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Inhaled corticosteroid treatment modulates ZNF432 gene variant's effect on bronchodilator response in asthmatics

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Abstract

Background—Single nucleotide polymorphisms (SNPs) influence a patient's response to inhaled corticosteroids and β_2-agonists, and the effect of treatment with inhaled corticosteroids is synergistic with the effect of β_2-agonists. We hypothesized that use of inhaled corticosteroids could influence the effect of SNPs associated with bronchodilator response.

Objective—To assess whether, among asthma subjects, the association of SNPs with bronchodilator response is different between those treated with inhaled corticosteroids vs. those on placebo.

Methods—A genome-wide association analysis was conducted using 581 white subjects from the Childhood Asthma Management Program (CAMP). Using data for 449,540 SNPs, we conducted a gene by environment analysis in PLINK with inhaled corticosteroid treatment as the environmental exposure and bronchodilator response as the outcome measure. We attempted to

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replicate the top 12 SNPs in the Leukotriene Modifier Or Corticosteroid or Corticosteroid-Salmeterol (LOCCS) Trial.

Results—The combined P-value for the CAMP and LOCCS populations was 4.81E-08 for rs3752120, which is located in the zinc finger protein gene *ZNF432*, and has unknown function.

Conclusions—Inhaled corticosteroids appear to modulate the association of bronchodilator response with variant(s) in the *ZNF432* gene among adults and children with asthma.

Clinical Implications—Clinicians who treat asthma patients with inhaled corticosteroids should be aware that the patient's genetic makeup likely influences response as measured in lung function.

Capsule Summary—Our study suggests that inhaled corticosteroids could influence the effect of multiple SNPs associated with bronchodilator response across the genome.

Keywords

asthma; bronchodilator response; lung function; inhaled corticosteroids; single nucleotide polymorphisms; zinc finger proteins; *ZNF432*

INTRODUCTION

Inhaled corticosteroids and β_2-agonists are the two most commonly used medications for asthma. Recent evidence suggest that inhaled corticosteroid treatment can restore β_2-adrenergic responsiveness of airway smooth muscle cells.[1] Additional studies have shown that corticosteroids increase β_2- adrenergic receptor (*ADRB2*) gene transcription in the lung[2] and that inhaled corticosteroids potentiate β_2-agonist-induced airway smooth muscle relaxation.[3]

Single nucleotide polymorphisms (SNPs) appear to influence a patient's response to inhaled corticosteroids and β_2-agonists. For example, a significant association between 8-week response to inhaled steroids and SNPs from the corticotrophin-releasing hormone receptor 1 (*CRHR1*) gene, which encodes the primary receptor mediating the release of adrenocorticotropic hormone, have been demonstrated.[4] One SNP, rs242941, in *CRHR1* (minor allele frequency ~30%) is associated with positive treatment response to corticosteroids in adult and pediatric clinical trials (p=0.025 and 0.006, respectively).[4,5] Furthermore, a *CRHR1* haplotype was associated with a 2-3 times greater short-term response to inhaled steroids.[4] Several *ADRB2* polymorphisms, including non-synonymous changes from arginine to glycine at position 16 (A16→G) and from glutamic acid to glutamine at position 27 (E27→Q) have been associated with asthma.[6] Other polymorphisms of this gene have been associated with response to inhaled [.beta]$_2$-agonist treatment [7] and bronchodilator response (BDR).[8,9] Additional genes that have been reported to be associated with BDR include corticotrophin-releasing hormone receptor 2 (*CRHR2*) [10], arginase 1 (*ARG1*)[11,12].

In addition to the genetic variants found to be associated with responses to either inhaled corticosteroid alone or to β_2-agonists alone, we have previously demonstrated that a non-synonymous SNP in adenylate cyclase 9 (*AC9*)[13] predicts degree of both cellular and clinical response to β_2-agonist medications and that this response was significantly enhanced in the presence of glucocorticoid medications.[13] Given this preliminary data and knowledge that the effect of treatment with inhaled corticosteroids is synergistic with the effect of β_2-agonists and a patient's response to both medications is genetically influenced, we hypothesized that inhaled corticosteroids could influence the effect of multiple SNPs associated with bronchodilator response across the genome.

