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Genetic predictors associated with improvement of asthma symptoms in response to inhaled corticosteroids

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Abstract

Background—To date, genome-wide association studies (GWASs) of inhaled corticosteroid (ICS) response in asthmatic patients have focused primarily on lung function and exacerbations.

Objective—We hypothesized that GWAS analysis could identify novel genetic markers predicting a symptomatic response to ICSs.

Methods—We analyzed differences in asthma symptoms in response to ICSs in 124 white children from the Childhood Asthma Management Program (CAMP) trial using scores from diary cards. Of the 440,862 single nucleotide polymorphisms (SNPs) analyzed, the top 100 ranked SNPs were pursued for replication initially in subjects from the pediatric Childhood Asthma Research and Education trials (77 white children) and then in subjects from the adult Asthma Clinical

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Research Network (110 white adults) and Leukotriene Modifier or Corticosteroid or Corticosteroid-Salmeterol trials (110 white adults).

Results—The lowest *P* value for GWAS analysis in the CAMP trial was 8.94×10^{-8} (rs2388639). Of the 60 SNPs available in the Childhood Asthma Research and Education Network trials, rs1558726 (combined $P = 1.02 \times 10^{-5}$), rs2388639 (combined $P = 8.56 \times 10^{-9}$), and rs10044254 (combined $P = 9.16 \times 10^{-8}$) independently replicated. However, these 3 SNPs were not additionally replicated in the adult asthmatic patients of the remaining trials. rs10044254 lies in the intronic region of F-box and leucine-rich repeat protein 7 (*FBXL7*) and is associated with decreased expression in immortalized B cells derived from CAMP participants.

Conclusions—We have identified a novel SNP, rs10044254, associated with both decreased expression of *FBXL7* and improved symptomatic response to ICSs in 2 independent pediatric cohorts. Our results suggest that there might be a specific genetic mechanism regulating symptomatic response to ICSs in children that does not carry over to adults.

Keywords

Asthma; child; glucocorticoid; pharmacogenomics; polymorphism

Asthma, a chronic airway inflammatory disease, is an important cause of morbidity and mortality worldwide.[1] Current guidelines recommend inhaled corticosteroid (ICS) treatment for the management of asthma.[2-4] The superior effectiveness of ICSs includes improvements in lung function, an increase in the number of symptom-free days, and reductions in exacerbations and hospitalizations.[2-5] Despite their general effectiveness, there is high interindividual variation in response to ICS treatment in asthmatic patients.[6,7] Using pharmacogenomic approaches, several investigators have identified promising candidate genes associated with response to ICSs.[8-13]

Recent advances have increased understanding of the complex nature of asthma characterized by asthma symptoms, variable airway obstruction, and airway hyperresponsiveness. The complexity of asthma suggests that there might be multiple biological pathways involving different genes. For example, we found that genetic predictors of a poor long-term response to ICSs differed markedly depending on the definition of outcome (exacerbation vs lung function).[14]

To date, pharmacogenomics studies of ICS response in asthmatic patients have focused primarily on identifying genes and single nucleotide polymorphisms (SNPs) associated with physiologic measures, including lung function,[8-10] and indirect measures, such as exacerbations.[11,12] Traditional measures (eg, self-reported symptoms) are important to diagnose and monitor response to asthma treatment.[2,3,15,16] However, there have been few pharmacogenomic studies focusing on self-reported asthma symptoms,[17,18] although self-reported asthma symptoms account for a substantial proportion of the clinical measures of treatment response.[19,20] Therefore we performed a genome-wide association study (GWAS) with the hypothesis that we could identify novel genetic markers predicting symptomatic response to ICSs in asthmatic patients. We initially tested our hypothesis by conducting a GWAS in white children randomly assigned to ICSs in the Childhood Asthma Management

Program (CAMP) trial.[21] Then we tested associations of the highest-powered SNPs in 3 independent populations drawn from the Childhood Asthma Research and Education (CARE) Network trials,[7,22] the Asthma Clinical Research Network (ACRN) trials,[23-25] and the Leukotriene Modifier or Corticosteroid or Corticosteroid-Salmeterol (LOCCS) trial (by the American Lung Association's Asthma Clinical Research Centers).[26]

Methods

Each study was approved by the institutional review board of the corresponding institution, and informed consent was obtained from all study participants. Detailed methods are described in the Methods section in this article's Online Repository at www.jacionline.org.

