Epithelial cell responses to rhinovirus identify an early-life–onset asthma phenotype in adults

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Background: The study of pathogenic mechanisms in adult asthma is often marred by a lack of precise information about the natural history of the disease. Children who have persistent wheezing (PW) during the first 6 years of life and whose symptoms start before age 3 years (PW^+) are much more likely to have wheezing illnesses due to rhinovirus (RV) in infancy and to have asthma into adult life than are those who do not have PW (PW^-).
Objective: Our aim was to determine whether nasal epithelial cells from PW^+ asthmatic adults as compared with cells from PW^- asthmatic adults show distinct biomechanistic processes activated by RV exposure.
Methods: Air-liquid interface cultures derived from nasal epithelial cells of 36-year old participants with active asthma with and without a history of PW in childhood (10 PW^+ participants and 20 PW^- participants) from the Tucson Children's Respiratory Study were challenged with a human RV-A strain (RV-A16) or control, and their RNA was sequenced.
Results: A total of 35 differentially expressed genes involved in extracellular remodeling and angiogenesis distinguished the PW^+ group from the PW^- group at baseline and after RV-A stimulation. Notably, 22 transcriptomic pathways showed PW-by-RV interactions; the pathways were invariably overactivated in PW^+ patients, and were involved in Toll-like receptor– and cytokine-mediated responses, remodeling, and angiogenic processes.
Conclusions: Asthmatic adults with a history of persistent wheeze in the first 6 years of life have specific biomolecular alterations in response to RV-A that are not present in patients without such a history. Targeting these mechanisms may slow the progression of asthma in these patients. (J Allergy Clin Immunol 2022;150:604-11.)

Key words: *Early-onset childhood asthma, persistent wheeze, transcriptome, RNA sequencing, rhinovirus, airway remodeling, Toll-like receptor pathways, interleukin response, blood vessel remodeling, apoptosis, protein processing*

Asthma is a complex disorder that affects 25 million Americans and results in more than 400,000 hospitalizations annually.[1] Asthma is a highly heterogeneous condition, and the mechanisms of the disease vary greatly between different asthma phenotypes. Most studies attempting to characterize the molecular basis of these phenotypes in adults have been cross-sectional.[2] Although important advances have been made, it is difficult to determine from these studies which molecular pathways are involved in inception of the disease and which are the consequence of the chronic inflammatory process that characterizes it. Longitudinal studies, and especially those in which follow-up was started before disease inception, may provide new insights into the endotypes that underlie asthma heterogeneity.

A large proportion of cases of asthma begin in childhood, particularly during the preschool years.[3] Several longitudinal studies have identified a phenotype of asthma in which children have wheezing episodes during the first 3 years of life and are still having such episodes at age 6 years or have a diagnosis of asthma at that age.[4-6] These children with persistent wheezing (PW^+) have 14 times higher odds of having asthma that persists from childhood into adult life and 4 times higher odds of having newly diagnosed asthma in adult life than do children who do not wheeze during the first 6 years of life.[7] A distinguishing characteristic of PW^+ children is that rhinovirus (RV) infections are 10 times more likely than PW^- children to be identified during wheezing lower respiratory tract illnesses in the first 3 years of life.[5,6] Although gene variants present in chromosome 17q have been found to be associated with PW^+ status,[8] more recent studies suggest that these variants are not associated with any specific wheezing phenotype in early life.

We hypothesized that although most adult patients with asthma are susceptible to developing exacerbations during RV infections,[9] PW^+ asthmatic patients would have a specific, altered molecular response to RV that would differentiate them from patients who have adult asthma but no history of PW in early life (PW^-). To test this hypothesis, we studied enrollees in the Tucson Children's Respiratory Study (TCRS), a birth cohort that was started in 1980-1984 and assessed periodically since then and up to when the cohort members were 36 years old. We hoped to identify molecular processes that might underlie the PW trajectory and phenotype, thereby providing targets to slow the progression of early-onset asthma.

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Abbreviations used
ALI: Air-liquid interface
DEG: Differentially expressed gene
DIP: Differentially interacting pathway
FDR: False discovery rate
GO: Gene Ontology
GOBP: Gene Ontology biologic process
PAI-1: Plasminogen activator inhibitor-1
PW: Persistent wheezing
RNA-seq: RNA sequencing
RV: Rhinovirus
TCRS: Tucson Children's Respiratory Study

METHODS

Design and subjects

We recruited participants from the TCRS, a longitudinal assessment of 1246 healthy nonselected infants enrolled at birth and continuing to present day.[10] We focused on those with adult asthma, defined as having a physician-diagnosis of asthma by age 36 years and asthma symptoms or asthmatic episodes at any time between the ages of 22 and 36 years. The terms *frequent asthma*, *wheeze*, and *cough* were previously defined in our cohort.[11] Frequent physician-diagnosed asthma and frequent wheeze were defined as 4 or more episodes during the past year. Frequent cough was defined as cough lasting more than a week at least twice during the past year. Lung function studies were performed when participants were 36 years of age, and the percent of predicted values were computed by using the equations provided by the Global Lung Function Initiative.[12] Methacholine studies were performed when participants were 22 old, and a positive bronchial response was defined as having a provocative dose (of methacholine) cause a 20% drop in FEV_1 from baseline (PD_{20}) value of 0.759 mg or less.[13] Bronchodilator response was calculated by using the formula $100 \times ([\text{post-}FEV_1 - \text{pre-}FEV_1)]/\text{pre-}FEV_1)$.

