate cohort from a previous study[15]. No associations were observed between genotype at rs1876829 and ΔFEV1%pred. However, the mean ACQ scores (±SE) in major allele homozygous participants and heterozygotes were 1.65±0.11 and 1.21±0.17, respectively following one month of montelukast treatment (p=0.049). ACQ scores following six months of treatment also trended in this same direction: 1.55±0.12 and 1.23±0.16, respectively (p=0.21).

One additional marker, rs3757016 in *HDAC2* (Figure 7) was associated with significant improvement in ΔFEV1%pred for montelukast therapy (Table 2). Compared to major allele homozygotes, heterozygotes and/or homozygotes for the minor allele were associated with improved ΔFEV1%pred in participants receiving montelukast therapy.

Discussion

Among the novel associations that we identified were three SNPs in *CHRM2* and a single SNP in *HSP8A* that associated with response to FP/Salm therapy, and five SNPs in *CRHR1* and a single SNP in *HDAC2* that associated with response to montelukast therapy. Five SNPs in *CRHR1* replicated results in previous studies with inhaled corticosteroids. Of particular interest was the observation that *CRHR1* variants were inversely associated with differential improvement in lung function following fluticasone and montelukast therapies (Figure 3 and 5). To our knowledge this study is the first to report that associations between genetic variants and response to ICS and LTRA are inversely related, thus potentially providing a genetic rationale for selecting one controller over the other.

FP/Salm Therapy—The strongest pharmacogenetic association we observed was between three SNPs in *CHRM2*, which are in linkage disequilibrium (see Figure E1), and ACQ slope following treatment with FP/Salm (Figure 1A). Autonomic control of airway tone in humans is primarily mediated by acetylcholine released from parasympathetic nerves[26]. Acetylcholine exerts its effect via stimulation of muscarinic receptors (mAChRs; M1–M5) expressed on airway smooth muscle, submucosal glands, blood vessels and nerves[27–29]. M2 mAChR expressed on prejunctional, postganglionic parasympathetic nerves, functions to attenuate synaptic acetylcholine release through negative feedback[30,31], and loss of this control contributes to increased airway tone and hyperreactivity in asthma and COPD[32,33]. Conversely, postjunctional M2 mAChR expressed on smooth muscle can inhibit β_2-adrenoceptor-induced bronchodilation[34–36]. M3 mAChR expressed on airway smooth muscle and submucosal glands, facilitates smooth muscle contraction[37–39] and mucus secretion[40–42], respectively. β-adrenoceptors and mAChRs expressed in human airway smooth muscle can influence each other through receptor crosstalk[43].

The use of ICS + LABA in asthma is controversial[44]; some studies report that continued LABA use even in the presence of ICS can make asthma symptoms worse[10,45,46]. Several studies have reported that individuals carrying the Arg16 allele of the *ADRB2* are more susceptible to the negative effects of continuous β_2-adrenoceptor stimulation compared to carriers of the Gly16 allele[47–49]. We observed no association between ACQ slope and *ADRB2* variants in participants taking FP/Salm. However, given the potential of receptor crosstalk between β_2-adrenoceptors and M2 mAChR in airways, and the association between ACQ scores and *CHRM2* variants, it is important to replicate our findings in future studies.

Fluticasone Therapy—The linked *CRHR1* SNPs rs1876831, rs1876828, rs739645, rs1876829 were strongly associated with ΔFEV1%pred in patients receiving fluticasone (8.2-fold higher for minor allele homozygotes, Figure 5). rs242941 was associated with a negative ΔFEV1%pred compared to major allele homozygotes and heterozygotes (Figure 3). These data replicate results of our previous studies in asthma[8,50] and in COPD, at least for rs242941[51], indicating that *CRHR1* is an important gene in modulating the effects of ICS. SNPs in *CRHR1* have also been associated with markers of inflammation and endothelial dysfunction in elderly males with asthma and/or COPD[52].

Corticotropin-releasing hormone (CRH) binds to *CRHR1* and exerts its anti-inflammatory effects by mediating the release of ACTH and promoting the production of cortisol[53]. Thus, variants of *CRHR1* could affect CRH binding and subsequently ACTH release, airway inflammation, and responsiveness to ICS[8]. Alternatively, the associations between ICS responsiveness in asthma and *CRHR1* variants may be related to a large inversion polymorphism[25].

Montelukast Therapy—The association between *CRHR1* SNPs rs1876831, rs1876828, rs739645, rs1876829 and ΔFEV1%pred in patients receiving montelukast was the inverse of that observed for patients receiving fluticasone. We were not able to replicate this association in a cohort of participants who received montelukast in a previous study (LODO[15]). Interestingly, ACQ scores for heterozygotes at rs1876828 in LODO participants taking montelukast were lower following one month of therapy compared to placebo (1.21±0.17 vs 2.00±0.19; ANOVA p=0.012, two-sided Dunnet p=0.007), while ACQ scores in major allele homozygotes were not significantly different from placebo (1.65±0.11 vs 1.88±0.14; ANOVA p=0.41). This pattern was not however evident following six months of treatment (data not shown). These data are not consistent with our findings in the present study.