METHODS

Populations and Measures

Our primary test population is composed of subjects from the Childhood Asthma Management Program (CAMP), a clinical trial that followed 1,041 asthmatic children for four to six years and randomized subjects to budesonide, nedocromil, or placebo. This study methodology and design have been described previously.[13] For the current work, CAMP subjects in the budesonide arm were considered to be exposed to ICS while subjects in the nedocromil and placebo groups were not exposed to ICS. We chose to study BDR at the conclusion of the CAMP clinical trial, 48 months after randomization in order to study BDR after maximal exposure to ICS.[13] Of the 581 Caucasian subjects who had available DNA for genotyping, 30% (172) were in the inhaled corticosteroid (budesonide) group, 29% (171) were in the nedocromil group, and 41% (238) were in the placebo group. For the purposes of this analysis, we combined the nedocromil and placebo groups.

Replication was conducted in subjects in the Leukotriene Modifier Or Corticosteroid or Corticosteroid-Salmeterol Trial (LOCCS).[14] At the baseline visit, subjects were asked, "Over the past 6 months, on average how often did you use inhaled corticosteroids (e.g. Beclovent, Pulmicort, Flovent, etc)?" Subjects who responded "daily" or "2-6 times per week" were considered to be in the inhaled corticosteroid group, whereas subjects who responded "1-2 times per month," "less than 1 time per month," or "never" were placed in the non-inhaled corticosteroid group. All subjects were started on an inhaled corticosteroid for four weeks and then BDR was measured at the randomization visit. Subjects were later randomized to fluticasone, montelukast, or fluticasone/salmeterol combination therapy.

In both populations, BDR was based on prebronchodilator and postbronchodilator measurements. After two puffs (180 ug/puff) of albuterol by metered dose inhaler with spacer were administered, at least 10 minutes elapsed before the postbronchodilator spirometry was performed. BDR was calculated as the percent difference between the prebronchodilator and postbronchodilator FEV1 value [BDR=100 × (post FEV1-preFEV1/ preFEV1)]. Institutional Review Board approval was obtained at each institution. Informed consent was obtained for all study participants.

Genotyping and Quality Control

Genome-wide SNP genotyping was performed on the CAMP sample using Illumina's HumanHap550 Genotyping BeadChip by Illumina, Inc (Illumina, Inc., San Diego, CA) or Illumina's Infinium HD Human610-Quad BeadChip at the Channing Laboratory. Before frequency and genotyping pruning, there were 516,512 SNPs. SNPs were excluded for missing in more than 5% of subjects (n=26,070), having MAF less than 5% (n=41,621), having Hardy-Weinberg equilibrium p-values among controls less than 0.001 (n=547). After frequency and genotyping pruning, there were 449,540 SNPs. The average genotyping completion rate for each subject was 99.8%.

Genotyping of LOCCS was conducted at the Riken Center for Genomic Medicine using the Illumina Infinium HD Human610-Quad BeadChip. We studied 12 SNPs that were identified from the screening stage in CAMP in the LOCCS population. The average genotyping completion rate for each subject was 99.7%.

Statistical Methodology

We performed a genome-wide study to examine the interaction of genetic variants and treatment with inhaled corticosteroids on bronchodilator response. In the CAMP population, we conducted a gene by environment analysis in PLINK[12] with inhaled corticosteroid

treatment as the environmental exposure and bronchodilator response (BDR) as the outcome measure using genome-wide data. SNPs were excluded from the analysis if they were missing in more than 5% of subjects, having minor allele frequency (MAF) less than 5%, having Hardy-Weinberg equilibrium p-values among controls less than 0.001. We then replicated our top SNPs that had P-value <1E-05 in LOCCS. We calculated combined P-values for CAMP and LOCCS using Fisher's Method.[15]. We adjusted for age and gender in our analyses. We conducted a stratified analysis of ICS and placebo groups using genetic linear association analysis with BDR as the outcome and adjusting for age and gender. Local association plots were created using LocusZoom (http://csg.sph.umich.edu/locuszoom).

Expression Quantitative Trait Analysis

To identify expression quantitative trait loci (eQTL) in response to ICS treatment, lymphoblastoid cell lines derived from 151 CAMP participants were treated with 10^{-6} M dexamethasone (a corticosteroid). After six hours, expression levels were measured using the Illumina HumanRef8 v2 BeadChip (Illumina, San Diego, CA). After arrays and probes filtering, vst transformation, and quantile normalization, 21,175 gene probes were kept. We limited eQTL analysis to 117 Caucasian individuals who were randomized to ICS treatment in the CAMP clinical trial and for whom GWAS genotyping data was available.