From this group of adult asthmatic patients, we successfully recruited participants with or without PW a history of PW in childhood (10 PW^+ and 20 PW^- participants) and sampled their nasal epithelia to develop *in vitro* air-liquid interface (ALI) cultures. We then challenged individual cultures with a mock control and a human strain of RV-A (RV-A16). This specific strain RV-A16 was chosen because of its clinical relevance. RV-A16 is derived from a human viral isolate associated with upper respiratory tract infections. *In vivo* viral challenge of RV-A16 causes symptoms in healthy volunteers, and the strain is widely used in *in vitro* culture studies to investigate RV-A pathophysiology.[14] We compared RV binding, replication, and the epithelial transcriptomic response before and after RV-A16 challenge between the PW^+ and PW^- groups. Informed consent was obtained from all subjects and approved by the institutional review board (institutional review board no. 7500000005).

Nasal samples and development of ALI cultures

Under local anesthesia, a 1-cm^2 nasal sample was taken from the middle turbinate; the cells were dissociated, and plated onto Primaria plates (Corning, Corning, NY). These primary cells were then seeded onto transwell culture inserts and differentiated at the air-liquid-interface (ALI) by using PneumaCult media (STEMCELL Technologies, Cambridge, Mass). ALI differentiation was confirmed through visual inspection of motile cilia, and transepithelial electric resistance measurements greater than 2000 Ω/cm^2.

Cellular RV-A16 provocation assay

For each biologic sample, duplicate ALI cultures were differentiated. The human RV-A strain RV-A16, diluted in Dulbecco modified Eagle medium plus nutrient mixture F12, was used to infect 1 culture at a concentration of 1×10^5 RNA copies per well; the cultues were incubated for 4 hours and then rinsed. This procedure was repeated with Dulbecco modified Eagle medium plus nutrient mixture F12 alone on the second culture as a control (no virus). Forty-eight hours after infection, RNA was isolated by using the RNEasy column kit and high-quality RNA samples, as measured by using the Bioanalyzer 2100 used for library preparation.

RNA-seq analyses

The mRNA was obtained from total RNA and cDNA libraries for RNA sequencing (RNA-seq) prepared using the KAPA Stranded RNA-Seq Kit (Roche, Basel, Switzerland). Libraries were sequenced by using the Illumina HiSeq 3000 using a 1×50-bp run. Data quality control was conducted with the Illumina SAV, and demultiplexing was performed using the Illumina Bcl2fastq2 version 2.17 program. The fastq files were aligned to the human genome (TopHat software[15]) and mapped to genes using the University of California, Santa Cruz genome browser (version hg38,[16] using Bowtie2, version 2.1.0[17]); gene expression levels were then estimated by using RSEM, version 1.2.15.[18] The quality of mapping was excellent: for any given sample, anywhere between 91% and 98% of reads aligned (average reads mapped 96%).

To perform stringent RNA-seq analyses and allow for replication testing, we divided our samples into a discovery group and a validation group, which were matched by sex and age. In the discovery cohort, 22 unique patients were collected from the PW^+ (n = 6) and PW^- (n = 16) groups, and in the validation cohort, 8 unique patients were divided into the PW^+ (n = 4) and PW^- (n = 4) groups. In both cohorts, each patient had 2 aliquots: control (no virus) and post–RV-A16 infection. We then removed mapped genes with low counts (cpm <30) by using NOISeq library,[19] and we normalized batch effects and systematic variations using the *ARSyNseq*[20] and TMM normalization[21] functions in the R package software. By analyzing our data in discovery and validation sets and correcting for batch effects, we filtered data variability unrelated to the PW phenotype or the RV-A response. Differentially expressed genes (DEGs) were identified by pairwise comparisons between the PW^+ and PW^- groups at baseline and after RV-A infection by using a moderate *t* test through the LIMMA program[22] at Benjamini-Hochberg– (false discovery rate [FDR])-adjusted *P* value thresholds less than .05. Raw and normalized expression data are available in the Gene Expression Omnibus (GSE149273).

