RESEARCH

Open Access

Check for updates

Peripheral blood miRNAs are associated with airflow below threshold in children with asthma

Anshul Tiwari^{1,2}, Brian D. Hobbs^{1,3}, Rinku Sharma¹, Jiang Li¹, Alvin T. Kho^{1,4}, Sami Amr⁵, Juan C. Celedón⁶, Scott T. Weiss¹, Craig P. Hersh^{1,2}, Kelan G. Tantisira⁷ and Michael J. McGeachie^{1,8*}

Abstract

Background MicroRNAs (miRNAs) are crucial post-transcriptional regulators involved in inflammatory diseases, such as asthma. Poor lung function and airflow issues in childhood are linked to the development of chronic obstructive pulmonary disease (COPD) in adulthood.

Methods We analyzed small RNA-Seq data from 365 peripheral whole blood samples from the Genetics of Asthma in Costa Rica Study (GACRS) for association with airflow levels measured by FEV1/FVC. Differentially expressed (DE) miRNAs were identified using DESeq2 in R, adjusting for covariates and applying a 10% false discovery rate (FDR). The analysis included 361 samples and 649 miRNAs. The two DE miRNAs were further tested for association with airflow obstruction in a study of adult former smokers with and without COPD.

Results We found 1 upregulated and 1 downregulated miRNA in participants with airflow below the threshold compared to those above it. In the adult study, the same miRNAs were upregulated and downregulated in individuals with FEV1/FVC < 0.7 versus those with FEV1/FVC > 0.7, showing suggestive statistical evidence. The target genes of these miRNAs were enriched for PI3K-Akt, Hippo, WNT, MAPK, and focal adhesion pathways.

Conclusions Two differentially expressed miRNAs were associated with airflow levels in children with asthma and airflow obstruction in adults with COPD. This suggests that shared genetic regulatory systems may influence childhood airflow and contribute to adulthood airflow obstruction.

Keywords miRNAs, Asthma, Airflow obstruction, COPD, Differential expression, FEV1/FVC ratio

*Correspondence:

Michael J. McGeachie remmg@channing.harvard.edu

¹Channing Division of Network Medicine, Brigham and Women's Hospital,

Harvard Medical School, Boston, MA, USA

²Department of Molecular Physiology and Biophysics, Vanderbilt

University, Nashville, TN, USA

³Division of Pulmonary and Critical Care Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA Medicine, Cambridge, MA, USA ⁶Division of Pediatric Pulmonary Medicine, UPMC Children's Hospital of Pittsburgh, University of Pittsburgh, Pittsburgh, PA, USA ⁷Division of Pediatric Respiratory Medicine, University of California San

Division of Pediatric Respiratory Medicine, University of California San Diego and Rady Children's Hospital, San Diego, CA, USA ⁸Channing Division of Network Medicine, Harvard Medical School, 181

Longwood Avenue, Room 539, Boston, MA 02115, USA



© The Author(s) 2025. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creative.commons.org/licenses/by-nc-nd/4.0/.

⁴Computational Health Informatics Program, Boston Children's Hospital, Boston, MA, USA ⁵Translational Genomics Core, Mass General Brigham Personalized

Introduction

Asthma affects around 23 million people in the United States and over 300 million people globally [1]. The characteristic of asthma is an obstruction of airflow that is often, but not always, reversible. A fixed airflow obstruction that is not completely reversible is a characteristic of chronic obstructive pulmonary disease (COPD). The term "airflow obstruction" refers to the observation of a reduced expiratory airflow relative to the total volume of air exhaled. This is indicated by a decline in the ratio of forced expiratory volume in 1 s (FEV₁) to forced vital capacity (FVC) [2]. The Dutch Hypothesis [3] proposed a genetic (or genomic) connection between asthma in childhood and COPD. Large-scale genome-wide association studies (GWASs) of asthma and COPD have illustrated an overall genetic correlation between asthma and COPD as well as multiple specific overlapping genomic loci with a concordant direction of effect [4]. However, despite strong evidence of partially overlapping genetic susceptibility to asthma and COPD, little research has centered on shared non-genetic omics drivers of airflow limitation or obstruction.

Recent findings have shown that mRNA regulation by microRNAs (miRNAs) may form part of the common genomic basis of asthma and COPD [5]. miRNAs are noncoding RNA molecules of 21-23 nucleotides that regulate gene expression by binding to 3' untranslated target mRNA regions and triggering translation degradation or inhibition [6]. miRNAs play significant regulatory roles in immunological and inflammatory responses in several tissues and are thus potential therapeutic targets in asthma and COPD [7]. Earlier studies of miRNAs in asthma centered on asthma itself, such as circulating miRNA expression in children with asthma compared with healthy controls, regulation of IL-5 expression by miRNA, differential expression of miRNA in asthmatics and healthy controls, and differential expression of miRNA in epithelial and airway cells in asthmatics and healthy controls [8-11]. We have previously demonstrated shared miRNA regulation of asthma and COPD exacerbations [12]; however, exacerbations generally result from a complex interplay of external stimuli such as allergens or respiratory infections, social or economic factors influencing the availability and cost of exigent hospital care, and predisposing internal biology. This work identified five miRs (451b; 7-5p; 532-3p; 296-5p and 766-3p) associated with childhood asthma exacerbations and adult COPD exacerbations. Complex multigenic disorders such as asthma have had hundreds of DNA variants associated [13], and the genomic regulation is similarly complex and multi-genic [14]. Previous genetic studies [15, 16] of airflow obstruction and of asthma exacerbations have identified disparate loci, leading to separate insights into the underlying pathogenesis of asthma [17]. Peripheral blood miRNAs have not been investigated in airflow limitation or obstruction in children with asthma and makes an intriguing follow-up to prior work investigating exacerbations. Therefore, we hypothesized that peripheral blood miRNA, separate from those that regulate exacerbations, would regulate immunological and inflammatory responses associated with airflow levels in children with asthma and that these effects would be observable in adults with COPD. We tested this hypothesis in two well-characterized cohorts, one including children with asthma and another including current and former smoking adults with and without COPD.