Histone deacetylase 2 (*HDAC2*) SNP rs3757016 was associated with a significant improvement in ΔFEV1%pred for patients receiving montelukast (Figure 7). *HDAC2* contributes to suppression of inflammatory gene expression and is thought to play a major role in corticosteroid resistance[15,54,55], which potentially may be reversed by increasing *HDAC2* activity[56]. The mechanism underlying the association between rs3757016 and ΔFEV1%pred in participants taking montelukast is not clear. However, if rs3757016 is associated with gain of *HDAC2* function, then attenuation of inflammatory gene expression

would increase with allele dosage, conceivably leading to increased effectiveness of montelukast.

Our study has several limitations including small numbers of participants which limited our power to detect associations between response and SNPs with minor allele frequencies <0.1. To avoid population stratification, we limited our study to Caucasians because of the small number of African Americans and other ethnicities who participated. We have limited access to other pharmacogenetic studies which impeded our ability to replicate our novel associations.

In conclusion, we believe the results of our study strongly support the inclusion of pharmacogenomics in comparative effectiveness research and personalized medicine.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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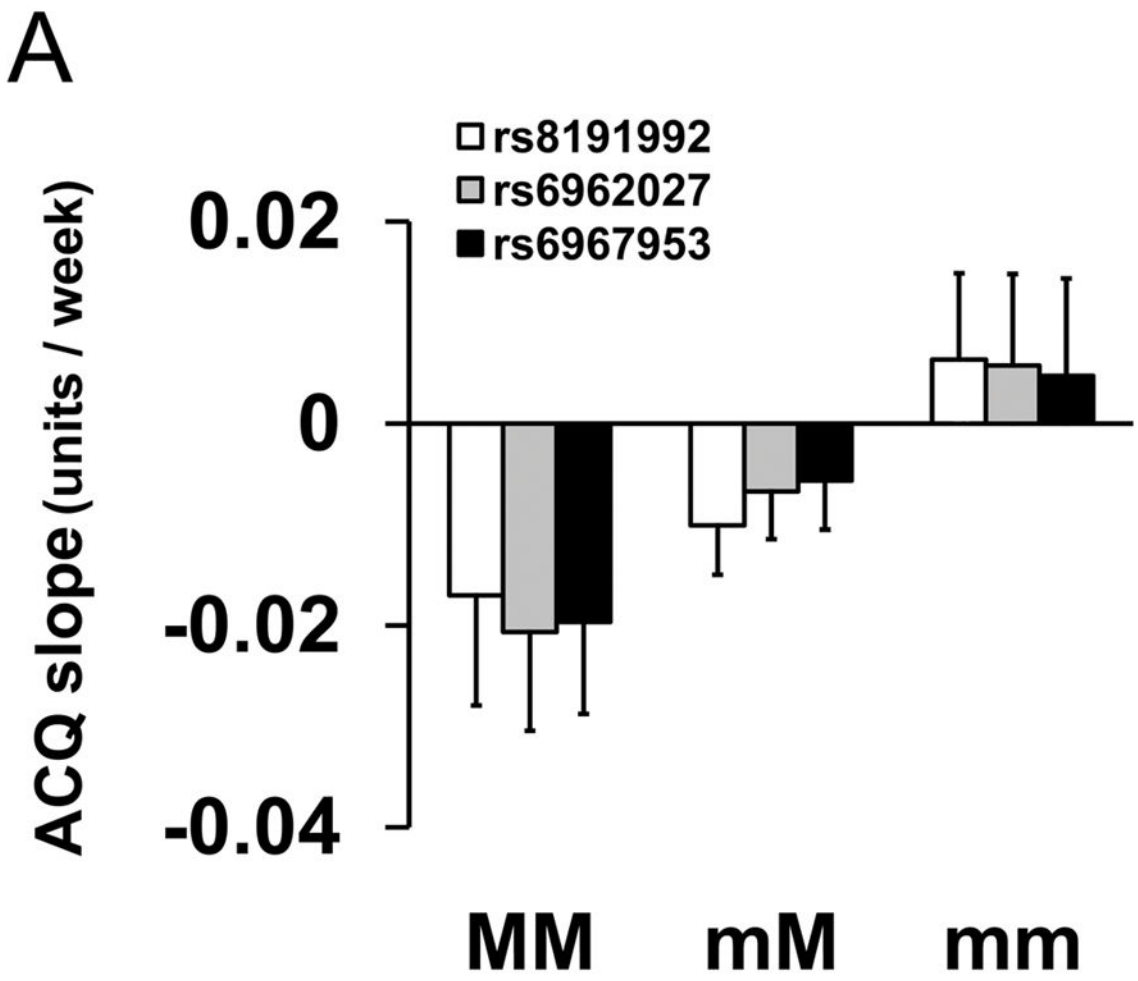
At a Glance Commentary

Genetic determinants that influence response to the three most common asthma controller therapies, inhaled corticosteroids, the combination of inhaled corticosteroids and long acting β_2-agonists, and leukotriene receptor antagonists, are identified in 26 candidate genes. Our data support continued pharmacogenomic studies in asthma and the use of genetic variants to personalize asthma treatment.