GO analyses

We performed Gene Ontology (GO) analyses to assign biologic relevance to the DEGs associated with the PW phenotype at baseline and after RV-A infection. In our analyses, we calculated the overlap of DEGs identified in the PW phenotype at baseline and after RV-A infection compared with the number of genes associated with the GO term. Significant GO terms associated with DEGs were prioritized at an FDR less than 5% by using the single-protein analysis of network method,[23-26] with use of STRING molecular interactions[27] and visualization with Cytoscape software.[28] Functional analyses of overrepresented DEGs in pathways (gene sets) were conducted by using the Fisher exact test's enrichment statistics[15] in gene sets from GO biologic processes[29] (GOBPs) and controlled by FDR. Network visualizations were conducted with Cytoscape.[28]

DIP analyses

We assessed the differentially interacting pathways (DIPs) activated by RV-A in PW^+ patients with asthma as compared with the DIPs in PW^- asthmatic patients. DIP analyses require integration of multiple gene expressions into a single pathway score for each subject. This substantial dimension reduction allowed us to identify DIP statistical interactions modeled between 2 factors: RV response (RV-A vs control) and PW phenotype (PW^+ vs PW^-). Functional Analysis of Individual Microarray Expression[30] was used to translate subjects' RNA expression of GO sets into pathway-scale scores in the discovery set at an FDR less than 5% and also in the validation set at a Bonferroni threshold less than 5%. Functional similarities between GO pathways were calculated by

TABLE I. Characteristics of asthmatic adults with or without a history of persistent wheeze

Characteristic		Persistent wheeze		
		Yes (n = 10)	No (n = 20)	*P* value
Sex	% Male	60.0	45.0	.70
Age (y)	Mean (SD)	35.2 (1.0)	35.2 (0.9)	.99
Race/ethnicity	% Non-Hispanic White	60.0	60.0	
	% Hispanic White	10.0	25.0	
	% Other	30.0	15.0	.50
History of parental asthma	% Yes	33.3 (3/9)	50.0 (9/18)	.68
Maternal smoking at enrollment	% Yes	0.0	15.0	.53
Paternal smoking at enrollment	% Yes	33.3 (3/9)	30.0 (6/20)	.99
Birth weight (g)	Mean (SD)	3667 (613)	3421 (538)	.27
Age at first physician-diagnosed active asthma (y)	Mean (SD)	8.0 (3.5)	13.9 (8.1)	.01
Skin test result at age 6 y	% Positive	44.4 (4/9)	57.9 (11/19)	.69
Alternaria test result at age 6 y	% Positive	33.3 (3/9)	42.1 (8/19)	.99
Eosinophil count ≥4% at age 6 y	% Positive	57.1 (4/7)	29.4 (5/17)	.36
Total serum IgE level at age 6 y	GM (95% CI) [n]	235 (74-747) [8]	43.2 (17.3-107) [16]	.02
V_{max}FRC at age 6 y	Ln mean, (SD) [n]	7.08 (0.5) [5]	6.95 (0.2) [15]	.43
eNO level at age 22 y	*z* score, mean (SD) [n]	-0.17 (1.5) [8]	0.11 (1.3) [14]	.65
Methacholine PD_{20} at age 22-26 y	% Positive	71.4 (5/7)	66.7 (10/15)	.99
BDR ≥12% at age 22-36 y	% Positive	11.1 (1/9)	20.0 (4/20)	.56
Urgent care/ER Visit for asthma at age 22-36 y	% Positive	40 (4/10)	10 (2/20)	.14
Concurrent at age 36 y				
Frequent wheeze	% During past year	20.0	35.0	.68
Frequent cough	% During past year	10.0	30.0	.37
Frequent asthma	% During past year	20.0	26.3	.99
Respiratory symptom score	% Any, past year	20.0	50.0	.24
Smoking	% No	40.0	50.0	
	% Current	10.0	5.0	
	% Former	50.0	45.0	.99
Total serum IgE level	GM, (95% CI) [n]	66.5 [30.9-143] (10)	43.3 (19.1-98.1) [19]	.48
BMI	Mean (SD)	32.5 (7.2)	36.0 (12.9)	.43
FEV_1 % predicted	Mean (SD)	95.8 (16.2)	92.7 (11.6)	.55
FVC % predicted	Mean (SD)	103.7 (15.5)	100.9 (13.3)	.60
FEV_1/FVC % predicted	Mean (SD)	92.3 (10.4)	91.9 (7.8)	.89
FEF_{25-75} % predicted	Mean (SD)	90.3 (42.3)	77.8 (21.4)	.41
Prescription for asthma medication in past year	% Yes	60.0 (6/10)	50.0 (10/20)	.71
Short-acting bronchodilator	% Yes	55.6 (5/9)	50.0 (10/20)	.99
Long-acting bronchodilator	% Yes	11.1 (1/9)	5.0 (1/20)	.53
Inhaled steroid	% Yes	22.2 (2/9)	15.0 (3/20)	.63
Oral steroid	% Yes	0.0 (0/9)	10.0 (2/20)	.99

