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

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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 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. (J Allergy Clin Immunol 2014;133:664-9.)

Key words: *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

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Abbreviations used

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

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