Methods

Genetics of asthma in costa rica study

Subject recruitment and the study protocol for the Genetics of Asthma in Costa Rica Study (GACRS) have been previously reported [18, 19]. In brief, the GACRS included 1,165 asthmatic Costa Rican children with asthma aged 6 to 14 years who were recruited between February 2001 and July 2011. Asthma was defined as physician-diagnosed asthma and having either at least two respiratory symptoms (wheezing, coughing, or dyspnea) or a history of asthma attacks in the previous year. All study participants also had a high probability of having six great-grandparents born in Costa Rica's Central Valley, as defined by a genealogist based on each parent's paternal and maternal last names. Following American Thoracic Society guidelines, spirometry was performed using a Survey Tach Spirometer (Warren E. Collins, Braintree, Mass). Written parental informed consent and the child's assent were obtained for all study participants. The study was approved by the Institutional Review Boards of the Hospital Nacional de Niños (San José, Costa Rica) and Brigham and Women's Hospital (Boston, MA).

COPDGene

The study design and protocol for the COPDGene trial (NCT00608764 on ClinicalTrials.gov) were previously reported in detail [20]. In brief, COPDGene is a prospective study of both non-Hispanic white and African American current and former smokers with at least 10 pack-years of smoking, with and without spirometry-defined COPD. We included 439 individuals from the COPDGene 5-year follow-up visit with available peripheral whole blood small RNA sequencing data.

Primary outcome

In GACRS spirometry was performed at the initial visit according to ATS guidelines. Forced expiratory volume in one second (FEV₁) and forced vital capacity (FVC) were measured. Each subject performed spirometry tests

to satisfaction three times, with the best being retained. These were converted to percent predicted FEV₁ and FVC using Hankinson et al. [21] equations based on healthy Mexican Americans, which assesses airflow relative to a person's age, sex, height, and race. Their ratio was used to define airflow level. In this cohort, the threshold for airflow was considered to be a predicted FEV₁/FVC ratio of 100%. We chose this threshold primarily to increase statistical power: clinical airflow obstruction was rare in GACRS so choosing a threshold such as FEV₁/FVC <70% would lead to unbalanced cases vs. controls. Participants with an airflow A predicted FEV₁/FVC of 100% represent the average for a healthy person of the appropriate age, sex, height, and race. This was treated as a binary variable and our primary outcome.

In the COPDGene study, post-bronchodilator spirometry was collected at the 5-year follow-up visit. In this cohort, we defined airflow obstruction as raw FEV₁/ FVC<0.7, as it is the most relevant division in COPD, and this threshold is used as part of the diagnosis of COPD.

Other clinical and demographic data were compared between cases and controls using Chi-squared tests for discrete variables and Student's t-test for continuous variables.

miRNA sequencing data and quality control

We used small RNA sequencing data previously sequenced on whole blood from 374 GACRS samples and 450 COPDGene samples [12]. GACRS blood samples were acquired at the time of phenotype assessment, between 2001 and 2005, and were stored at -80 degrees C until sequencing began in 2019. Small RNA-seq libraries were prepared using the NEXTflex Small RNA-Seq Kit v3 (PerkinElmer's, Waltham, MA, USA), which has a maximum input of 10 uL (10 ng to 250 ng). First, adapters were ligated to the 3' and 5' ends of the RNA. Reverse transcription was then carried out to generate cDNA from the ligated RNA. Following reverse transcription, cDNA yield was amplified by PCR, utilizing a distinct barcoded PCR primer for each sample. Finally, the PCR product entered size selection using magnetic beads for libraries in the range of 140–160 bp, resulting in the isolation of the indexed miRNA libraries. Libraries were then pooled and sequenced on an Illumina NextSeq 550 high output flow cell, with a run length of 75 bp single reads, to generate ~ 10 M reads per sample. Briefly, we applied blockers for hemolysis-linked miRNAs hsa-miR-486-5p, hsa-miR-92a, and hsa-miR-451a (PerkinElmer, Waltham, MA) that were added to the input sample before library creation [22]. For quality control (QC) of the RNA-seq data, the COMPSRA [23] and BCBio small RNA-seq (https://github.com/bcbio/bcbio-nextgen) pipelines were implemented. miRNAs with less than five mapped reads in at least 50% of participants were removed before analysis. Raw and processed GACRS miRNA data is available in the Gene Expression Omnibus, accession GSE244036. No passing miRNA had any sample more than three standard deviations from the mean (log scale). In GACRS, we utilized the guided Principal Component Analysis (gPCA) [24] package to find batch effects. The PCA of COPDGene samples revealed an outlier batch that was eliminated from further analysis.