BDR, Bronchodilator response; *eNO*, exhaled nitric oxide; *FEF*, forced expiratory flow; *GM*, geomertic mean; *Ln*, natural logarithm; *PD_{20}*, provocative dose (of methacholine) causing a 20% drop in FEV_1 from baseline; *$V_{max}FRC$*, maximal flow at funcational residual capacity.
The Fisher exact test was used when indicated. The group sample sizes for specific medications are n = 9 for PW^+ patients and n = 20 for PW^- patients.

using information theoretic similarity methods to identify common interacting biologic processes.[31-33]

Specific details of RNA sequencing and analyses are described in the Supplementary Methods (available in the Online Repository at www.jacionline.org).

RESULTS

Characteristics of subjects with the PW^+ and PW^- asthma phenotypes

All participants had active adult asthma identified in the TCRS cohort, and there were no significant differences between the 2 phenotype groups in terms of sex, age, or race/ethnicity. In early life, participants with a history of PW^+ in childhood were, as expected, more likely to be diagnosed with asthma at a younger age (at age 8.0 years) than PW^- participants (at age 13.9 years). At age 6 years, the PW^+ participants were also more likely to have higher blood eosinophilia and significantly higher serum IgE than the PW^- participants. There were no significant differences in skin prick test response to individual allergens (house dust mite, Bermuda olive, careless weed, *Alternaria*, mesquite, and mulberry) at age 6 in the PW^+ and PW^- groups. In detailed analyses of the TCRS cohort members as adults (at ages 22-36 years), we did not identify any differences in active asthma symptoms, use of asthma medications, methacholine challenge study results at age 26 years, or lung function levels between the PW^+ and PW^- groups in the past year (Table I).

Assessment of DEGs

Our primary goal for this study was to identify biologic pathways and genes that were specifically activated by RV-A in PW^+ adult patients with asthma as compared with in PW^- adult patients with asthma. Because of the limited numbers of participants that we were able to include in this study (largely owing to proximity), we did not have enough power to detect this

interaction by using single DEGs. Nevertheless, it was still important to determine whether there were DEGs that were overexpressed or underexpressed in PW^+ patients versus in PW^- patients either at baseline or after RV-A stimulation. RNA-seq analysis showed that at baseline (unstimulated ALI cultures), 133 DEGs were significantly different between the unstimulated PW^+ (n = 6) and PW^- (n = 16) cultures identified in the discovery group at an FDR less than 5%; of these, 72 DEGs were confirmed by using a Bonferroni correction less than 5% in the validation group (n = 4 in each of the 2 groups). All 72 DEGs were dysregulated in the same direction in the discovery and validation groups: 23 DEGs were downregulated and 49 DEGs were upregulated when PW^+ and PW^- cultures were compared at baseline (see Tables E1 and E2 in the Online Repository at www.jacionline.org). These genes were associated with 6 GOBPs at an FDR less than 5%: (1) heterophilic cell-cell adhesion via plasma membrane cell adhesion molecules (GO:0007157), (2) extracellular matrix organization (GO:0030198), (3) cell-substrate adhesion (GO:0031589), (4) extracellular structure organization (GO:0043062), (5) leukocyte migration (GO:0050900), and (6) negative regulation of ERK1 and ERK2 cascade (GO:0070373).

We next assessed whether there were significant differences in gene expression between the PW^+ and PW^- ALI cultures after challenging them with a human RV-A strain. The supernatant was removed to exclude any unbound virus, and RV-A16 RNA titer was quantified by quantitative PCR 48 hours after infection in the PW^+ (n = 0) and PW^- (n = 20) ALI cultures. There were no significant differences in RV-A16 viral replication between the 2 groups (data not shown), suggesting a similar viral load. There were 336 DEGs in the discovery group that were significantly different between the PW^+ and PW^- cultures at an FDR less than 5%, and 135 of these were confirmed in the validation group at a Bonferroni correction less than 5%. All 135 DEGs were dysregulated in the same direction in the discovery and validation groups. Of these genes, 93 DEGs were downregulated and 42 DEGs were upregulated in the PW^+ group compared with in the PW^- group (see Tables E3 and E4 Online Repository at www.jacionline.org). These genes were enriched in 4 GOBPs at an FDR less than 5%: (1) regulation of angiogenesis (GO:0045765), (2) positive regulation of angiogenesis" (GO:0045766), (3) regulation of vasculature development (GO:0001944), and (4) positive regulation of vasculature development (GO:0045766).