To detect SNPs associated to the variations of gene expression levels, we applied a general linear model (GLM), in which the predictor is the genotype of a SNP using additive coding and the outcome variable is the pre-processed expression level of a gene probe from dexamethasone-treated cell lines. For each gene probe, we considered all SNPs within 50 KB of both end of the gene in the cis-eQTL analysis.

RESULTS

Descriptive Statistics

Table I provides baseline demographic characteristics measured in our study population. Our initial study population included a total of 808 Caucasian subjects, including 581 Caucasian subjects from CAMP. The replication population included 227 Caucasian subjects from LOCCS who had available genotype information. The mean age was 8.87 years [SD 2.14 years] in CAMP and 40.8 years [14.1 SD] in LOCCS. Sixty percent of the CAMP population was male while 37% of the LOCCS population was male. The mean bronchodilator response while on inhaled corticosteroids was 8.7% [SD 8.1%] in CAMP and 6.5% [SD 6.8%] in LOCCS.

Gene by Environment Analysis

The corresponding quantile-quantile (Q-Q) plot (Figure 1 in Online Repository) demonstrates that the SNPs with the lowest P-values deviate from what is expected for a null distribution, suggesting that some may reflect true associations with bronchodilator response that are modulated by exposure to inhaled corticosteroids. The genomic inflation factor was 1.03, demonstrating minimal population stratification. The Manhattan plot (Figure 1) for this analysis shows that the regions of SNPs that are most significantly associated with bronchodilator response while accounting for inhaled corticosteroid treatment are in chromosomes 19 and 8.

Table II shows the top SNPs (gene by environment interaction P-value < 1E-05) in CAMP and LOCCS. We attempted to replicate these 12 SNPs in the LOCCS population. The strongest associations are on chromosome 19. One combined P-value for the CAMP and LOCCS populations reached genome-wide significance: 4.81×10^{-8} for rs3752120, which was in linkage disequilibrium with rs3450 (R^2=0.82 in CAMP and 0.75 in LOCCS,

combined P=7.56 × 10^{-9}) and rs12460587 (R^2=0.84 in CAMP and 0.84 in LOCCS, combined P=2.43 × 10^{-9}). These three SNPs are in or near *ZNF432* gene.

Figure 2 depicts the effect of treatment with ICS versus no ICS and genotype on the outcome of BDR for rs3752120. The x-axis shows the number of genotypes. There are 3 possibilities for the number of copies of rs3752120: 0 copy, 1 copy, or 2 copies. The Y-axis shows the BDR. This figure demonstrates that having two copies of the mutant allele and not being treated with ICS produces a higher BDR than having two mutant alleles and being treated with ICS. In addition, rs2288884 is located near a second gene on chromosome 19, *ZNF614* (combined P=5.14 ×10^{-8}) and rs11666341 is located near *ZNF841* (combined P=1.42 × 10^{-9}). Figure 3 depicts a plot of the regional association results from our genome-wide association study on chromosome 19, in the region of rs10411428. The plot demonstrates the magnitude of association of SNPs in this region in addition to the pairwise linkage disequilibrium patterns associated with rs10411428. Multiple SNPs in this region are in linkage disequilibrium and are associated with bronchodilator response while modulated by ICS.

We also conducted individual regression models for BDR as an outcome for the ICS group alone and for the placebo group alone, while adjusting for age and gender. Our results are depicted in Table III and show that the beta estimates are in opposite directions for the ICS and placebo groups, suggesting that ICS modulates the effect of SNPs on bronchodilator response in a direction distinct from placebo. Analysis of microarray data from lymphoblastoid cell lines from a subset of CAMP subjects determined that the variant rs11666341 is associated with variable gene expression of *ZNF432* (p=0.046). Results are presented in Table 1 of the Online Repository. Cells from subjects who were homozygous for the major allele, rs11666341, had lower expression levels under dexamethasone-treated conditions (Figure 2 in Online Repository).