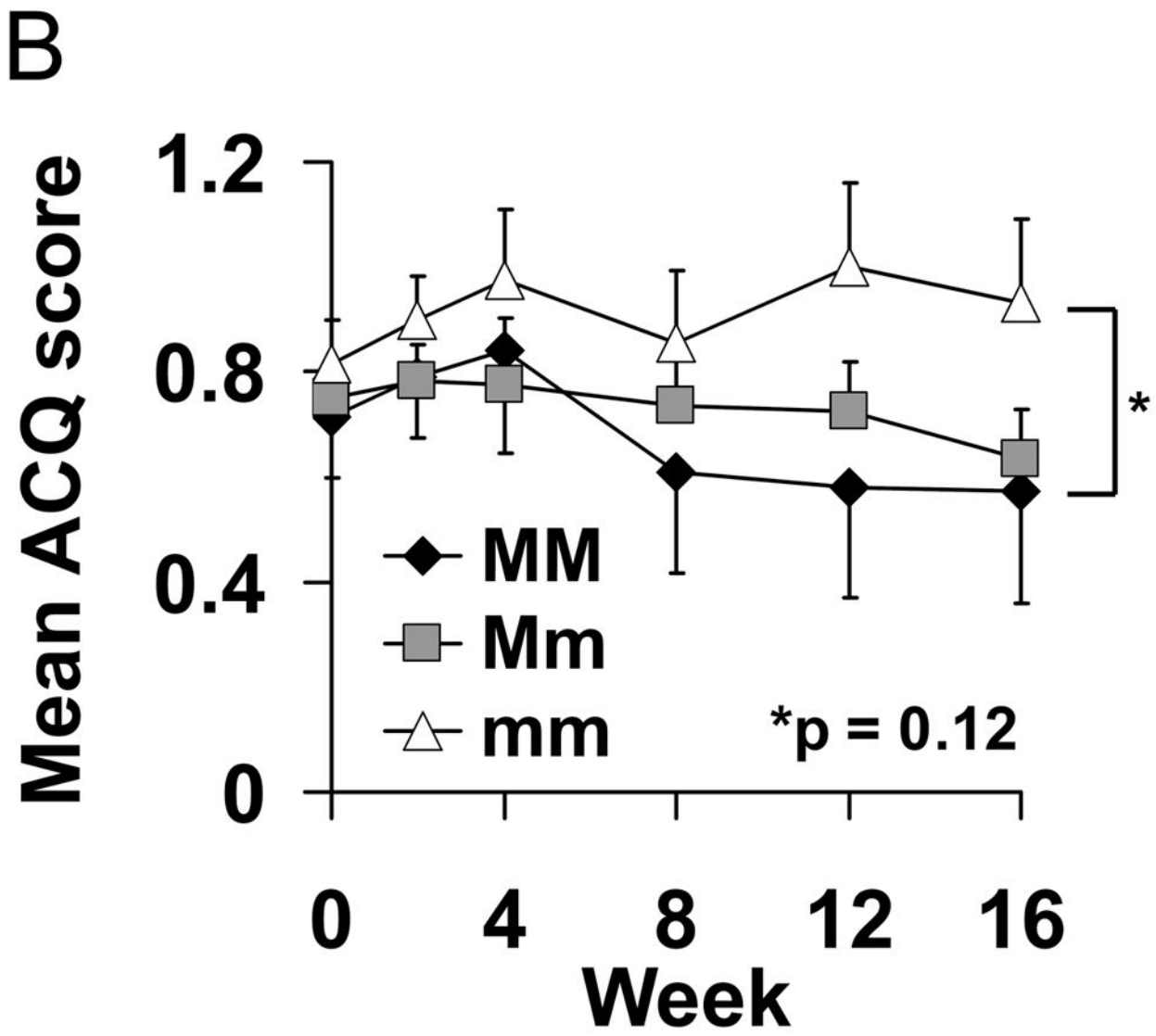


Figure 1.

Association between Genotype of Markers in *CHRM2* and Asthma Control Questionnaire Scores for FP/Salm Treatment. (Panel A) Mean slopes of the regression lines of scores from the Asthma Control Questionnaire[16] (±SE) vs. time were compared by genotype for rs8191992 (□), rs6962027 (◘) and rs6967953 (■) in participants taking fluticasone and salmeterol combination for 16 weeks. (Panel B) Mean scores from the Asthma Control Questionnaire[16] (±SE) as a function of time between randomization (zero time) and week 16 by genotype. MM refers to homozygous for the major alleles (✦); mM refers to heterozygotes (◘); and mm refers to homozygotes for the minor allele (✧) of each SNP.