A total of 35 DEGs were dysregulated in the PW^+ cultures compared with in the PW^- cultures both at baseline and after RV-A challenge. Of these genes, 23 were part of the STRING interaction data network, allowing us to calculate the overlap between genes and enriched GO terms. Five genes (*DUSP6, FBLN1, TIMP2, PHLDB2*, and *ICAM1*) were associated with overrepresented GO terms enriched at baseline, 1 gene (*SPHK1*) was associated with overrepresented GO terms after RV-A challenge, and 3 genes (*SERPINE1*, *ITGA5*, and *WNT5A*) were associated with overrepresented GO terms enriched at baseline and after RV-A challenge (Fig 1).

Biologic pathway analysis of gene expression interactions between PW phenotype and RV-A challenge

Given that we were greatly more powered to find statistical interactions at the biologic pathway than at the DEG level owing to the substantial dimension reduction (~3000 GOBPs studied rather than >25,000 transcripts), we assessed gene pathways that were differentially activated by RV-A in PW^+ asthmatic patients than in PW^- asthmatic patients. As compared with DEG analyses, determination of differential pathway expression first requires integration of multiple gene expression in 1 pathway score for each subject (pathway expression transformation). These normally distributed pathways scores can then be analyzed for differential expression and statistical interactions. Our goal was to focus on GOBPs showing statistical interaction between RV-A exposure and PW phenotype (ie, those that were significantly and differentially expressed in the PW^+ group after RV-A infection but not at baseline). Similar to in the gene-based analysis, we utilized the moderate *t* test implemented in the LIMMA program to derive those GOBPs whose Functional Analysis of Individual Microarray Expression–derived and normally distributed pathway expression scores codiscriminated between phenotypic groups at an FDR less than .05. In our discovery cohort, we found 31 PW-by–RV-A interactive GOBP terms. To remove falsely prioritized GOBP terms that are computational hierarchic signal inheritance artifacts occurring in GO enrichment studies,[34] we used the GO module software[35] that prioritized 24 GOBP terms as true positive for further evaluation in the validation cohort. We replicated 22 of these interactive GOBP terms in the validation cohort (conservative Bonferroni-corrected threshold < 0.05) (see Fig E1 Online Repository at www.jacionline.org). These 22 pathways differentially activated by RV-A in PW^+ and PW^- asthmatic patients were grouped into 5 different functional modules based on their functional similarity; they included (1) Toll-like receptor and pattern recognition receptor signaling, (2) interferon and interleukin production, (3) blood vessel remodeling, (4) apoptosis, and (5) regulation of protein processing (Fig 2).

DISCUSSION

A major goal of precision medicine for a heterogenous disease such as asthma is the development of new therapies that treat the specific molecular pathways underlying different asthma phenotypes. Defining such phenotypes in adult life, however, is a complex task because prospective data regarding the natural course of the disease are usually unavailable. In these circumstances, defining which factors are causes or consequences of the disease process and which are truly relevant for its pathogenesis is problematic. In a large proportion of adults with asthma, inception of the disease occurs in childhood,[4,7] but retrospective data collected when patients are adults are often inaccurate and biased. The TCRS offers a unique opportunity to address these challenges. We have been following a large, unselected cohort enrolled at birth for the past 4 decades, during which time we obtained detailed information about their asthma symptoms at several in-depth surveys. In this population, we identified (25 years ago) a group of children that we called persistent wheezers (PW^+) because they had wheezing illnesses during the first 3 years of life and were still wheezing at age 6 years.[36] We later showed that PW^+ children were at high risk of having asthma as adults,[7] and others have shown that they are up to 10 times more likely to wheeze during RV infection in the first 3 years of life.[5] We thus postulated that in adult asthmatic patients who were PW^+ as children, altered responses to RV infection would play a critical role in asthma pathogenesis and would thus