Identification of differentially expressed miRs

DESeq2 [25] version 1.30.0 (R version 4.0.3) with a Benjamini-Hochberg false discovery rate (FDR) correction for multiple testing was used to identify differentially expressed miRNAs (upregulated and downregulated miRNAs) between those with airflow above and below threshold in GACRS. DESeq2 uses raw count data and performs internal normalization and then negative binomial regression, a test appropriate to RNA-Seq count data [25]. A significance threshold of 10% FDR was used. This FDR threshold indicates that 10% of our declared significant miRNAs are likely to be false positives. The analysis was adjusted for age, sex, use of inhaled corticosteroids (ICS) in the prior year, maternal smoking, BMI, genotype PCs, and sequencing batch. Logistic regression was used to obtain estimates of effect size (Odds Ratios) for a doubling of miR counts. For consistency with the differential expression analysis, data for logistic regression was normalized using the DESeq2 method, which is based on the geometric mean of samples per gene [25]. Additionally, we conducted a permutation test to assess the difference in mean expression levels of top miRs between groups with airflow above and below the threshold. The permutation test randomly shuffled the miRNA values while preserving the group structure, allowing us to generate a null distribution of differences in means. This null distribution was obtained by repeating the permutation process ten thousand times. Subsequently, we compared the observed difference in means, calculated from the original data to this null distribution to determine its statistical significance. This approach enabled a robust assessment of group differences in top miRNA expression levels.

Similarly, in the COPDGene study, DESeq2 was used to assess the association of top DE miRNAs with airflow obstruction (FEV1/FVC < 0.70) while adjusting for age, sex, smoking history (current vs. former), pack-years of cigarette smoking, self-reported race (either non-Hispanic white or African American), and sequencing batch.

Functional annotation of differentially expressed miRNAs

The Micro T-CDS [26], TarBase [27], and Target Scan [28] databases were used to identify target mRNA transcripts for top DE miR between the above and below

threshold airflow using the default parameters of the multiMiR package version 1.12 [29]. The clusterProfiler package version 3.18.1 was used to analyze the Kyoto Encyclopedia of Genes and Genomes (KEGG) [30] pathway using the union of each miR's targets [31]. To show substantial enrichment of targeted genes for a pathway, we used an adjusted p-value threshold of 0.05 and a gene count of 3 or more.

The miRNA-target gene network and enrichment analysis for the top DE miRNAs were generated using the web-based tool miRNet 2.0 [32]. For network construction, miRNet takes the list of DE miRNAs and retrieves the predicted and validated putative gene targets from Tarbase-8.0 and miRTarBase-8.0. Functional enrichment was done using the KEGG database and a hypergeometric test, with an FDR threshold of 0.1 considered significant (Fig. 1).

The transcription factor (TF)-miRNA-Gene regulatory network for Hippo, PI3K-Akt, and MAPK signaling pathway-related validated target genes of DE miRNAs was reconstructed. The network contains three relations: TF - DE miRNA, TF - Target gene, and DE miRNA target genes. We used TRRUST v2 [33] for TF - Target gene interactions, TransmiR v2.0 [34] for TF-miRNA interactions, and miRTarBase 7.0 [35] for experimentally validated (reporter assay or western blot) miRNAtarget gene interactions. The transcription factors (TFs) were limited to those involved in the Hippo, PI3K-Akt, and MAPK signaling pathways, with genes shown that are jointly regulated by at least two miRs or transcription factors.

Results

Cohort characteristics

Peripheral whole-blood samples were available for 365 of the 1,165 children with asthma in the GACRS (31.33%). Children with airflow below the threshold were of similar age and weight to those above the threshold. Children below the threshold differed from those above thresholds mostly in spirometry, with greater FVC and lower FEV₁, both pre-and post-bronchodilator administration (Table 1). Characteristics of participants undergoing small RNA sequencing in COPDGene are shown in Table 2.

Differentially expressed miRNAs

We had 361 samples and 649 miRNAs for DE analysis comparing the groups with airflow above (n = 220) and below (n = 141) threshold after quality control, filtering, and normalization.

In participants with airflow below threshold, we found one miRNA (let-7e-5p, p = 0.0001, FDR = 0.054; negative binomial regression odds ratio = 0.75, CI (0.64–0.87)) with higher expression and one miRNAs (miR-342-3p, *p*=0.0002, FDR=0.054; negative binomial regression odds ratio=1.15, CI (1.07–1.23)) with lower expression (Table 3; Figs. 2 and 3). These two miRNAs were then tested for association with airflow obstruction (FEV₁/ FVC ratio < 0.7) in the COPDGene study, in which let-7e-5p was upregulated (*p* < 0.064) and miR-342-3p (*p* < 0.085) was downregulated in participants with FEV₁/FVC < 0.7 (*n* = 196) compared to those with FEV₁/ FVC > 0.7 (*n* = 243) (Table 4).

Statistical significance of miR-342-3p and let-7e-5p were further assessed using a permutation test, with both showing strong evidence of no chance associations with airflow above or below threshold (miR-342-3p permutation p < 1e-04, let-7e-5p permutation p = 0.058). This test indicates that the observed difference in mean expression levels of miR-342-3p and let-7e-5p between the groups is extremely unlikely to have occurred by chance alone under the null hypothesis of no difference (Fig. 4).

To investigate potential pharmacogenomic effects on these two miRNAs, we then performed an analysis stratified by ICS use, and one also by SABA (Short-acting beta agonists) usage. We found that let-7e-5p was differentially expressed between higher and lower FEV1/FVC in the groups reporting regular SABA usage (p < 0.004) and the group reporting no recent ICS usage (p < 0.008), in each case being overexpressed in the higher FEV1/FVC group, consistent with our whole-cohort analysis.

These two miRNAs, therefore, showed the same direction of effect with airflow below threshold in childhood asthma (GACRS) and airflow obstruction in current and former smoking adults (COPDGene study).