Study population and phenotyping

The primary group of subjects consisted of white children from the CAMP trial. For the replication analysis, white children enrolled in 2 of 5 CARE trials and white adults from 3 of 6 ACRN trials and an arm of the LOCCS trial with ICS monotherapy were included. For each day of the study, all participants were asked to rate and score their asthma symptoms during the past 24 hours on a diary card. Similar questions were used, and the symptom scores ranged from 0 (absent) to 3 (severe) in all trials. The change in asthma symptom scores from baseline was defined as follows:

Average symptom score of the last week on ICS treatment – Average symptom score of 1 week before ICS treatment start.

Participants whose symptom scores were available at least 4 days in every week of the trials were included in the present study. Detailed characteristics of each of the clinical trials and the phenotyping methods are described in the Methods section in this article's Online Repository.

Genotyping

CAMP subjects were genotyped on the HumanHap550v3 BeadChip or Infinium HD Human610-Quad BeadChip (Illumina, San Diego, Calif), whereas the CARE and ACRN subjects were genotyped on the Affymetrix 6.0 chip (Affymetrix, Santa Clara, Calif) as part of the National Heart, Lung, and Blood Institute's Share Asthma Resource Project (http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000166.v1.p1). LOCCS subjects were genotyped on the Infinium HD Human610-Quad BeadChip (Illumina). All SNPs that were included in the GWAS had a completion rate of greater than 95%, a minor allele frequency (MAF) of greater than 0.05, and a Hardy-Weinberg equilibrium (HWE) *P* value of greater than .0001. Complete genotype information was available for a total number of 421 subjects from all study cohorts (124 from CAMP, 77 from CARE, 110 from ACRN, and 110 from LOCCS).

Functional assessment

We evaluated relationships between rs10044254 and dexamethasone-mediated changes in F-box and leucine-rich repeat protein 7 (*FBXL7*) gene expression in immortalized B-cell lines

derived from 70 of 124 CAMP subjects. Expression profiles were measured after stimulation for 6 hours with 10^{-6} mol/L dexamethasone or a sham treatment with the use of the HumanRef-8v2 BeadChip, as previously detailed.[9] Data adjusted for background were log transformed and then underwent variance stabilization and normalization.

Statistical analysis

The association of SNPs with changes in asthma symptom scores was measured with a linear regression model, as implemented in PLINK,[27] by using 3 different genetic models (additive, dominant, and recessive). The regression models were adjusted for age, sex, baseline symptom scores, and 4 significant principal components. SNPs were considered to have significant associations if they possessed a nominal *P* value of less than .05. For these SNPs, a combined *P* value was calculated from the 1-sided *P* values of the replication populations by using the Stouffer z-transform test[28] with R (version 2.15.2) software (www.r-project.org).

Results

Table I summarizes the characteristics of screening and replication populations. In each trial the average asthma symptom score significantly decreased after ICS treatment for 4 to 8 weeks. However, the large SDs in each population suggested a wide individual variability in response. The genomic inflation factor for the CAMP, CARE, ACRN, and LOCCS subjects was 1.001, 1.000, 1.000, and 1.058, respectively, suggesting minimal population stratification (see Fig E1 in this article's Online Repository at www.jacionline.org). A primary GWAS of the change in asthma symptom scores related to ICS treatment was performed on 440,862 SNPs in the pediatric CAMP subjects. Of the top 100 SNPs (ranked by *P* values in CAMP) from the 3 different genetic models, 60 SNPs had been genotyped in the pediatric CARE cohorts and then were tested for replication. Table II shows the 3 SNPs (rs1558726, rs2388639, and rs10044254 from CAMP) that were also significantly associated with changes in asthma symptom scores in the pediatric CARE subjects. These SNPs were obtained with the same genetic model. The combined *P* values of rs2388639 and rs10044254 for the pediatric CAMP and CARE subjects were 8.56×10^{-9} and 9.12×10^{-8}, respectively, which meet the threshold for conventional genome-wide significance. However, we were unable to replicate these significant associations in the adult cohorts (ACRN and LOCCS subjects).