DISCUSSION

Our study has several key findings. Treatment with inhaled corticosteroids appears to modify the effect of SNPs on bronchodilator response. Our analysis, was conducted in a pediatric population, and replicated in an adult population, suggesting that our results are generalizable across age groups. Secondly, we have identified a region of association on chromosome 19 that contains multiple zinc finger protein genes. Variation in this region could influence the effect of inhaled steroids on bronchodilator response. Finally, synergistic effects observed between inhaled corticosteroids and β_2 agonists caused by specific genes.

Previous studies have suggested that the anti-inflammatory effects of corticosteroids increase airway response to β_2 agonists by up-regulating β_2 adrenergic receptor expression and increasing cyclic AMP production by airway epithelial cells. [2,16,17] This leads to the synergistic effect that steroids have on bronchodilator response to β_2 agonists.[2,16,17] A study by Jin et al assessed for effect modification by use of inhaled corticosteroids and examined whether SNPs in the dual-specificity phosphatase 1, *DUSP1* gene, which are associated with BDR, are modified by concurrent use of ICS medication. The authors found that the *DUSP1* polymorphisms did modulate the effect between ICS use and BDR.[18] Thus, our study is consistent with previous studies in suggesting that genetic factors could mediate the relationship between inhaled corticosteroid use and BDR. The clinical implications of a variant that predicts BDR while on ICS are unclear. Patients with a variant that leads to higher BDR while on ICS may benefit by preserving or restoring smooth muscle function; on the other hand, patients with the variant may need to avoid ICS because a higher BDR while on ICS may signify the ICS is not working. Further in vitro and in vivo studies are necessary to elucidate the clinical implications of our results.

We have identified a region on chromosome 19 that appears to influence the effect of inhaled steroids on asthma. We focused on the gene that coincides with the peak, the zinc finger protein gene, *ZNF432*, which is located on chromosome 19. The results from the eQTL analysis suggested that the variant rs11666341 is associated with variable gene expression of *ZNF432*, further supporting the finding that *ZNF432* modulates the effect of inhaled corticosteroids in adults and children with asthma. Cells from subjects who were homozygous for the major allele had lower expression levels under dexamethasone-treated conditions, supporting our hypothesis that this variant modulates bronchodilator response. While the function of *ZNF432* is unknown, other zinc fingers have been found to play a role in asthma. For example, Zfp35 (*ZNF271*) appears to influence the pathogenesis of airway inflammation and hyperresponsiveness in asthma by controlling Th2 cell generation and Th2cytokine expression.[19] Many zinc finger proteins have been demonstrated to be involved in transcription.[20]*ZNF432* has been found to be associated with inflammatory bowel disease, at $P= 8.3 \times 10^{-5}$.[21] Thus, further study of these zinc finger proteins may provide insight on the mechanisms by which inhaled corticosteroids influence bronchodilator response.

Despite the strengths of our study, a few limitations deserve mention. First, our study is relatively small, with only 808 subjects in total. However, we did not have access to data for larger populations because few pharmacogenetic trials have genetic data on subjects who are taking both inhaled corticosteroids and have short acting bronchodilator response measurements. We relied on patient report of inhaled corticosteroid use in our replication population. It is possible that subjects who are randomized to an inhaled corticosteroid group in a clinical trial such as CAMP may be more likely to be taking inhaled corticosteroids than subjects who report taking inhaled corticosteroids that are prescribed by their physicians during the run-in period of a trial such as LOCCS. In neither trial is ICS use directly monitored. Furthermore, we were unable to conduct functional studies to study whether corticosteroids modulate the zinc finger proteins we identified; nevertheless the eQTL analyses do support our findings.

In conclusion, inhaled corticosteroids appear to influence the effect of genetic information on bronchodilator response. A zinc finger protein, *ZNF432*, appears to modulate the effect on inhaled corticosteroids on bronchodilator response in adults and children with asthma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

AC9	adenylate cyclase 9

ADRB2	β_2-adrenergic receptor
BDR	bronchodilator response
CAMP	Childhood Asthma Management Program
CRHR1	corticotrophin-releasing hormone receptor 1
ICS	inhaled corticosteroid
eQTL	expression quantitative trait loci
Q-Q	quantile-quantile
LOCCS	Leukotriene Modifier Or Corticosteroid or Corticosteroid-Salmeterol Trial
SNP	single nucleotide polymorphism
SPATS2L	spermatogenesis associated serine-rich 2-like gene