Functional assessment of differentially expressed miRNAs

We performed a biological pathway enrichment analysis of putative gene targets of the 2 DE miRNAs using the clusterProfiler R package [31] (Fig. 5A). Phosphatidylinositol 3-kinase (PI3K) – protein kinase B (Akt), Hippo, Wingless-related integration site (WNT), Mitogenactivated protein kinase (MAPK), and focal adhesion signaling pathways were among the topmost enriched pathways. We also separately considered the targets of only the two miRNAs that were also associated with FEV_1/FVC in the COPDGene study, where PI3K-Akt, MAPK, and Hippo signaling pathways were among the top five most enriched pathways (Fig. 5B). The targets of these two miRNAs are shown in Fig. 1, with a highlighting of genes participating in enriched pathways previously associated with asthma.

Transcription factor-miRNA-gene regulatory network reconstruction for specific pathways

To further understand the regulatory relationship between DE miRNAs, target genes, and transcription factors (TF) related to Hippo, PI3K-Akt, and MAPK



Fig. 1 Network of two differentially expressed miRs between children with airflow above and below threshold, and their gene targets. Nodes with different colors indicate genes in selected enriched KEGG pathways

signaling pathway, the TF-miRNA-Gene regulatory network was re-constructed as shown in Fig. 6 (A, B, and C; images abbreviated to show genes regulated by at least two elements). DE miRNA let-7e-5p had the highest connectivity in all three pathway-specific networks (Fig. 6). Moreover, the two DE miRNAs were predicted to have common targets in all three pathways, for example, *CCND1*, *CCND2*, and *LIMD1* genes in the Hippo signaling pathway, *IGF1R* gene in MAPK signaling pathway, and (*THBS1*, *MDM2*, *LAMC1*, *IGF1R*, *CCND1*, *CCND2*) genes in PI3K-Akt signaling pathway, were found to be common targets for both DE miRNAs. Particularly, the

Table 1	Baseline	epidemio	logic and	clinical	characteristics o	f
the GACF	RS					

	Below threshold (N=220)	Above threshold (N=141)	<i>p</i> -value
Gender			
Male	92 (41.8%)	57 (40.4%)	0.879
Female	128 (58.2%)	84 (59.6%)	
Age (years)			
Mean (SD)	9.23 (1.76)	8.96 (1.98)	0.194
Median [Min, Max]	8.88 [6.12, 13.3]	8.90 [6.271, 12.9]	
Height (cm)			
Mean (SD)	133 (11.2)	132 (12.5)	0.53
Median [Min, Max]	131 [109, 167]	131 [103, 163]	
Weight (kg)			
Mean (SD)	33.1 (11.8)	31.9 (11.4)	0.356
Median [Min, Max]	30.9 [18.0, 81.6]	28.9 [15.0, 66.5]	
BMI			
Mean (SD)	18.3 (4.06)	17.8 (3.73)	0.27
Median [Min, Max]	17.5 [11.3, 41.4]	16.6 [12.5, 29.4]	
% predicted Pre-BD FEV1			
Mean (SD)	96.7 (15.3)	104 (16.2)	< 0.001
Median [Min, Max]	95.9 [46.2, 146]	102 [56.5, 180]	
% predicted Post-BD FEV1			
Mean (SD)	102 (14.2)	107 (15.7)	0.00186
Median [Min, Max]	101 [51.2, 152]	106 [57.3, 163]	
% predicted Pre-BD FVC			
Mean (SD)	105 (14.8)	98.7 (16.0)	< 0.001
Median [Min, Max]	104 [63.2, 162]	98.2 [52.5, 174]	
% predicted Post-BD FVC			
Mean (SD)	106 (14.2)	101 (16.1)	0.00434
Median [Min, Max]	105 [70.8, 167]	100 [53.1, 157]	
% predicted FEV1/ FVC Post-BD			
Mean (SD)	96.5 (6.25)	106 (4.54)	< 0.001
Median [Min, Max]	97.4 [73.5, 113]	106 [92.5, 114]	
FEV1/FVC Post-BD			
Mean (SD)	85.8 (5.76)	94.4 (4.04)	< 0.001
Median [Min, Max]	86.6 [66.4, 99.9]	94.2 [83.6, 100]	
FEV1/FVC Pre-BD			
Mean (SD)	82.1 (6.11)	94.0 (3.67)	< 0.001
Median [Min, Max]	83.4 [61.8, 90.5]	93.4 [87.8, 100]	
BD Response as % of baseline FEV1			
Mean (SD)	6.39 (9.63)	3.37 (7.78)	0.00119
Median [Min. Max]	4.41 [-11.8.48.6]	1.95 [-16.3. 35.3]	
Inhaled Steroids			
No	103 (46.8%)	62 (44.0%)	0.673
Yes	117 (53.2%)	79 (56.0%)	

% predicted Pre-BD FEV1: Percent predicted pre-Bronchodilator Forced expiratory volume in one second. % predicted pre-BD FVC: Percent predicted pre-Bronchodilator Forced vital capacity. % predicted FEV1/FVC post-BD: Percent predicted post-Bronchodilator FEV1/ FVC ratio. BD Response: Bronchodilator response as a percentage of pre-bronchodilator FEV1. Inhaled Steroids: respondents indicated the use of inhaled corticosteroids in the previous year. P-values computed with Chi-Square or Student's t-Test