The SNP rs10044254 lies in the intronic region of the *FBXL7* gene (Entrez Gene ID: 23,194), whereas rs1558726 and rs2388639 lie within intergenic regions of chromosomes 12 and 16, respectively. For rs10044254, subjects in the CAMP trial who carried 2 variant alleles (n = 3; median, 1.14; interquartile range [IQR], 1.08-1.28) showed increases in asthma symptom scores even after ICS treatment, whereas subjects who were homozygous for the reference allele (n = 73; median, −0.28; IQR, −0.57 to 0) showed decreases (Fig 1). For the CARE subjects, those who were homozygous for the variant allele (n = 5; median, 0.28; IQR, 0-0.36) versus the reference allele (n = 48; median, −0.26; IQR, −0.57 to 0.11) showed the same trend (Fig 1). In summary, subjects in the CAMP and CARE trials who carried 2 variant alleles of rs10044254 showed significantly poorer symptom responses to

ICS treatment compared with subjects homozygous or heterozygous for the reference allele. Immortalized B cells derived from subjects with the variant allele showed a significantly lower *FBXL7* expression in response to dexamethasone treatment (median, −0.03; IQR, −0.08 to 0.01) compared with those from subjects with the reference allele (median, −0.01; IQR, −0.04 to 0.03; $P = .048$, allelic model; Fig 2). *FBXL7* expression measured in immortalized B-cell lines after stimulation with dexamethasone showed a trend toward positive correlation with asthma symptom score improvement after ICS treatment but did not reach statistical significance (data not shown).

For rs1558726 and rs2388639, subjects who were homozygous for the variant alleles also showed poorer symptom responses to ICS treatment compared with subjects with the other genotypes in both the CAMP and CARE trials (see Fig E2 in this article's Online Repository at www.jacionline.org). However, those 2 SNPs did not affect dexamethasone-induced expression of genes adjacent to them in immortalized B-cell lines (data not shown).

Discussion

In this study we identified 60 SNPs that were significantly associated with changes in asthma symptom scores after administration of ICSs in the pediatric CAMP subjects, of which 3 SNPs were replicated in an independent cohort of pediatric asthmatic patients. Despite the replication in children, these SNPs were not associated in adults. Two of the 3 SNPs achieved genome-wide significance. Notably, despite an overall improvement in symptoms while taking ICS medications, subjects with 2 copies of the variant alleles for rs10044254, located within *FBXL7*, showed worsening of their symptoms. We confirmed the potential functional relevance of rs10044254 by demonstrating that this SNP is associated with changes in dexamethasone-induced *FBXL7* expression in immortalized B cells derived from the CAMP subjects in whom the association study was performed.

Previous studies on pediatric and adult asthmatic patients have shown that the perception of asthma symptoms (eg, dyspnea, wheezing, and cough) might be influenced by age, sex, asthma severity, and use of medication.[29-31] To date, no study has evaluated differences in the perception of asthma symptoms in pediatric and adult asthmatic patients. However, for patients with childhood asthma, it was reported that adolescents (13-18 years) were more accurate in perceiving symptoms than school-age children (6-12 years),[32] and the accuracy for perceiving symptoms increased with age in children age 7 to 17 years.[33] These findings showing that perception of asthma symptoms increases with age suggest that perceived symptomatic response to ICS treatment is also likely to differ between pediatric and adult asthmatic patients. In the present GWAS we found 3 SNPs showing significant associations with improvements in asthma symptom scores responding to ICSs in children from both the CAMP and CARE trials. However, these associations were not further replicated in adults from the ACRN and LOCCS trials, which suggest that there might be a specific genetic mechanism regulating symptom response in children that does not carry over to adults. Interestingly, exhaled nitric oxide (eNO) as an associated biomarker of pulmonary response to ICSs showed a similar phenomenon.[34] eNO is a predictor of ICS response in a clinical trial with pediatric asthmatic patients (Characterizing Response to Leukotriene Receptor Antagonist and Inhaled Corticosteroids [CLIC] study)[7] but not in a clinical trial with adult

