Vanin-1 expression and methylation discriminate pediatric asthma corticosteroid treatment response

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Background: There is considerable heterogeneity in asthma treatment response.
Objective: We sought to identify biomarkers of corticosteroid treatment response in children with asthma and evaluate the utility and mechanistic basis of these biomarkers.
Methods: Children (5-18 years) presenting to the emergency department with an acute asthma exacerbation were recruited and followed during hospitalization. Nasal epithelial cells were collected on presentation to the emergency department (T_0) and 18 to 24 hours later (T_1), and T_1/T_0 gene expression ratios were analyzed to identify genes associated with good and poor corticosteroid treatment response phenotypes. The utility of these genes in discriminating between systemic corticosteroid treatment response groups was then tested prospectively in a new cohort of patients. A gene candidate (vanin-1 *[VNN1]*) that consistently distinguished good versus poor response phenotypes was further studied in an experimental asthma model, and *VNN1* promoter methylation was measured by means of bisulfite pyrosequencing in patients.
Results: *VNN1* mRNA expression changes were associated with systemic corticosteroid treatment response in children with acute asthma, and *VNN1* was required for optimal response to corticosteroid treatment in an experimental asthma model. A CpG site within the *VNN1* promoter was differentially methylated between good versus poor treatment response groups, and methylation at this site correlated with *VNN1* mRNA expression.
Conclusions: We have identified a biological basis for poor corticosteroid treatment response that can be used to distinguish a subgroup of asthmatic children who respond poorly to systemic corticosteroid treatment. *VNN1* contributes to corticosteroid responsiveness, and changes in *VNN1* nasal epithelial mRNA expression and *VNN1* promoter methylation might be clinically useful biomarkers of treatment response in asthmatic children. (J Allergy Clin Immunol 2015;136:923-31.)

Key words: *Childhood asthma, biomarker, vanin-1, treatment response, phenotype*

Asthma affects 25.7 million persons in the United States, including 7 million children.[1] Although asthmatic patients share similar clinical symptoms, the disease is heterogeneous.[2] This heterogeneity contributes to the difficulty in both studying and treating asthma. Nearly two thirds of asthmatic children reported at least 1 attack in the past year,[3] highlighting the suboptimal management of childhood asthma.[4] The frequency of absent or incomplete efficacy in asthma treatment has been estimated to be 40% to 70%.[5]

Currently, systemic corticosteroid treatment is considered the most effective medication for control of chronic asthma and rescue of acute exacerbation. Transcriptional profiling of individual host responses is a necessary and fundamental next step to better understand this individual variation and identify biomarkers of systemic corticosteroid treatment response. This approach has been used successfully to classify subphenotypes of asthma, including treatment response phenotypes.[6-9] Previous studies have often used samples requiring bronchoscopy or induced sputum collection, which is not always feasible in clinical practice, especially in children with an acute asthma exacerbation (AAE). In the present study we used genome-wide expression profiling of nasal epithelial cells to identify genes with temporal expression patterns (before and after treatment) that consistently and reliably discriminated between systemic corticosteroid treatment response groups among children hospitalized for asthma exacerbations. Nasal epithelial cells can be readily sampled safely during an asthma attack[10] and reflect changes observed in the bronchial airways of asthmatic children.[11] We identified and replicated a gene, vanin-1 *(VNN1)*, the mRNA expression of which consistently discriminated between good and poor responders to systemic corticosteroid treatment. We pursued mechanistic studies in an experimental asthma model and in human samples.

METHODS

Subjects

After institutional review board approval, children given a diagnosis of asthma at the age of 5 to 18 years who presented to the Cincinnati Children's Hospital Medical Center (CCHMC) emergency department (ED) with an AAE were recruited. Exclusion criteria are listed in the Methods section in this article's Online Repository at www.jacionline.org. Of the 57 subjects

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Abbreviations used
AAE: Acute asthma exacerbation
AHR: Airway hyperresponsiveness
BALF: Bronchoalveolar lavage fluid
CCHMC: Cincinnati Children's Hospital Medical Center
CTCF: CCCTC-binding factor
DMSO: Dimethyl sulfoxide
ED: Emergency department
HCK: Tyrosine protein kinase
HDM: House dust mite
ICS: Inhaled corticosteroid
LOS: Length of stay
PPARγ: Peroxisome proliferator–activated receptor γ
qRT-PCR: Quantitative real-time PCR
SOD2: Superoxide dismutase 2
SRGN: Serglycin
VNN1: Vanin-1
WT: Wild-type

consented, 21 were hospitalized for asthma exacerbation, and 15 had genome-wide mRNA expression data for both time points. These 15 patients were used as a discovery cohort to test the association between gene expression and systemic corticosteroid treatment response. A replication cohort of 25 children hospitalized for asthma were recruited to further validate the findings from the discovery cohort. Eighteen children hospitalized for AAEs were recruited for methylation studies, 5 of whom overlapped with the replication cohort.