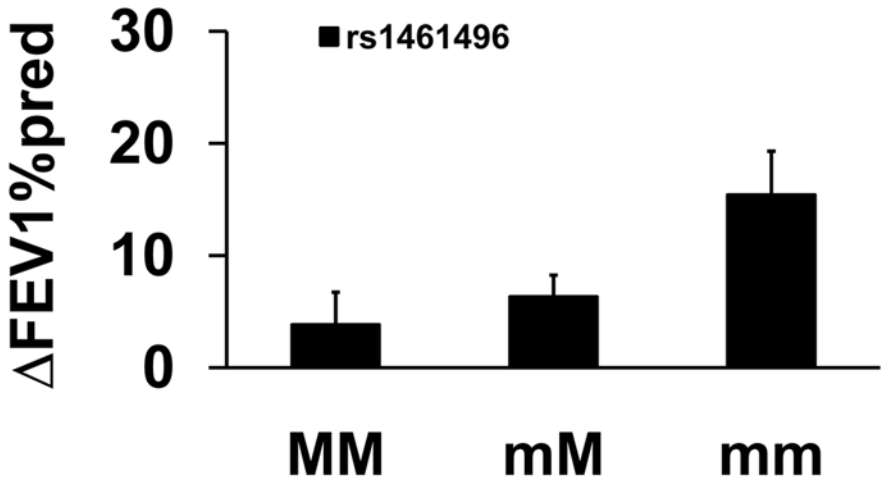


Figure 2.

Association between Genotype for rs1461496 in *HSP8A* and Pulmonary Function for FP/Salm Treatment. Mean percent changes in percent predicted FEV1 (±SE) were calculated between week 16 and visit 1 (randomization) in participants taking fluticasone and salmeterol treatment by genotype. MM refers to homozygous for the major allele; mM refers to heterozygotes; and mm refers to homozygotes for the minor allele.

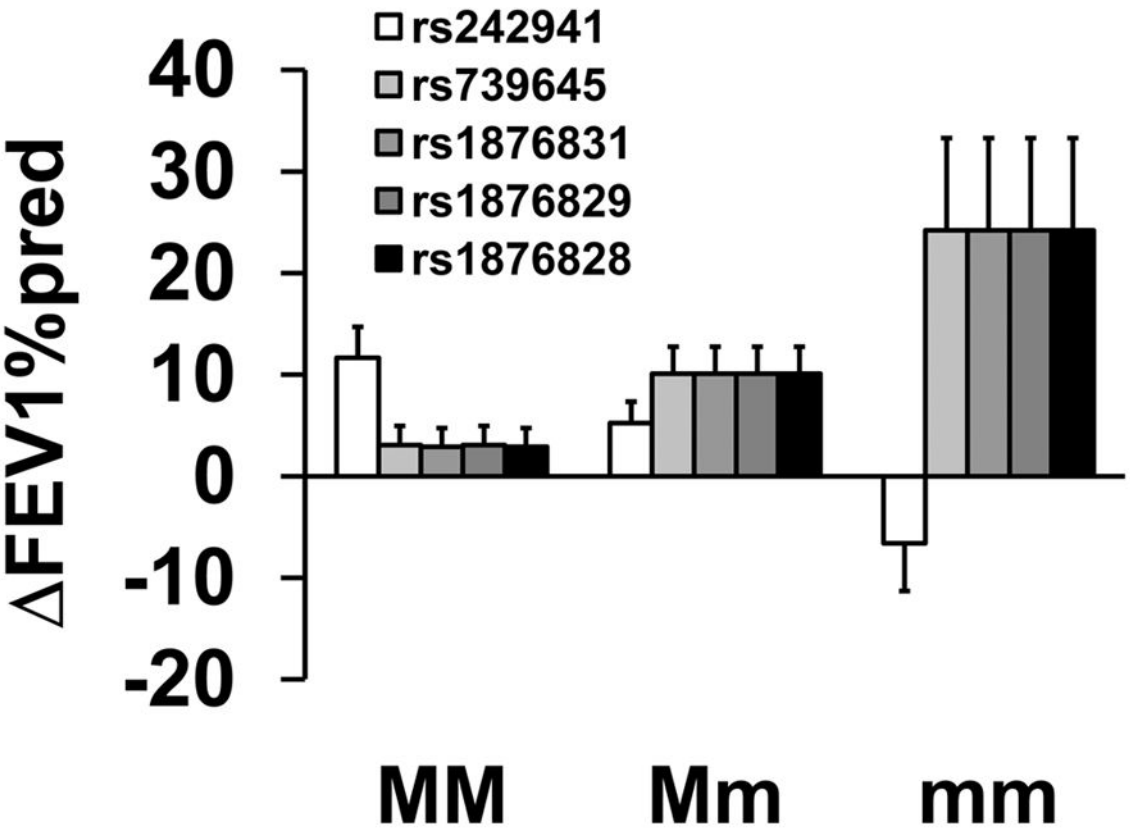


Figure 3.

Association between Genotype of Markers in *CRHR1* and Pulmonary Function for Fluticasone Treatment. Mean percent changes in percent predicted FEV1 (±SE) were calculated between week 16 and visit 1 (randomization) in participants taking fluticasone treatment by genotype for rs242941 (□); rs739645 (▫); rs1876831 (▪); rs1876829 (▪); and rs1876828 (■). MM refers to homozygous for the major alleles; mM refers to heterozygotes; and mm refers to homozygotes for the minor alleles.