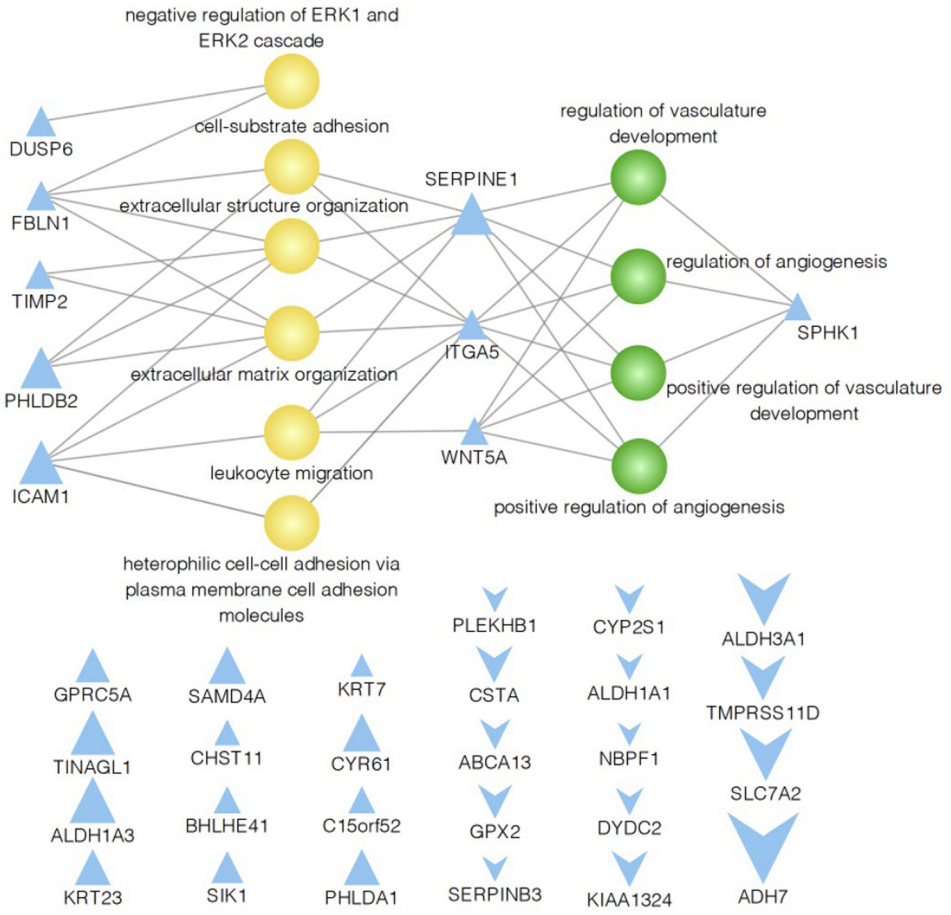

FIG 1. DEGs and their relation to overrepresented GO terms that are dysregulated in PW^+ cultures compared with in PW^- cultures at baseline and after RV-A challenge. A total of 35 dysregulated genes were dysregulated in PW^+ cultures compared with in PW^- cultures at baseline and after RV-A challenge. Upregulated genes (*blue triangles*) and downregulated genes (*blue chevrons*) are sized according to fold change level (mean between baseline and RV-A–stimulated conditions). Overrepresented GO terms enriched in the DEGs of PW^+ versus in the DEGs of PW^- cultures at baseline (*yellow circles*) and overrepresented GO terms enriched in the DEGs of PW^+ versus in the DEGs of PW^- cultures after RV-A challenge (*green circles*). Lines between the GO terms and genes denote GO-derived associations between the genes and GO terms. Five genes (*DUSP6, FBLN1, TIMP2, PHLDB2*, and *ICAM1*) were associated with overrepresented GO terms enriched at baseline, 1 gene (*SPHK1*) was associated with overrepresented GO terms after RV-A challenge, and 3 genes (*SERPINE1, ITGA5,* and *WNT5A*) were associated with overrepresented GO terms enriched at baseline and after RV-A challenge.

distinguish them phenotypically from PW^- patients. Here, we show that when compared when cells obtained from PW^- patients with asthma, ALI cultures of nasal epithelial cells obtained from adults with asthma who were PW^+ during their preschool years show significant and specific differences in their gene expression patterns both before and after *in vitro* exposure to RV-A.

We first determined whether single genes showed significant differences in expression between PW^+ and PW^- asthmatic patients. At baseline (ie, before RV-A exposure), PW^+ asthmatic patients showed increased expression of GO processes underlying airway remodeling, including genes associated with extracellular matrix formation, cell adhesion, migration, activation, and proliferation. Airway remodeling is a major determinant of airflow limitation,[37,38] which in turn is associated with persistence of asthma from childhood into adult life.[39] Interestingly, in this same population we previously showed that level of airway function in adult life was strongly associated with the age at inception of asthma symptoms: among asthmatic patients, the FEV_1/FVC ratio was lowest in those whose symptoms started before age 6 years.[7] After RV exposure, PW^+ asthmatic patients showed increased expression of GO processes underlying angiogenesis and vasculature development, processes that have been shown to be highly associated with airway remodeling.[40]

In an exploratory analysis, we found 35 common transcripts in the PW^+ group that were dysregulated at baseline and also differentially activated by RV-A16. ICAM1 is the main receptor of RV-A,[41] and its expression has been found to be increased in the airway epithelia of asthmatic patients.[42] ICAM1 expression is also found on endothelial cells and is critical for allowing migration of leukocytes from the circulation to sites of airway inflammation to induce both innate and adaptive antiviral immune responses.[43] Several *ICAM1* single-nucleotide polymorphisms have been associated with reduced childhood asthma risk, and a murine knockout model has been shown to be