Table 2	Baseline epidemiologic and clinical characteristics of
COPDGe	ne

Stratified by FEV1/FVC Ratio < 0.7			
	Ratio > 0.7	Ratio < 0.7	р
n	243	196	
Age (mean (SD))	62.64 (7.98)	68.81 (8.55)	< 0.001
Female Gender (%)	137 (56.4)	98 (50.0)	0.22
African American Race (%)	68 (28.0)	44 (22.4)	0.23
Current Smoking (%)	88 (36.2)	72 (36.7)	0.99
Pack Years Smoking (mean (SD))	37.82 (21.74)	50.82 (25.98)	< 0.001
FEV1pp (mean (SD))	90.37 (17.52)	63.84 (23.14)	< 0.001
FEV1/FVC Ratio (mean (SD))	0.78 (0.05)	0.56 (0.11)	< 0.001
WBC Count (mean (SD))	6.86 (2.18)	7.23 (1.94)	0.06
neutrophil % (mean (SD))	57.59 (10.16)	61.83 (10.41)	< 0.001
lymphocyte % (mean (SD))	31.41 (9.76)	26.28 (9.09)	< 0.001
monocyte % (mean (SD))	7.73 (2.23)	8.51 (2.92)	0.00
eosinophil % (mean (SD))	2.60 (1.89)	2.69 (1.94)	0.63
miRSeqBatch (%)			0.30
plate1	48 (19.8)	43 (21.9)	
plate2	48 (19.8)	36 (18.4)	
plate3	44 (18.1)	46 (23.5)	
plate4	59 (24.3)	33 (16.8)	
plate6	44 (18.1)	38 (19.4)	

MYC transcription factor regulates let-7e-5p expression in all three pathways.

Discussion

We report that 2 miRNAs (let-7e-5p; miR-342-3p) are suggestively associated with airflow in a study of Costa Rican children with asthma. Of these two miRNAs, one was downregulated, and one was upregulated in participants with airflow below the threshold. miR-342-3p and let-7e-5p were observed to have the same direction of effect for differential expression related to airflow obstruction (FEV₁/FVC ratio < 0.7) in COPDGene study participants, with suggestive statistical evidence.

Our choice of predicted FEV1/FVC ratio dichotomization at 100% represents a threshold chosen around the midpoint of the spectrum of observed airflow obstruction, or lack thereof, in GACRS. In healthy individuals, we would expect half of the subjects to be above 100% FEV1/FVC percent predicted. This contrasts with the 70% threshold chosen for replication in COPD, which represents a clinically meaningful level of obstruction in that disease. The GACRS cohort of children with mild asthma has low levels of fixed airflow obstruction, making a threshold of mild or no obstruction appropriate. Research has shown that poor lung function in childhood asthma can lead to adult COPD [36, 37], and while we don't expect to see the same level of airflow obstruction in children as in COPD, identifying those with relatively poorer airflow compared to other children with asthma

Table 3 Significant up- and down-regulated miRNAs in GACKS. Mean counts: normalized mean counts in the reference group.
Log2 FC: base-2-fold change from infrequent to frequent exacerbators. Odds ratio: computed with logistic regression. 95% CI: 95%
confidence interval for odds ratio. P-value: for differential expression between above and below-threshold groups, computed with
DESeq2. FDR: false discovery rate adjusted p-values, with FDR < 0.10 considered significant

miR	Mean Counts	log2 FC	Odds Ratio	95% CI	<i>p</i> -value	FDR
hsa-let-7e-5p	92.7	0.426	0.75	(0.64 – 0.87)	0.0001	0.054
hsa-miR-342-3p	9580	-0.201	1.15	(1.07–1.23)	0.0002	0.054



Fig. 2 Differential expression of peripheral blood miRNA between airflow above and below threshold in the GACRS. Benjamini-Hochberg adjusted p-values are shown. The mean expression is shown in the log2 scale. The vertical dotted lines correspond to a fold change up and down; the horizontal line represents an FDR < 10%

identifies those at greater risk of future clinical airflow obstruction.

We sought to generalize our childhood asthma miRNA airflow association to adult current and former smokers in the COPDGene study. Asthma is a major risk factor for COPD. However, airflow obstruction defined by reduced

 Table 4
 Replication of up-and down-regulated miRNAs

 between above and below EEV1/EVC threshold in COPDGene

Setticen above and Sciott FETIVITE threshold in cor Bidene					
miR	baseMean	log2FC	pvalue		
hsa-let-7e-5p	1476	0.155	0.064		
hsa-miR-342-3p	10,460	-0.094	0.086		

FEV₁/FVC can occur through a variety of pathophysiologic mechanisms. Our work shows that airflow below the threshold in asthma and obstruction in adult current and former smokers share some regulatory precursors, which agrees with research showing that asthma and COPD have overlapping causes [3, 4]. These two miRNAs associated with airflow (FEV₁/FVC) in both childhood asthma and current and former smoking adults have been previously associated with inflammation [38-40]. let-7e-5p has been linked to a proinflammatory role in asthma [41], and previously connected to the PI3-AKT, MAPK, Hippo, and Wnt signaling pathways, crucial regulatory pathways in asthma etiology [42-44]. In a murine model, miR-342-3p was linked to allergic airway disease, and it was also found to decrease inflammation in human macrophage THP-1 cells [40, 45]. Airflow obstruction in asthma is primarily linked to a distinctive form of airway inflammation that includes an increase in T cells (mainly CD4+) and eosinophils, as well as a thicker reticular layer of the epithelial basement membrane [46].



Fig. 3 let-7e-5p (left) and miR-342-3p (right) are distributed above or below the airflow threshold in GACRS. X-Axis: FEV1/FVC ratio above (1) and below (0) threshold



Fig. 4 Distribution of label-shuffling permutation test values for differential expression of hsa-miR-342-3p (a) and hsa-let-7e-5p (b). Red line: True test value for differential expression between above and below threshold FEV1/FVC ratio



Fig. 5 KEGG pathways enriched for target genes of two differentially expressed miRs between children with airflow above and below threshold (342-3p and let-7e-5p). Gene targets for miRs were identified using microT-CDS Diana, Target Scan & TarBase databases. *Gene Ratio*: genes of interest in the gene set over the total genes of interest

Some colleagues have previously investigated the association of serum miRNAs and FEV1/FVC in childhood asthma [10]. In a smaller study using 160 North American children with asthma on inhaled corticosteroids, Kho et al. [10]. identified 22 miRs associated with airway obstruction. We did not find an overlap between these 22 and our results here, but significant technical variations, as well as population differences and issues of statistical power, may have led to these results.