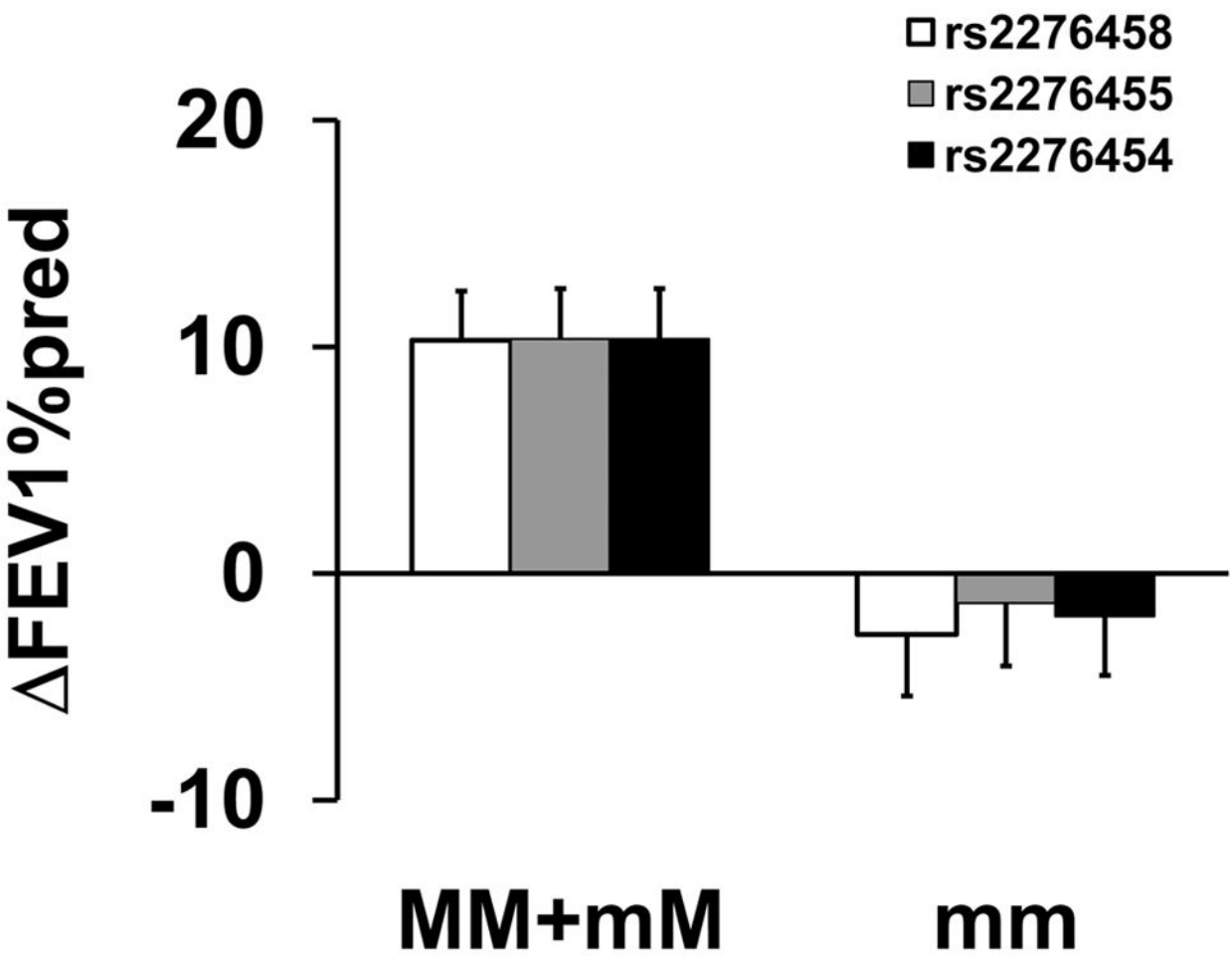


Figure 4.

Association between Genotype of Markers in *COL2A1* and Pulmonary Function for Fluticasone Treatment. Mean percent changes in percent predicted FEV1 (±SE) were calculated between week 16 and visit 1 (randomization) in participants taking fluticasone treatment by genotype for rs2276458 (□); rs2276455 (▩); and rs2276454 (■). MM refers to homozygous for the major alleles; mM refers to heterozygotes; and mm refers to homozygotes for the minor alleles.