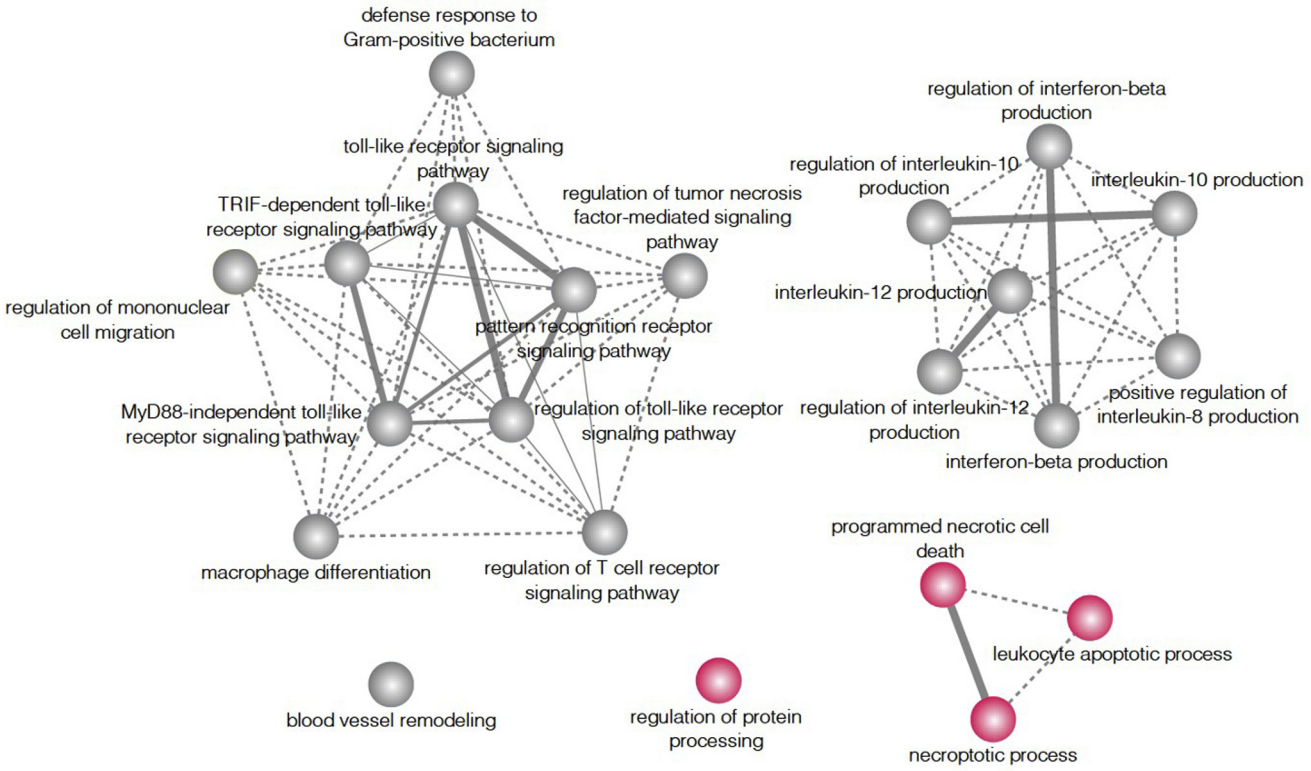

FIG 2. Functional Similarities of Biologic Processes Interactions between PW and RV-A response. A total of 22 GOBPs were increased after RV-A exposure in PW^+ patients as compared with after RV-A exposure in PW^- patients. Red nodes represent GOBPs that were increased at baseline and then disproportionately increased after RV-A exposure in PW^+ patients versus after RV-A exposure in PW^- patients. Gray nodes represent GOBPs that were increased significantly only after RV-A exposure in PW^+ patients and not after RV-A exposure in PW^- patients. These GOBPs were grouped into 5 modules based on their information theoretic similarity (ITS) score. Interactions between the GOBPs are reflected by their ITS scores, as follows: dotted line indicates an ITS score greater than 0.30 but less than 0.69 (mild similarity); thin line indicates an indicates an ITS score greater than 0.70 but less than 0.79 (moderate similarity); medium-thickness line indicates an ITS score greater than 0.80 but less than 0.89 (high similarity); and thick line indicates an ITS score greater than 0.90 but less than 1.00 (nearly identical biologic functions and gene sets).

protective against asthma.[44] Once RV-A binds to ICAM1, the ERK1/2 MAPK pathway is activated; this pathway is a trigger for epithelial-mesenchymal transitions that contribute to the airway remodeling seen in asthma.[45] *TIMP-2* is an inhibitor of matrix metalloproteinases, which play a role in modulating inflammation, wound repair, and airway remodeling. Alterations of matrix metalloproteinase-to-TIMP ratios have been associated with smoking and asthma severity.[46] *SERPINE1* encodes for plasminogen activator inhibitor-1 (PAI-1); it is a protein critical in airway remodeling processes. Clinically, PAI-1 levels have been found to be increased in nasal and sputum fluids of asthmatic patients after common colds, suggesting a potential mechanism related to airway remodeling and tissue repair following respiratory viral infections.[47] In animal models, PAI-1–deficient mice exhibit reduced extracellular matrix deposition in experimental asthma models.[48] Together, these findings suggest that increased SERPINE1 expression in PW^+ cultures at baseline and after RV-A infection may represent an overactivated inflammatory process in those asthmatic patients with early-onset wheeze. These results need to be interpreted cautiously, however, because these individual genes were assessed at the summative level, and although their combined effect was significant, there could be differences at the individual subject level. Moreover, we were underpowered to identify DEGs showing significant PW-by–RV-A interaction.

