Background: Variation in response to the most commonly used class of asthma controller medication, inhaled corticosteroids, presents a serious challenge in asthma management, particularly for steroid-resistant patients with little or no response to treatment.

Objective: We applied a systems biology approach to primary clinical and genomic data to identify and validate genes that modulate steroid response in asthmatic children.

Methods: We selected 104 inhaled corticosteroid–treated asthmatic non-Hispanic white children and determined a steroid responsiveness endophenotype (SRE) using observations of 6 clinical measures over 4 years. We modeled each subject’s cellular steroid response using data from a previously published study of immortalized lymphoblastoid cell lines under dexamethasone (DEX) and sham treatment. We integrated SRE with immortalized lymphoblastoid cell line DEX responses and genotypes to build a genome-scale network using the Reverse Engineering, Forward Simulation modeling framework, identifying 7 genes modulating SRE.

Results: Three of these genes were functionally validated by using a stable nuclear factor κ-light-chain-enhancer of activated B cells luciferase reporter in A549 human lung epithelial cells, IL-1β cytokine stimulation, and DEX treatment. By using small interfering RNA transfection, knockdown of family with sequence similarity 129 member A (FAM129A) produced a reduction in steroid treatment response (P < .001).

Conclusion: With this systems-based approach, we have shown that FAM129A is associated with variation in clinical asthma steroid responsiveness and that FAM129A modulates steroid responsiveness in lung epithelial cells. (J Allergy Clin Immunol 2018;142:1211-1221.)

Key words: Inhaled corticosteroids, steroid response endophenotype, genomics, FAM129A, Reverse Engineering, Forward Simulation modeling, systems biology

Asthma affects approximately 23 million persons in the United States and approximately 300 million persons worldwide. Inhaled corticosteroids (ICSs) are the most commonly prescribed medications to control asthma. However, not all patients with asthma respond to these treatments; some have symptoms that are well controlled by ICSs, whereas others require additional or alternative medications.

Although asthma is a heterogeneous disease, even among a relatively similar cohort of children with mild-to-moderate asthma, there are noticeable differences in the efficacy of ICSs at maintaining asthma control. Several genetic markers have been found to be associated with the efficacy of ICS therapy, such as glucocorticoid-induced transcript 1 (GLCCI1) and corticotropin-releasing hormone receptor 1 (CRHR1).

Although these variants have fairly large effect sizes, in the case of GLCCI1, a genome-wide association study (GWAS) indicated up to 6% of variation in response to ICS treatment, but attempts at clinical validation have produced mixed results. These findings suggest that additional genes might be important for determining ICS response in asthmatic patients.
Abbreviations used

CAMP: Childhood Asthma Management Program
DEX: Dexamethasone
EDARADD: Ectodysplasia A receptor–associated death domain
FAF1: FAS-associated factor 1
FAM129A: Family with sequence similarity 129 member A
FAM174B: Family with sequence similarity 174 member B
FICD: FIC domain containing
GLCCI1: Glucocorticoid-induced transcript 1
GWAS: Genome-wide association study
ICS: Inhaled corticosteroid
LCL: Immortalized lymphoblastoid cell line
MDS: Multidimensional scaling
NF-κB: Nuclear factor κ-light-chain-enhancer of activated B cells
QC: Quality control
REFS: Reverse engineering, forward simulation methodology
siRNA: Small interfering RNA
SNP: Single nucleotide polymorphism
SRE: Steroid responsiveness endophenotype
YY1: Yin yang 1

We hypothesized that it should be possible to integrate the ICS responsiveness signal measured in clinical trial subjects with the dexamethasone (DEX) response signal measured in immortalized lymphoblastoid cell lines (LCLs; LCL DEX response) from those subjects to create an informative steroid response network. Thus we expected to determine network edges that are indicative of interactions in steroid biology. Furthermore, we hypothesized that this integrative network will not just represent steroid biology but that secondary sources of variation in LCL DEX response are due to variation in the donor’s genotypes and SRE. Thus we expected to determine network edges indicative of variations in genotypes and SRE if relevant signals were detected with sufficient power and for the first neighbors of SRE to be genes or SNPs that modulate the ICS response.