asthmatic patients (Predicting Response to Inhaled Corticosteroid Efficacy [PRICE] study).[23] Accordingly, measurements of eNO before beginning treatment might be useful in predicting response to ICS therapy in pediatric but not in adult asthmatic patients.

Two of the 3 associated SNPs were located in intergenic regions. However, the third SNP, rs10044254, was located within the *FBXL7* gene. *FBXL7* encodes for a member of the F-box protein family, which constitutes one of the 4 subunits of ubiquitin protein ligase complex called SKP1-cullin-F-box.[35,36] SKP1-cullin-F-box belongs to the families of E3 ubiquitination ligases and is involved in linking ubiquitin chains to target proteins.[35,37] The ubiquitin proteolytic pathway is responsible for the degradation of most intracellular proteins, including membrane-surface receptors.[38] There are 2 possible mechanisms for *FBXL7* in the pathogenesis of asthma.

First, F-box protein might abrogate airway inflammation by facilitating degradation of cytokine receptors.[38] Notably, it was reported that F-box protein FBXL19–mediated ubiquitination and degradation of the receptor for IL-33 limited pulmonary inflammation.[39]

Second, F-box protein might be involved in dyspnea perception through degradation of the a subunit of hypoxia-inducible factor 1 (HIF),[38] which is negatively regulated by an FBW7-mediated degradation pathway during hypoxia.[40] To further explore the potential effects of *FBXL7* on HIF-1α regulation, we conducted a pathway analysis specifically focusing on potential *FBXL7-HIF1A* interactions using the program GeneMania (http://genemania.org/; see Fig E3 in this article's Online Repository at www.jacionline.org). Although inconclusive, the results indicate that *FBXL7* is coexpressed with *HIF1A* and therefore might directly or indirectly interact with HIF-1α. The regulation of HIF-1α by *FBXL7* might partly account for child-specific symptomatic responses because it was suggested that dyspnea perception might be influenced by age, like pain perception.[41] In addition, ubiquitination of inducible nitric oxide synthase was required for its degradation,[42] and FBXO45, a member of the F-box protein family, was identified as a novel and direct nitric oxide synthase 2 interactor in human airway epithelial cells.[43] Thus F-box protein might also contribute to the different role of eNO in predicting ICS response between child and adult asthmatic patients.

There are known differences in symptoms, lung function, and exacerbations, and these factors, although loosely correlated, do not strongly predict one another.[44-47] This is also a primary reason that symptoms, lung function, and exacerbation are all independent metrics of asthma control in the current asthma guidelines.[2,3] It is reasonable to expect that different genes will control response to medications for each of these components. Therefore it is not surprising that *FBXL7* was not found in the previous studies[8-13] interrogating the genetic mechanisms underlying asthma exacerbations or lung function in response to ICSs.

Our study has several limitations. First, our genotyping platforms differ among the 4 cohorts, and SNPs genotyped on the Illumina and Affymetrix platforms overlap poorly, thereby increasing the likelihood that SNPs that were significantly associated with symptomatic response to ICSs were not genotyped across the cohorts. To partially overcome this limitation, we could have performed 1000 Genomes imputation of our SNP data set, which was limited to 440,862 markers. Although we are likely to find additional

significantly replicated SNPs from the imputed genotype data, we were able to replicate 3 SNPs, 2 of which achieved genome-wide significance, without imputation. Furthermore, the probabilistic nature of imputed SNPs presents challenges when testing for association of those SNPs.[48]

Second, for children in the CAMP and CARE trials, completion of diary card data required parental participation, thereby introducing an additional source of potential error into the measurement of the self-reported symptoms. For children between the ages of 3 and 7 years, parents tend to underreport their children's asthma symptoms.[49] However, because the mean ages of enrolled children in the CAMP and CARE trials were 8 and 10 years, communication with their parents regarding asthma symptoms likely was not a confounder among this age group.