To address the main goal of this study, we tested the hypothesis that sets of genes involved in specific GOBPs could be differentially activated by RV-A in the 2 groups. We found that 22 GOBPs were consistently overactivated by RV-A exposure in PW^+ patients versus in PW^- patients and these pathways could be further reduced to 5 major modules. Of these modules, 2 (Toll-like receptor and pattern recognition receptor signaling and interferon and interleukin production) underlie immune responses to the virus and 3 (blood vessel remodeling, apoptosis, and regulation of protein processing) are most likely associated with airway remodeling processes (Fig 2). Taken together with our results reported earlier in this article, these findings suggest that, compared with the airway epithelium of other patients with adult asthma, the airway epithelium of those whose symptoms start in early childhood have concurrent upregulation of 2 critical molecular pathways: excessive inflammatory responses to RV and overactive airway remodeling mechanisms. The copresence of these 2 endotypes may explain why schoolchildren who were at high risk for asthma and had wheezing illnesses due to RV in the first 3 years of life had

significantly lower lung function during the school years than did those children who had wheezing illnesses due to other viruses[49] and were at high risk for developing asthma in adult life.[4,50]

Our study suggests that a specific RV-induced response in asthmatic patients might contribute to persistent wheeze in childhood, as well as to the pathogenesis of adult asthma. Clinically, this is relevant, as RV infections are the most common causes of asthma exacerbations. These data suggest that targeted antiviral therapies in early life may slow the progression of asthma in children with persistent wheeze. As 1 example, the use of omalizumab in a randomized controlled trial has been shown to reduce asthma exacerbations in asthmatic children.[51] By design, the TCRS cohort allowed us to ascertain differences between adult asthmatic patients with or without a history of persistent wheeze in childhood without the effects of recall bias. Our use of RNA-seq allowed the comparison of the epithelial transcriptome response between ALI cultures to define genes and biologic processes that distinguished PW^+ and PW^- samples at baseline and after RV-A challenge. An alternative strategy could be to target the downstream signals associated with antiviral immune responses and airway remodeling seen after RV-A infection in PW^+ cultures.

There are several limitations of our study. This study was designed as an epidemiologic study, and thus, many of the subjects were not under our direct clinical care. Therefore, we were unable to provide specific clinical information as to the nature of their asthma or exacerbations. Although all of our participants were diagnosed with adult asthma by a physician by age 36 years, not all of the participants were symptomatic over the past year. However, two-thirds of adult asthmatic patients had a positive methacholine challenge result after 22 years. Our sample size was relatively small owing to the challenges of recruiting adult patients who met our stringent persistent wheeze and adult asthma phenotype back to our center for nasal biopsy studies. However, because we utilized discovery and validation groups that were matched by sex and age, as well as single-subject analyses from each patient of the control (no virus) and post–RV-A16 infection groups, we were able to normalize batch effects and systematic variations to reduce the noise to signal ratio and thereby identify significant genes and pathways unique to the specific PW phenotype and RV-A response. Similar gene expression studies in asthmatic patients identifying endotype-phenotype association studies have been performed with equivalent sample sizes.[52] It is impossible to tell whether the transcriptome changes that we identified in cultures derived from adults truly reflect the PW childhood response from ages 3 to 6 years. However, by challenging these cultures with RV-A, we were able to replicate a common viral epithelial response–associated with PW in children. The fact that we were able to identify significant transcriptome differences suggests that the PW childhood phenotype after RV-A infection can be distinguished even when those participants are adults. The most important limitation of our study was the lack of replication of our findings in a large set of other samples of adult patients with asthma. We are unaware of any other study at this time that has followed participants from birth until the fourth decade of life to replicate our findings. Identifying the PW^+ phenotype in retrospective studies is a very difficult task, especially because adults have a paucity of information regarding events occurring during the preschool years.

In summary, we found that remodeling mechanisms and inflammatory processes were concurrently overactivated after RV-A exposure in nasal epithelial cells of adult patients with asthma and a history of persistent wheeze in the first 6 years of life as compared with in those whose symptoms started thereafter. Our results suggest that therapeutic approaches inhibiting these 2 major disease mechanisms in early life may play a major role in the prevention and treatment of early-onset asthma.

Clinical implications: Targeted approaches to inhibiting rhinovirus-induced upregulation of airway remodeling and immune responses in early life may play a major role in the prevention and treatment of early-onset asthma.

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