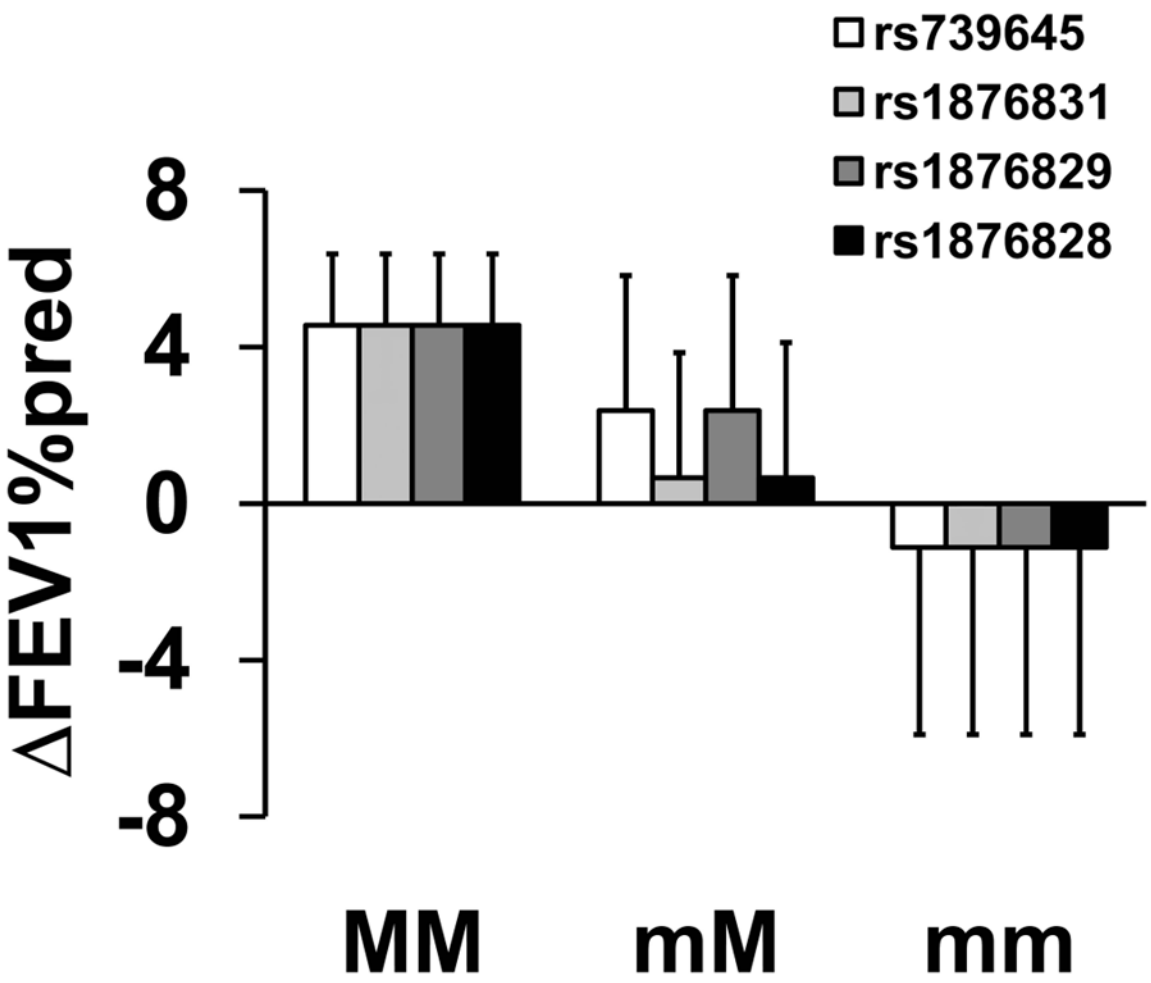


Figure 5.

Association between Genotype of Markers in *CRHR1* and Pulmonary Function for Montelukast Treatment. Mean percent changes in percent predicted FEV1 (±SE) were calculated between week 16 and visit 1 (randomization) in participants taking montelukast by genotype for rs739645(□); rs1876831 ( ); rs1876829 ( ); and rs1876828 (■). MM refers to homozygous for the major alleles; mM refers to heterozygotes; and mm refers to homozygotes for the minor alleles.

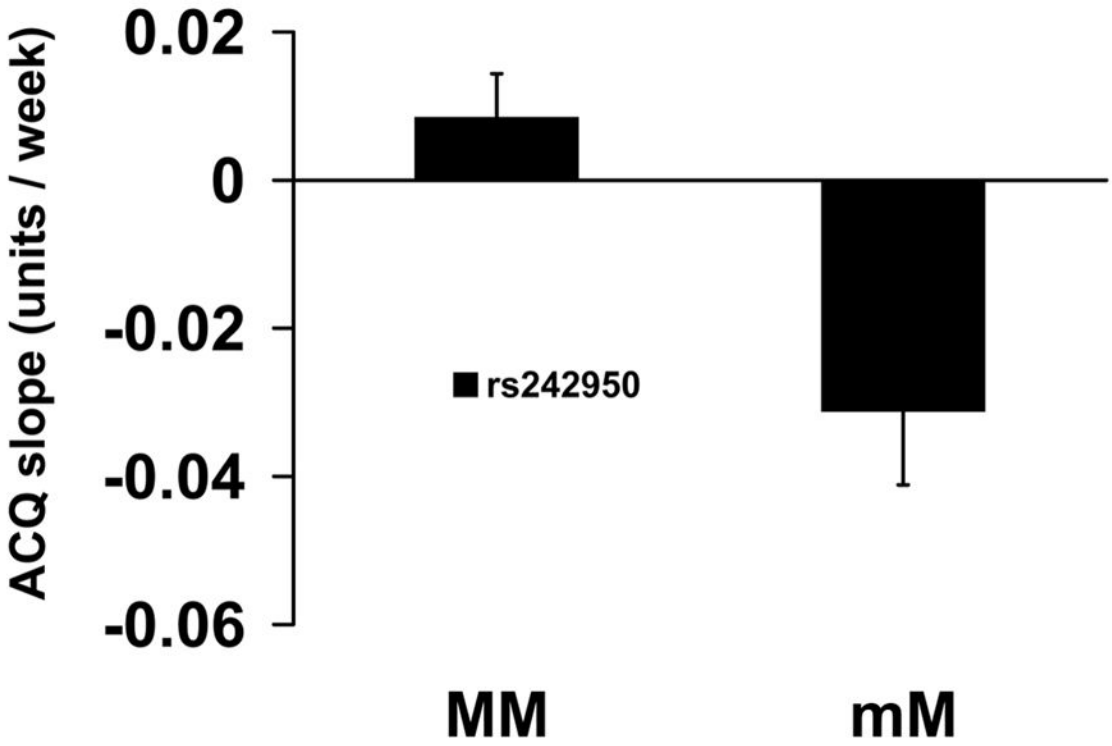


Figure 6.