Finally, although our results would suggest that rs10044254 is an expression quantitative trait locus for *FBXL7* and might therefore represent a functional SNP, further mechanistic studies are needed to clarify the precise role of the SNP and the *FBXL7* in the response to ICS treatment.

In conclusion, we have identified 3 pharmacogenetic SNPs, including one of potentially functional significance, that are associated with an improvement in childhood asthma symptoms in response to ICSs. Our findings have special significance given that genetic variants are one of the promising biomarkers toward personalized care for children with asthma, as reviewed elsewhere.[50] Additional work, including investigations into possible adult-specific loci, is warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ACRN	Asthma Clinical Research Network
CAMP	Childhood Asthma Management Program
CARE	Childhood Asthma Research and Education network
CLIC	Characterizing Response to Leukotriene Receptor Antagonist and Inhaled Corticosteroids
eNO	Exhaled nitric oxide
FBXL7	F-box and leucine-rich repeat protein 7
GWAS	Genome-wide association study
HIF	Hypoxia-inducible factor
HWE	Hardy-Weinberg equilibrium
ICS	Inhaled corticosteroid

IQR	Interquartile range
LOCCS	Leukotriene Modifier or Corticosteroid or Corticosteroid-Salmeterol
MAF	Minor allele frequency
PACT	Pediatric Asthma Controller Trial
PRICE	Predicting Response to Inhaled Corticosteroid Efficacy
SNP	Single nucleotide polymorphism
SOCS	Salmeterol or Corticosteroids Study

Clinical implications: Our results suggest that there might be a specific genetic mechanism regulating the symptomatic response to ICSs in children that does not carry over to adults.

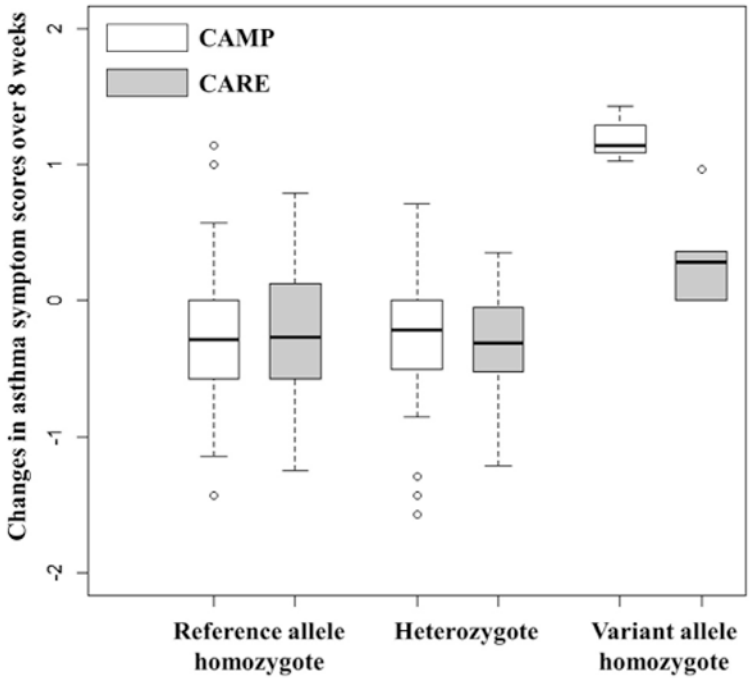

Fig 1.

Changes in asthma symptom scores after ICS treatment in the 2 pediatric asthma populations (CAMP and CARE) genotyped for rs10044254 and stratified by genotype status. Subjects in the CAMP and CARE trials who carried 2 variant alleles showed increases in asthma symptom scores after ICS treatment, whereas subjects that were homozygous for the reference allele and heterozygous showed decreases. Data represent medians (IQRs), and *small circles* represent subjects who were outliers from the first and the third quartiles.