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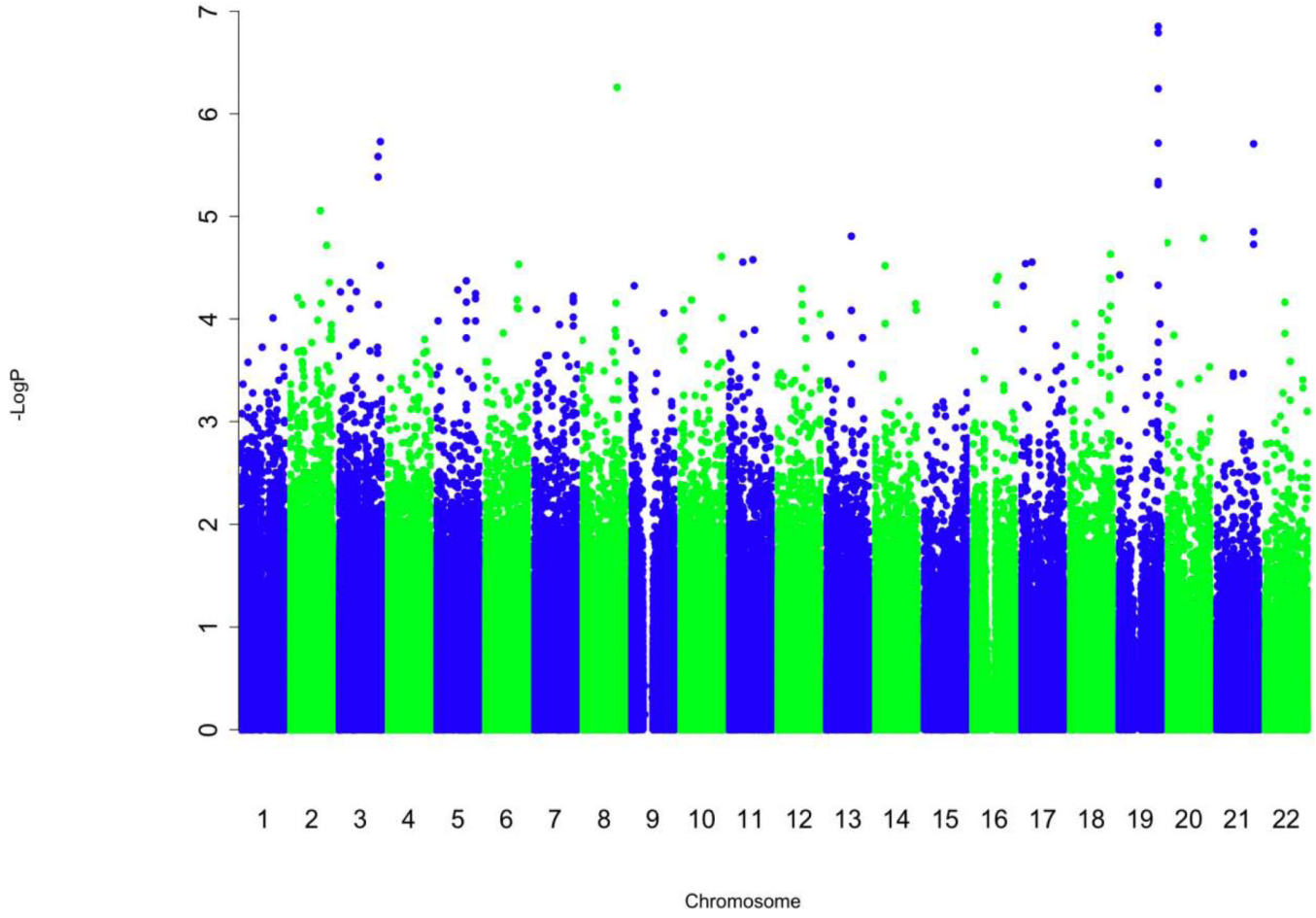

Figure 1.
Manhattan Plot of –log10 (P-value) for the analysis in CAMP. This figure demonstrates that multiple SNPs in chromosome 19 may be associated with bronchodilator response and modulated by inhaled corticosteroid treatment.