Association between rs242950 in *CRHR1* and Asthma Control Questionnaire Scores for Montelukast Treatment. Mean slopes of the regression line of scores from the Asthma Control Questionnaire (±SE) vs. time were compared by genotype for rs242950 in participants taking montelukast for 16 weeks. MM refers to homozygous for the major allele; mM refers to heterozygotes.

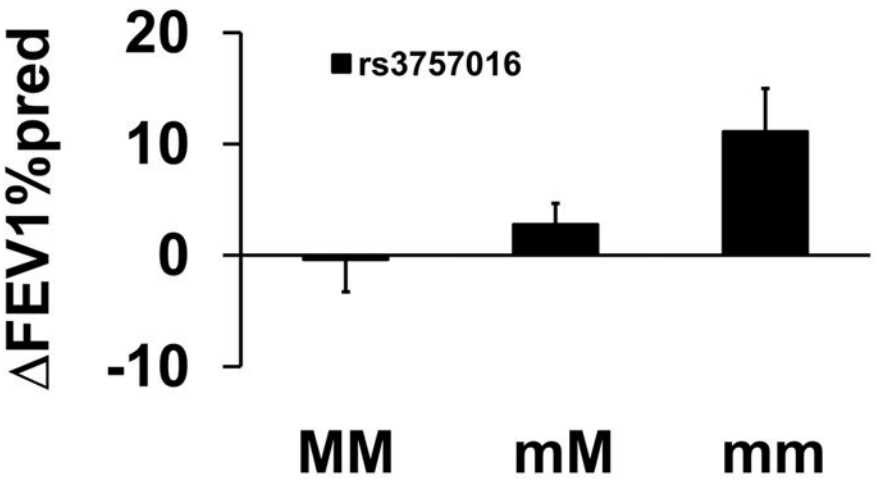


Figure 7.

Association between Genotype of *HDAC2* and Pulmonary Function for Montelukast. Mean percent changes in percent predicted FEV1 (±SE) were calculated between week 16 and visit 1 (randomization) in participants taking montelukast by genotype for rs3757016 in *HDAC2*. MM refers to homozygous for the major alleles; mM refers to heterozygotes; and mm refers to homozygotes for the minor alleles.

Table 1

Baseline Characteristics of Study Participants

	Treatment Assignment*			
	F/S	F	M	P-value†
Number of participants	64	65	60	0.46
Mean age at randomization (years; SD)	34.3(15.4)	31.5(14.9)	36.4(15.6)	0.20
Male (%)	33	38	38	0.77
Smokers (> 20 packs per lifetime, %)	38	11	22	0.001
Mean pack-years (years; SD)	5.7(5.2)	8.6(6.0)	5.8(4.3)	0.41
Asthma characteristics				
Mean age at onset of asthma (years; SD)	17.6(18.7)	20.5(27.1)	17.8(19.2)	0.71
≥1 emergency visits for asthma in the previous year	38	26	27	0.27
≥1 course of oral corticosteroids in the previous year	33	21	25	0.31
Use of rescue inhaler >1 time per week (%)	59	70	68	0.41
On inhaled corticosteroids at baseline (%)	27	27	28	0.98
Asthma Control Scores (Mean±SD)‡				
ACQ at enrollment	1.77(0.76)	1.74(0.73)	1.8(0.90)	0.87
ACQ at randomization	0.75(0.38)	0.70(0.34)	0.72(0.41)	0.71
ASUI at randomization	0.89(0.08)	0.88(0.09)	0.88(0.11)	0.72
Pulmonary Function (Pre-Bronchodilator, Mean±SD)§				
Peak Flow at enrollment (L/min)	407(124)	382(111)	400(108)	0.45
Peak Flow at randomization (L/min)	412(109)	398(105)	422(109)	0.48
FVC at enrollment (L)	3.88(1.04)	3.74(1.11)	3.86(1.13)	0.72
FVC at randomization (L)	4.03(1.07)	3.94(1.09)	4.05(1.20)	0.83
FEV1 at enrollment (L)	2.82(0.79)	2.75(0.78)	2.80(0.75)	0.86
FEV1 at randomization (L)	3.01(0.80)	3.00(0.78)	3.05(0.79)	0.92
% Predicted FEV1 at enrollment‖	84.1(12.0)	84.1(12.8)	83.2(11.2)	0.89
% Predicted FEV1 at randomization‖	89.4(9.7)	91.7(8.9)	90.8(10.2)	0.38

[*] F/S, combination of fluticasone and salmeterol ; F, fluticasone; M, montelukast

[†] P-values were derived from a Pearson's X^2 goodness-of-fit test for categorical variables and from ANOVA for continuous variables[21].