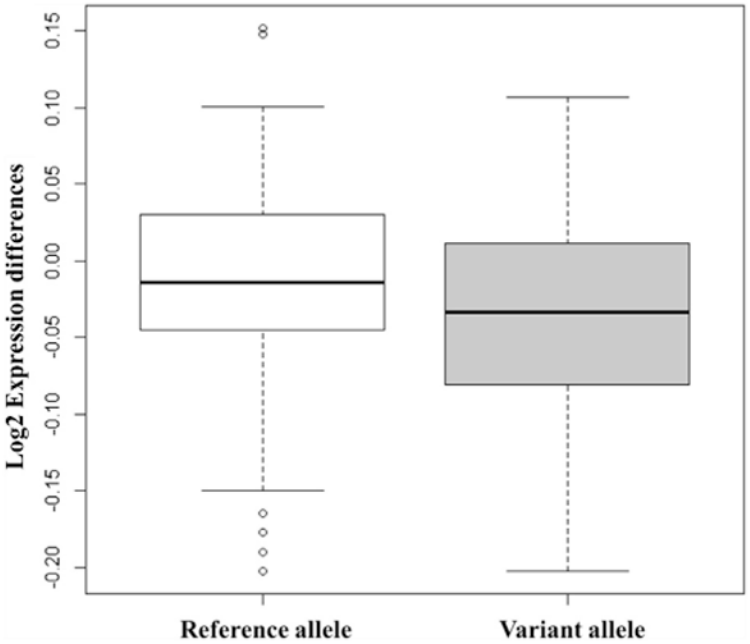

Fig 2.

FBXL7 gene expression in response to dexamethasone stimulation in immortalized B cells of the CAMP subject stratified by rs10044254 genotype. *Reference* represents expression data of B cells from the subjects with the reference allele, and *Variant* indicates expression data of B cells from the subjects with the variant allele (allelic model). Data represent medians (IQRs), and *small circles* represent subjects who were outliers from the first and the third quartiles.

Table I
Characteristics of screening and replication cohorts

	CAMP	CARE	ACRN	LOCCS
No.	124	77	110	110
Age (y), mean ± SD	8.9 ± 2.2	10.4 ± 3.1	34.1 ± 11.8	33.2 ± 14.5
Sex (male)	58.0%	63.6%	39.1%	40.0%
Race (white)	100%	100%	100%	100%
Baseline symptom score, mean ± SD	0.69 ± 0.48	0.54 ± 0.37	0.42 ± 0.50	0.39 ± 0.47
Duration of ICS treatment (wk)	8	8	6	4
Mean symptom score changes after ICS, mean ± SD	−0.24 ± 0.55	−0.24 ± 0.47	−0.17 ± 0.36	−0.17 ± 0.36

Table II

SNPs significantly associated with mean change in asthma symptom scores after ICS treatment

	rs1558726		**rs2388639**		**rs10044254**	
MAF*	0.09		0.19		0.18	
Gene†	*RMST*		*LOC728792*		*FBXL7*	
Model	Additive		Recessive		Recessive	
	β	*P* value	β	*P* value	β	*P* value
CAMP	0.358	5.16×10^{-4}	0.878	4.50×10^{-7}	1.190	1.02×10^{-5}
CARE	0.305	2.63×10^{-3}	0.503	2.73×10^{-3}	0.532	1.24×10^{-3}
ACRN	0.063	.233	0.006	.481	0.011	.472
LOCCS	−0.059	.177	−0.186	.036	−0.053	.280
Combined *P* value‡		1.02×10^{-5}		8.56×10^{-9}		9.16×10^{-8}

*MAF in CAMP subjects.

†The nearest gene, except rs10044254: *RMST*, rhabdomyosarcoma 2 associated transcript; *LOC728792*, hypothetical LOC728792.

‡The combined association *P* values for the CAMP and CARE (pediatric asthma) populations using the Stouffer z-transform test.