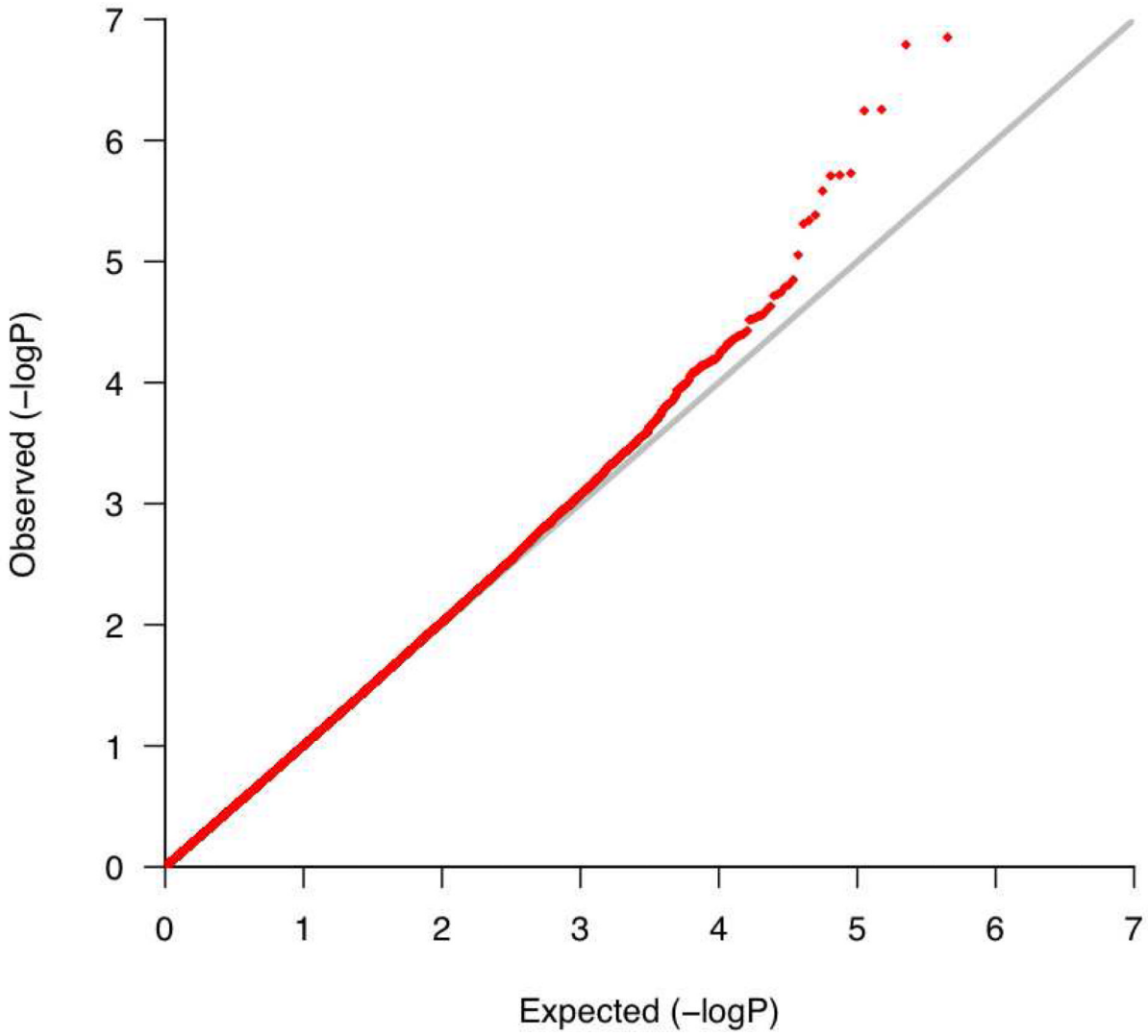

Figure 2.

Depiction of the effect of treatment with inhaled corticosteroids (ICS) versus no ICS and genotype on the outcome of bronchodilator response (BDR) for rs3752120. The x-axis shows the number of genotypes. There are 3 possibilities for the number of copies of rs3752120: 0 copy, 1 copy, or 2 copies. Y axis shows the BDR. This figure demonstrates that having two copies of the mutant allele and not being treated with ICS produces a higher BDR than having two mutant alleles and being treated with ICS.