Fig. 6 Transcription factor (TF)-miRNA-Gene regulatory networks related to (A) Hippo, (B) PI3K-Akt and (C) MAPK signaling pathways, with regulation by both miR-342-3p and let-7e-5p

In vitro and in vivo studies have demonstrated that exogenous over-expression of miR-342-3p induced apoptosis and inhibited tumorigenicity, cell growth, invasion, and migration [47], cellular phenotypes which may be related to airway remodeling and lead to reduced FEV₁/FVC. Another study reported the downregulated expression of miR-342-3p in COPD cases [48].

The Hippo pathway, which regulates organ size in the embryonic stages of development by encouraging apoptosis and regulating cell proliferation, is evolutionarily conserved throughout Drosophila melanogaster to humans [49, 50]. Yes-associated protein 1 (YAP1 or YAP) and transcriptional coactivator with PDZ-binding motif (TAZ), the main hippo effectors, are transcriptional coactivators that bind to transcription factors such as TEAD, SMAD, or TP73 when activated to influence the expression of various genes [51–53]. It was recently reported that YAP1 is abundantly expressed in embryonic and mature lung respiratory epithelial cells. Hippo/ YAP1 signaling controls epithelial cell proliferation and differentiation, as well as embryonic lung development and postnatal airway homeostasis [54]. Furthermore, it has been revealed in mice that YAP1 is dynamically regulated during airway epithelial regeneration following lung damage, indicating a potential function for Hippo/YAP1 signaling in the etiology of acute and chronic lung disorders [55].

It is well accepted that YAP1 and TAZ function as downstream effectors of the Hippo pathway. They have emerged as important translational co-activators of a wide range of biological processes, and they play an important role in lung development and function. The YAP1/TAZ signaling pathway is dysregulated, which contributes to the development and progression of chronic lung conditions such as asthma, COPD, and lung infection. Therefore, owing to its critical functions, Chaulk et al. reported that cell density-mediating nuclear expression of YAP1/TAZ is required for high levels of Dicer to occur in MCF-10 A cell lines. Mechanistically, let-7 family members, which are well-validated as tumor suppressors, are significantly attenuated by nuclear YAP1/ TAZ levels. Increased nuclear YAP1/TAZ can suppress the process of pre-miRNA conversion into mature let-7 miRNA by mediating the Dicer enzyme. However, let-7 biogenesis is dependent on cell density, rather than the expression of YAP1/TAZ [56, 57].

Genetic polymorphisms in FRMD6, an upstream activator of the Hippo pathway, have been linked to asthma and lung function [58]. Furthermore, genetic variations in the BIRC5 gene (also known as survivin), one of YAP1's target genes, increased the risk of inflammatory disorders such as asthma. Furthermore, FRMD6 mRNA levels in sputum were considerably lower in asthmatic patients than in healthy controls, although BIRC5 levels were greater [58-60]. let-7e-5p and miR-342-3p, gene targets were enriched with Age-RAGE signaling pathway proteins, and this pathway is a major culprit in diabetic complications. Numerous researchers have discovered that the Receptor for Advanced Glycation End-products (RAGE) pathway expression is strongest in the lungs, and it has been identified as a driving factor for inflammation in pulmonary pathophysiology and associated with COPD [61-63]. Research has demonstrated a critical role of RAGE throughout the development of the Th-2 high allergic airway disease [64]. Taken together with our results, this supports a role for miRNA regulation of these RAGE-mediated processes leading to airway inflammation, airway remodeling, and later airflow obstruction.

The MAPK and PI3K-Akt signaling pathways, which have previously been related to asthma, were enriched in both gene targets of the two DE miRNAs and gene targets of the two miRNAs impacting both asthma and COPD lung function. The relationship between these two pathways to asthma was recently discussed in our previous study [12], which linked changes in miRNA expression to asthma exacerbations. The miRNA identified in that work also had mRNA targets significantly enriched for MAPK and PI3K-Akt pathway genes, and much of that discussion is also relevant to airway obstruction in asthma and COPD. MAPK and PI3K-Akt dysregulation can result in airway remodeling which can result in obstruction as well as a predisposition for increased exacerbations.

In addition to Th2-high eosinophilic asthma and Th2low neutrophilic asthma, MAPK signaling may also play a role in COPD [46]. Smoke from cigarettes can activate the MAPK pathway in small airway epithelial cells, which results in the release of chemokines and inflammatory cytokines [65]. In Th2-low neutrophilic asthma and COPD, this kind of activation can result in airway remodeling, which lowers lung function and increases the chance of an exacerbation [66]. In allergic asthma, the PI3K-Akt pathway plays a regulatory role [67]. When PI3K-Akt is activated, other signaling molecules are activated downstream, one of which is NF-kB, a transcription factor that promotes inflammation. Inhibition of PI3K-Akt reduces the expression of proinflammatory cytokines IL-4, IL-6, and IL-8, as well as Tumor Necrosis Factor-alpha (TNF-a) and Immunoglobulin E (IgE); additionally, this pathway is regulated by other miRNA [68]. PI3K-Akt has also been implicated in inflammation in COPD and has been suggested as a possible therapeutic target in COPD [69].