[‡] ACQ, Asthma Control Questionnaire; ASUI, Asthma Symptom Utility Index

[§] FVC, forced vital capacity; FEV1, forced expiratory volume in 1 second

[//] Predicted values for pulmonary function were calculated as described by Hankinson et al.[17].

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Table 2

Summary of Significant Associations Between Outcomes and Genotypes of SNPs in Candidate Genes.

Treatment	Outcome*	Locus	Marker	N (MM, mM, mm)	Effect Size (SE)¶	P-value†	MAF	Function
Combination	ΔFEV1%pred§	*HSPA8*	rs1461496	61 (24, 30, 7)	8.41 (2.62)	$1.28x10^{-3}$	0.308	intron
	ACQ slope§	*CHRM2*	rs8191992	61 (18, 29, 14)	$2.45x10^{-2}$ ($6.26x10^{-3}$)	$8.92x10^{-5}$‡	0.435	untranslated-3'
		CHRM2	rs6962027	61 (17, 28, 16)	$2.52x10^{-2}$ ($6.04x10^{-3}$)	$2.98x10^{-5}$‡	0.431	near-gene-3'
		CHRM2	rs6967953	61 (17, 28, 16)	$2.56x10^{-2}$ ($6.11x10^{-3}$)	$2.81x10^{-5}$‡	0.420	unknown
		ABCC1	rs119774	61 (52, 9, 0)	$3.57x10^{-2}$ ($1.36x10^{-2}$)	$8.69x10^{-3}$	0.043	intron
Fluticasone	ΔFEV1%pred§	*CRHR1*	rs242941	64 (34, 26, 4)	−9.98 (3.24)	$2.07x10^{-3}$	0.341	intron
		CRHR1	rs739645	63 (36, 19, 8)	$1.03x10^{1}$ (2.82)	$2.61x10^{-4}$‡	0.156	intron
		CRHR1	rs1876831	64 (37, 19, 8)	$1.04x10^{1}$ (2.79)	$1.89x10^{-4}$‡	0.159	intron
		CRHR1	rs1876829	63 (36, 19, 8)	$1.03x10^{1}$ (2.82)	$2.62x10^{-4}$‡	0.156	intron
		CRHR1	rs1876828	64 (37, 19, 8)	$1.04x10^{1}$ (2.79)	$1.89x10^{-4}$‡	0.154	intron
		HAL	rs17024981	64 (56, 8, 0)	$2.15x10^{1}$ (6.22)	$5.46x10^{-4}$	0.078	intron
	ΔFEV1%pred//	*COL2A1*	rs2276458	64 (15, 37, 12)	$-1.71x10^{1}$ (6.00)	$4.25x10^{-3}$	0.347	intron
		COL2A1	rs2276455	63 (17, 34, 12)	$-1.71x10^{1}$ (6.00)	$4.25x10^{-3}$	0.400	intron
		COL2A1	rs2276454	64 (17, 34, 13)	$-1.71x10^{1}$ (6.04)	$4.61x10^{-3}$	0.412	coding-synon
Montelukast	ΔFEV1%pred§	*CRHR1*	rs739645	54 (34, 17, 3)	−6.26 (2.38)	$8.61x10^{-3}$	0.156	intron
		CRHR1	rs1876831	54 (34, 16, 4)	−6.26 (2.38)	$8.61x10^{-3}$	0.159	intron
		CRHR1	rs1876829	54 (34, 17, 3)	−6.26 (2.38)	$8.61x10^{-3}$	0.156	intron
		CRHR1	rs1876828	53 (34, 16, 3)	−6.26 (2.38)	$8.61x10^{-3}$	0.154	intron
		HDAC2	rs3757016	54 (17, 25, 12)	5.57 (2.12)	$8.63x10^{-3}$	0.460	untranslated-3'
	ACQ slope§	*CRHR1*	rs242950	55 (10, 45, 0)	$-3.54x10^{-2}$ ($1.21x10^{-2}$)	$3.57x10^{-3}$	0.126	intron

Abbreviations: MAF, minor allele frequency; N, number of patients; SE, Standard Error; synon, synonymous.

[*] ΔFEV1%pred, change in percent predicted FEV1[17] defined as (%predicted FEV1 (week 16) - %predicted FEV1 (enrollment) / %predicted FEV1 (enrollment)) × 100 (unit-less); ACQ slope, slope of the least squares regression line fit to a plot of Asthma Control Questionnaire[16] score versus time at weeks 0, 2, 4, 8, 12, 16 (ACQ units / week). The ACQ score ranges from 0 to 6, with lower values indicating less-severe asthma and 0.5 unit as the minimal clinically important difference[16].

[†] P-values were determined from general linear models correcting for age, gender, height, and height[2] (see Methods). Reported p-values for each treatment – phenotype combination are for the maximum set of associated markers for which less than one false positive is predicted by the method of Storey and Tibshirani[23];

[‡] associated markers that are significant by the Bonferroni multiple testing correction[57].

[§] Additive model;

[//] Recessive model.

[¶] Effect size is calculated as the slope of the line between the adjusted means of the response variable for each genotype group.