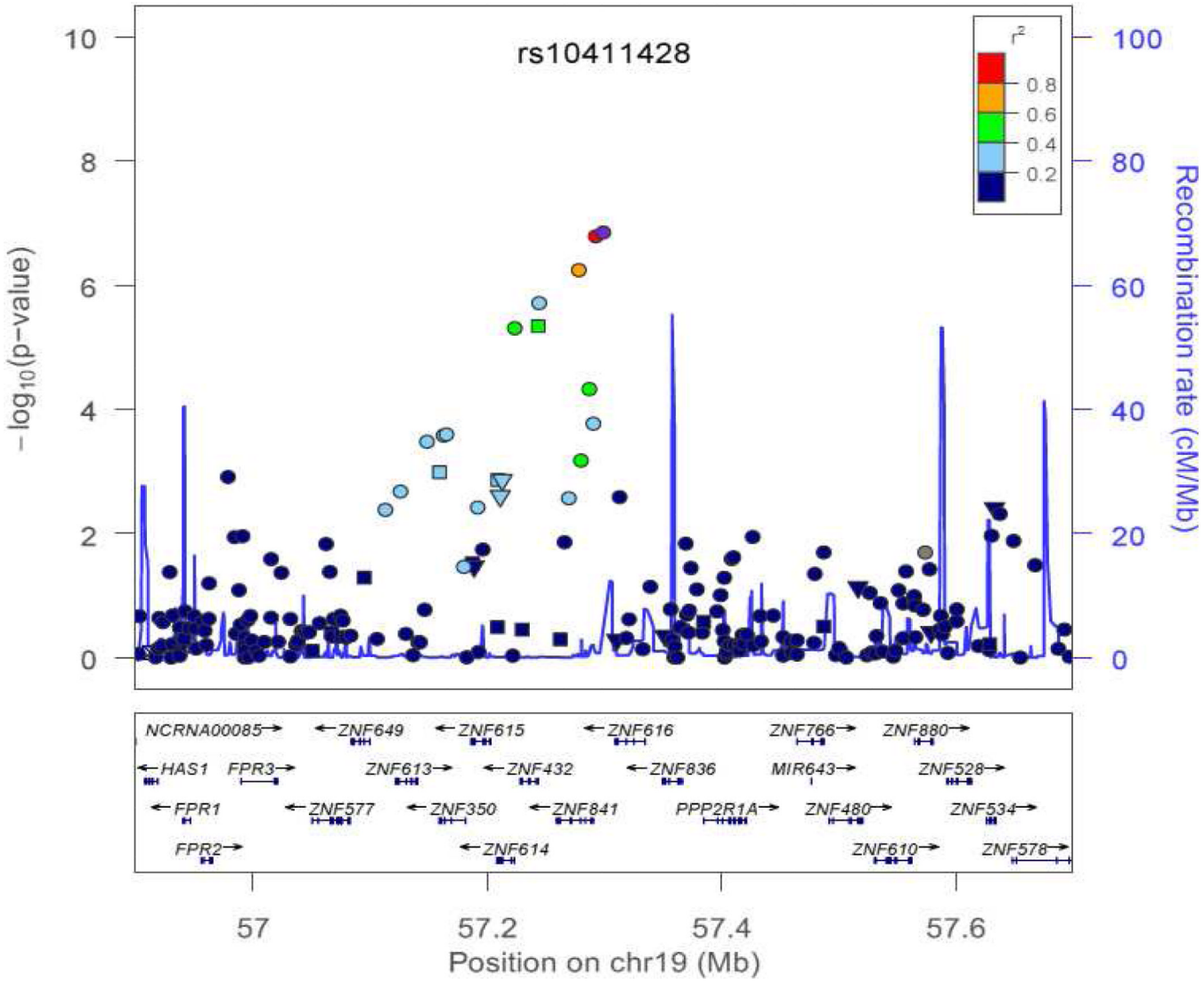

Figure 3.

Region of association near rs10411428 to bronchodilator response while modulated by treatment with inhaled corticosteroids. The x-axis denotes position along chromosome 19. The y-axis denotes –Log10 (P) corresponding to 1000GP imputed data P-values between the SNP, rs10411428, to each SNP in the plot is denoted in colors and was computed according to 1000GP June 2010 CEU data. Plot was created using LocusZoom.[22]

Table I

Baseline demographics of the CAMP and LOCCS populations.