let-7e-5p was also discovered to regulate an antiinflammatory gene, archetypal dual-specificity phosphatase (DUSP1), which is involved in a feedback system that regulates the harmful inflammatory response caused by the MAPK cascade [70, 71]. Recent studies showed that among various proliferation pathways, the PI3K pathway is the key signaling route in asthmatic airway smooth muscle (ASM) proliferation while the ERK(MAPK) pathway provides a complementary signal required for the full mitogenic response [72, 73]. PI3K pathway gene, Phosphatase, and tensin homolog (PTEN) which was previously reported to regulate the proliferation and migration of ASM cells in Asthma [74-76] was found to be regulated by let-7e-5p. DUSP1 and PTEN gene expression were found to be regulated by the same transcription factor, TP53 (Fig. 6). Both target genes share the same transcriptional and post-transcriptional regulators, revealing the existence of complex crosstalk between the pathways targeted by the DE miRNAs, fine-tuning the expression of target genes and transcription factors for precise cellular response in Asthma.

We have previously investigated the regulation of asthma exacerbations [12] and bronchodilator response (BDR) [77] by blood and serum, respectively, miRNA in children. The two replicated miRNAs here, miR-342-3p and let-7e-5p, were not identified in those studies, as they did not meet multiple testing criteria for statistical significance. However, miR-342-3p miRNA was nominally associated with BDR at a p < 0.05 significance level, indicating that miR-342-3p may have a broader impact on childhood asthma. Further, miR-342-3p was not identified in a recent study of miRNAs associated with increased SABA usage [78]. We further investigated the pharmacogenomic effects of these two miRNAs in stratified analysis finding that only let-7e-5p showed evidence of differential expression when restricted to the no-ICS group and the SABA-usage group. These results are inconclusive and potentially provocative and should

be investigated more in future work. We recognize that our study has several limitations. Our definition of airflow above or below the threshold in childhood asthma does not indicate clinical obstruction. Our definition was chosen because of concerns for statistical power in the GACRS cohort, where serious obstruction was rare. This expediency should bias us toward the null result of no miRNA associations. While the cohorts we interrogated to investigate childhood asthma and adult COPD have clinical differences, such as age, the history of smoking, and severity of disease, these differences would again bias toward the null hypothesis of no common miRNA associations. Because of differences in cohort demographics and causes of airway limitation in participants in GACRS and the COPDGene study, we would expect fewer miR-NAs to replicate from one study to the other. Similarly, there was no cell composition data for the GACRS samples, and including these as covariates may provide additional insight. Additionally, we were unable to establish a strong temporal connection between airflow below the threshold and related miRNAs since the GACRS is cross-sectional.

Conclusion

Two miRNAs were linked to airflow levels in childhood asthma. These two miRNAs, hsa-miR-342-3p and hsa-let-7e-5p, show suggestive generalization.

with FEV₁/FVC in current and former smoking adults. The miRNAs' targets were shown to be highly enriched in several established asthma and COPD-related pathways, such as PI3-AKT, Hippo, and MAPK signaling. These findings suggest that the biology of airflow limitation and obstruction may be partially due to shared microRNA gene regulatory mechanisms regardless of host factors or disease context.

Author contributions

A.T., M.J.M., A.T.K., S.T.W., C.P.H., B.D.H., J.C.C., and K.G.T. designed the study. A.T., J.L., B.D.H, and R.S. performed the analysis. S.A. performed small RNA sequencing. A.T. compiled data and wrote the initial manuscript. A.T. and M.J.M. wrote the manuscript. All authors critically reviewed the manuscript drafts and approved the final version.

Funding

This work received primary funding from NIH grants: R01 HL139634, R01 HL127332, R01 HL162570, R01 161362, P01 HL132825, R01 HL130512, U19 Al118608, R01 HL125734, K08 HL136928. Additional Grant support for COPDGene is from Award Number U01 HL089897 and Award Number U01 HL089856 from the National Heart, Lung, and Blood Institute. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Heart, Lung, and Blood Institute or the National Institutes of Health. COPDGene is also supported by the COPD Foundation through contributions made to an Industry Advisory Board that has included AstraZeneca, Bayer Pharmaceuticals, Boehringer-Ingelheim, Genentech, GlaxoSmithKline, Novartis, Pfizer, and Sunovion.

Data availability

Primary miRNA data can be downloaded from here https://www.ncbi.nlm.nih. gov/geo/query/acc.cgi?acc=GSE244036.

Declarations

Institutional review board statement

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of Brigham and Women's Hospital, IRB# 2017P001799, 7/29/2020. Additional approval for the Costa Rica cohort was obtained from the Hospital Nacional de Niños (San José, Costa Rica) and Brigham and Women's Hospital (Boston, MA, USA). COPDGene Institutional Review Board (IRB) approval was obtained at each of the participating study centers before study initiation.

Consent for publication

Not applicable.