METHODS

Our study design is shown in Fig 1. We selected a cohort from patients with mild-to-moderate childhood asthma who were receiving ICS therapy in the Childhood Asthma Management Program (CAMP) trial and for whom the LCL DEX response and genotypes were available. These data were filtered and combined in the REFS framework to identify genes associated with SRE. To guard against false-positive results, we carried genes forward for functional validation in human airway epithelial cells, in which we measured the effect of gene knockdown on nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) response to treatment with DEX and IL-1β.

CAMP

CAMP was a randomized clinical trial of the efficacy of long-term daily ICS treatments at managing asthma symptoms and improving lung function. CAMP enrolled 1041 children aged 5 to 12 years with mild-to-moderate persistent asthma over a 23-month period and followed them for an additional 4 years. Recruitment criteria and study characteristics have been described previously. During the original trial, CAMP participants were randomized into 3 treatment arms: budesonide (ICS), nedocromil, or placebo. We used 3 major types of data from selected CAMP participants: clinical data (from which we calculated SRE), genotype SNP data, and transcriptomic profiling from LCLs. CAMP participants were selected for the present study cohort, as described below.

Expression data, preprocessing, and quality control

After the main CAMP trial concluded, subjects were followed for at least yearly observation. CAMP participants were recontacted for an additional blood draw roughly 6 years after study conclusion. Of participants randomized to ICS treatment, 161 non-Hispanic white subjects provided blood samples from which CD4+ lymphocytes were isolated. These samples were immortalized and transcriptomically profiled under 2 treatment conditions: DEX treatment (10⁻⁶ mol/L) and a sham (ethanol) control. After 6 hours, expression levels were measured with the Illumina HumanRef8 v2 BeadChip (Illumina, San Diego, Calif). Bead-level data were stored by using BeadStudio (Illumina), and arrays that had low detection thresholds or low dynamic ranges were filtered out based on the Illumina HumanRef8 v2 BeadChip annotation package, and quantile normalized the data. We used the Bioconductor limma package to examine the multidimensional scaling (MDS) clustering of all arrays, including replicates.

Although studies reviewed in a recently published systematic review of more than 33 publications of ICS responsiveness have used particular asthma-related outcomes as proxies for ICS response, previously, we demonstrated that a combination of multiple clinical outcomes measures steroid responsiveness with higher accuracy, higher stability across populations, and higher robustness to missing data. We refer to this as the steroid responsiveness endophenotype (SRE), and it is a combination of 6 symptoms, 3 clinical outcomes measures steroid responsiveness endophenotype (SRE), and it is a combination of 6 clinical factors: baseline lung function (FEV₁); bronchodilator response (change in FEV₁ after administration of albuterol); frequency of asthma-related emergency department and hospital visits; frequency of supplemental courses of oral corticosteroids required to maintain asthma control; airway hyperreactivity measured by using a methacholine challenge test; and daily symptom diary cards. Integrating these factors provides a more complete picture of the response to ICS treatment, which might manifest differently in distinct patients with asthma and has been shown to measure steroid responsiveness more accurately than any of these clinical factors alone.

Genomic systems biology approaches, which are defined as those that take a holistic approach to disease understanding informed by multiple disciplines, genomics, and bioinformatic data sources, have previously been effective at identifying biological processes contributing to the cause of complex genetic diseases. Here we take a systems approach to address the genomics of ICS response in patients with asthma, building on our previous Bayesian network–based methodology of Reverse Engineering, Forward Simulation (REFS) modeling. This method uses patients’ genomic and outcome data to identify genes driving the associations of interest, enriching for likely causal importance of such associations. REFS uses Markov chain Monte Carlo sampling to construct a collection of Bayesian networks. This collection of networks is termed an ensemble, and by querying the ensemble as a whole, questions can be answered, such as the following: How do single nucleotide polymorphism (SNPs) and genes interact, and which genes have the greatest effect on patient outcomes?

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