N=808 **Mean [SD] or Percent (n)**	**CAMP (n=581)**	**LOCCS (n=228)**
Age in years [SD] (range)	8.9 [2.1] (5.2−13.2)	40.8 (14.1) (15−76)
Treatment group		
Budesonide (ICS)	172 (30%)	76 (33%) Fluticasone (ICS)
Nedocromil	171 (29%)	83 (36%) Fluticasone/salmeterol
Placebo	238 (41%)	69 (30%) Montelukast
Gender,		
Male	347 (60%)	85 (37%)
Female	243 (40%)	143 (63%)
PreFEV$_1$ at follow up,	1.91 [0.53]	2.89 [0.78]
Bronchodilator Response at follow up	0.097 [0.081]	0.065 [0.068]
FEV$_1$ percent predicted at follow up	95.68% [13.56%]	88% [16.7%]

Table II

Results of Gene by Environment Analysis.

CHR	SNP	Beta Estimate for SNP	SE	Beta Estimate for ICS Exposure	SE2	MAF	HWE p value	A1	A2	CAMP P_GXE	LOCCS P_GXE	Combined P_GXE
19	rs10411428	0.042	0.0099	−0.027	0.0087	0.41	0.17	T	C	$1.41x10^{-7}$	0.00036	$1.24x10^{-9}$
19	rs11666341	0.042	0.0098	−0.027	0.0087	0.45	0.17	A	G	$1.62x10^{-7}$	0.00036	$1.42x10^{-9}$
19	rs12460587*	0.035	0.0097	−0.029	0.0085	0.33	0.22	G	T	$5.69x10^{-7}$	0.00018	$2.43x10^{-9}$
19	rs3450	0.031	0.0099	−0.030	0.0082	0.18	0.72	C	T	$1.93x10^{-6}$	0.00017	$7.56x10^{-9}$
19	rs3752120**	0.032	0.0107	−0.031	0.0090	0.15	0.21	T	C	$4.58x10^{-6}$	0.00050	$4.81x10^{-8}$
19	rs2288884	0.033	0.0107	−0.031	0.0090	0.34	0.40	T	C	$4.91x10^{-6}$	0.00050	$5.14x10^{-8}$
8	rs6469488	0.072	0.0170	−0.032	0.0127	0.07	0.79	G	A	$5.54x10^{-7}$	0.77	$6.70x10^{-6}$
21	rs4919929	0.062	0.0153	−0.031	0.0122	0.07	0.42	T	C	$1.96x10^{-6}$	0.79	$2.22x10^{-5}$
3	rs9868563	0.099	0.0190	−0.012	0.0138	0.06	0.10	T	C	$2.62x10^{-6}$	0.60	$2.25x10^{-5}$
3	rs4686399	0.041	0.0094	−0.015	0.0068	0.29	0.41	G	A	$1.87x10^{-6}$	0.87	$2.33x10^{-5}$
3	rs1889261	0.084	0.0180	−0.018	0.0134	0.06	0.76	A	G	$4.14x10^{-6}$	0.45	$2.66x10^{-5}$
2	rs4233808	0.041	0.0100	−0.019	0.0089	0.16	0.69	G	A	$8.84x10^{-6}$	0.72	$8.20x10^{-5}$

This table summarizes the results of testing and replication in CAMP and LOCCS using the additive model sorted by combined population-based P values. The beta estimate for each SNP and for ICS exposure are provided. CHR=chromosome. SNP=single nucleotide polymorphism. SE= standard error. ICS=inhaled corticosteroid.

* located in *ZNF841*.

** located in *ZNF432*.

Table III

Individual regression models for bronchodilator as an outcome stratified by ICS or placebo groups in CAMP.

	ICS group (n=172)		Placebo group (n=409)	
SNP	**Beta-estimate**	**P**	**Beta-estimate**	**P**
rs4233808	0.041	8.25E−05	−0.020	0.026
rs9868563	0.099	7.13E−07	−0.012	0.37
rs1889261	0.084	5.98E−06	−0.018	0.19
rs4686399	0.04	3.28E−05	−0.014	0.040
rs6469488	0.072	2.71E−05	−0.032	0.012
rs2288884	0.032	0.0032	−0.030	0.00096
rs3752120	0.033	0.0030	−0.030	0.00096
rs3450	0.031	0.0019	−0.029	0.00047
rs12460587	0.035	0.00041	−0.028	0.0012
rs11666341	0.042	3.86E−05	−0.025	0.0042
rs10411428	0.042	4.12E−05	−0.026	0.0034
rs4919929	0.061	0.00011	−0.032	0.0094