Subjects provided demographic, environmental, asthma trigger, and personal and family allergy and asthma history data. Parental report of current inhaled corticosteroid (ICS) controller medication (eg, mometasone [Asmanex], Merck, Whitehouse Station, NJ; fluticasone [Flovent], GlaxoSmithKline, Research Triangle Park, NC; beclomethasone dipropionate [Qvar], Teva, Petah Tikva, Israel; budesonide [Pulmicort], AstraZeneca, London, United Kingdom; fluticasone/salmeterol [Advair], GlaxoSmithKline; mometasone/formoterol [Dulera], Merck; and budesonide/formoterol [Symbicort], AstraZeneca) was also collected. To assess baseline asthma symptom severity and control, a respiratory symptom score was calculated (based on frequency of wheeze, cough, shortness of breath, and chest tightness),[12] and the age-specific Asthma Control Test score was collected.[13]

Treatment protocol and treatment response definitions

Enrolled patients were treated according to the CCHMC evidence-based treatment protocol for inpatient asthma exacerbations.[14-16] The admitting physician determined the initial interval of albuterol treatments, which were subsequently spaced based on physician or respiratory therapist assessments. Patients received 2 mg/kg/d prednisone while hospitalized, and ICSs were continued through a mouthpiece. Length of stay (LOS) was calculated as the number of hours from the time the admission decision was made to the time the subject met clinical discharge criteria (see the Methods section in this article's Online Repository). Good responders were defined as those with an LOS of 24 hours or less, and poor responders were defined as those with an LOS of greater than 24 hours.

Nasal epithelial cell sample collection and processing

Nasal epithelial samples were collected at 2 time points from each subject: (1) in the ED (T_0) and (2) on the inpatient floor 18 to 24 hours after receiving corticosteroids in the ED (T_1). The procedure, characterization of cell types, sample processing, and RNA isolation have been described previously.[10] Nasal samples collected contained more than 90% epithelial cells, which is similar to our previous findings.[10] Expression profiles were generated on the Affymetrix Human Gene 1.0 ST platform (Affymetrix, Santa Clara, Calif). Quantitative real-time PCR (qRT-PCR) was used to validate and replicate candidate genes (see the Methods section and Table E1 in this article's Online Repository at www.jacionline.org). DNA isolation, bisulfite treatment, and pyrosequencing analysis of methylation levels of 5 CpG sites within the *VNN1* promoter for the methylation cohort are detailed in the Methods section and Table E2 in this article's Online Repository at www.jacionline.org.

Experimental asthma model

Vnn1$^{-/-}$ mice and age- and sex-matched wild-type (WT) BALB/c mice (see the Methods section in this article's Online Repository) were exposed to intratracheal doses of house dust mite (HDM; 20 μg in 50 μL of saline) or saline 3 times a week for 3 weeks, as previously described.[17] Mice were treated with intraperitoneal dexamethasone (3 mg/kg in dimethyl sulfoxide [DMSO]) or DMSO (100 μL) for the last 5 days of the 3-week model. Twenty-four hours after the last HDM challenge, airway hyperresponsiveness (AHR) was assessed (see the Methods section in this article's Online Repository), bronchoalveolar lavage fluid (BALF) was collected and processed, and inflammatory cells were quantified, as previously described.[17]

Statistical analysis

Detection of differentially expressed genes in the discovery set. To identify candidate genes, we performed sequential filtering to balance concerns of type I and II errors (Fig 1, *A*). First, we sought to identify genes reliably expressed in nasal cells (raw signal >100 in at least 2 samples). Next, we sought to identify genes responsive to treatment ($T_1/T_0 \geq 1.5$ or $T_1/T_0 \leq 0.66$; T_1/T_0 is defined as gene expression at T_1 relative to that at T_0). Then we identified genes with significant differences in T_1/T_0 ratio between the good and poor responder groups. A *P* value threshold of .05 was used because independent replication samples and complementary biologic studies minimize the risk of false-positive discovery to minimize the risk of missing true associations. We then identified those genes with a high rate of prediction accuracy (≥0.80) through linear discriminant analysis. To validate these results, we performed qRT-PCR.

Microarray data analysis. Microarray cell image files were analyzed with GeneSpring GX software (Agilent Technologies, Santa Clara, Calif). Probe-level measurements were subject to initial background correction and normalization by using GC-robust multi-array average. Transcript levels were normalized per chip to the 50th percentile and per gene to median intensity.

Association testing. In the discovery phase we used *t* tests (with log transformation) to identify genes between good and poor responders. Linear discriminant analysis[18] was applied to find genes that best discriminated between good and poor responders.

For replication, we first examined whether there were differences between the discovery and replication cohorts that might introduce bias. Time of admission was significantly different between the discovery and replication cohorts. Thus we matched our replication cohort to the discovery cohort based on month and T_0 and T_1 times by using propensity scores.[19] Importantly, gene expression profiles were not considered in the matching process. After matching, we performed *t* tests comparing the quantitative PCR results from good and poor responders. A linear regression model was fitted to examine the association between the *VNN1* mRNA expression change (T_1/T_0) and the continuous length of hospital stay (in hours) in the combined discovery and replication cohorts.

For the experimental asthma model, individual AHR, total BALF cell counts, and eosinophil percentages in mice treated with HDM plus dexamethasone were compared with and normalized by the corresponding mean value in the HDM-treated group. The difference between the WT and *Vnn1*$^{-/-}$ groups was determined by using the nonparametric Mann-Whitney test. A *P* value of less than .05 was considered significant. Percentage reduction was used to present the corticosteroid response results.

For methylation analysis, Pearson correlation was used to measure the correlation between changes in mRNA expression (T_1/T_0) and DNA methylation (mT_1-mT_0) of *VNN1*. The Fisher exact test was used to compare