Clinical trial number

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 13 September 2024 / Accepted: 13 January 2025 Published online: 24 January 2025

References

- Dharmage SC, Perret JL, Custovic A. Epidemiology of asthma in children and adults. Front Pediatr. 2019;7:246.
- Eschenbacher WL. Defining airflow obstruction. Chronic Obstr Pulm Dis. 2016;3(2):515–8.
- Postma DS, Weiss ST, van den Berge M, Kerstjens HAM, Koppelman GH. Revisiting the Dutch hypothesis. J Allergy Clin Immunol. 2015;136(3):521–9.
- Sakornsakolpat P, Prokopenko D, Lamontagne M, Reeve NF, Guyatt AL, Jackson VE, et al. Genetic landscape of chronic obstructive pulmonary disease identifies heterogeneous cell-type and phenotype associations. Nat Genet. 2019;51(3):494–505.
- Tiwari A, Li J, Kho AT, Sun M, Lu Q, Weiss ST, et al. COPD-associated mir-145-5p is downregulated in early-decline FEV(1) trajectories in childhood asthma. J Allergy Clin Immunol. 2021;147(6):2181–90.
- Wang Z, Lu Y, Han J. Peripheral blood microRNAs: a novel tool for diagnosing disease? Intractable Rare Dis Res. 2012;1(3):98–102.
- 7. De Smet EG, Mestdagh P, Vandesompele J, Brusselle GG, Bracke KR. Noncoding RNAs in the pathogenesis of COPD. Thorax. 2015;70(8):782–91.
- Chandan K, Gupta M, Sarwat M. Role of host and Pathogen-derived MicroR-NAs in Immune Regulation during Infectious and Inflammatory diseases. Front Immunol. 2020;10:3081.
- Tahamtan A, Teymoori-Rad M, Nakstad B, Salimi V. Anti-inflammatory MicroR-NAs and their potential for inflammatory diseases treatment. Front Immunol. 2018;9:1377.
- Kho AT, McGeachie MJ, Moore KG, Sylvia JM, Weiss ST, Tantisira KG. Circulating microRNAs and prediction of asthma exacerbation in childhood asthma. Respir Res. 2018;19(1):128.
- Rupani H, Sanchez-Elsner T, Howarth P. MicroRNAs and respiratory diseases. Eur Respir J. 2012;41(3):695–705.
- Tiwari A, Hobbs BD, Li J, Kho AT, Amr S, Celedon JC et al. Blood miRNAs are linked to frequent asthma exacerbations in Childhood Asthma and Adult COPD. Noncoding RNA. 2022;8(2).
- Gautam Y, Afanador Y, Ghandikota S, Mersha TB. Comprehensive functional annotation of susceptibility variants associated with asthma. Hum Genet. 2020;139(8):1037–53.
- Imboden M, Bouzigon E, Curjuric I, Ramasamy A, Kumar A, Hancock DB, et al. Genome-wide association study of lung function decline in adults with and without asthma. J Allergy Clin Immunol. 2012;129(5):1218–28.
- Herrera-Luis E, Ortega VE, Ampleford EJ, Sio YY, Granell R, de Roos E, et al. Multi-ancestry genome-wide association study of asthma exacerbations. Pediatr Allergy Immunol. 2022;33(6):e13802.
- Albert FW, Kruglyak L. The role of regulatory variation in complex traits and disease. Nat Rev Genet. 2015;16(4):197–212.
- Hernandez-Pacheco N, Vijverberg SJ, Herrera-Luis E, Li J, Sio YY, Granell R et al. Genome-wide association study of asthma exacerbations despite inhaled corticosteroid use. Eur Respir J. 2021;57(5).

- Kho AT, Sordillo J, Wu AC, Cho MH, Sharma S, Tiwari A, et al. CASTER: crosssectional asthma STEroid response measurement. J Pers Med. 2020;10(3):95.
- Hunninghake GM, Soto-Quiros ME, Avila L, Ly NP, Liang C, Sylvia JS, et al. Sensitization to Ascaris lumbricoides and severity of childhood asthma in Costa Rica. J Allergy Clin Immunol. 2007;119(3):654–61.
- 20. Regan EA, Hokanson JE, Murphy JR, Make B, Lynch DA, Beaty TH, et al. Genetic epidemiology of COPD (COPDGene) study design. COPD. 2010;7(1):32–43.
- Hankinson JL, Odencrantz JR, Fedan KB. Spirometric reference values from a sample of the general U.S. population. Am J Respir Crit Care Med. 1999;159(1):179–87.
- 22. LaBelle J, Bowser M, Brown A, Farnam L, Kho A, Li J, et al. Commercially available blocking oligonucleotides effectively suppress unwanted hemolysisrelated miRNAs in a large whole-blood RNA cohort. J Mol Diagnostics: JMD. 2021;23(6):671–82.
- Li J, Kho AT, Chase RP, Pantano L, Farnam L, Amr SS, et al. COMPSRA: a COMprehensive platform for small RNA-Seq data analysis. Sci Rep. 2020;10(1):4552.
- Reese SE, Archer KJ, Therneau TM, Atkinson EJ, Vachon CM, de Andrade M, et al. A new statistic for identifying batch effects in high-throughput genomic data that uses guided principal component analysis. Bioinf (Oxford England). 2013;29(22):2877–83.
- Love MI, Huber W, Anders S. Moderated estimation of Fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014;15(12):550.
- Paraskevopoulou MD, Georgakilas G, Kostoulas N, Vlachos IS, Vergoulis T, Reczko M et al. DIANA-microT web server v5.0: service integration into miRNA functional analysis workflows. Nucleic Acids Res. 2013;41(Web Server issue):W169–73.
- Karagkouni D, Paraskevopoulou MD, Chatzopoulos S, Vlachos IS, Tastsoglou S, Kanellos I, et al. DIANA-TarBase v8: a decade-long collection of experimentally supported miRNA-gene interactions. Nucleic Acids Res. 2018;46(D1):D239–45.
- Agarwal V, Bell GW, Nam J-W, Bartel DP. Predicting effective microRNA target sites in mammalian mRNAs. eLife. 2015;4:e05005.
- Ru Y, Kechris KJ, Tabakoff B, Hoffman P, Radcliffe RA, Bowler R, et al. The multiMiR R package and database: integration of microRNA-target interactions along with their disease and drug associations. Nucleic Acids Res. 2014;42(17):e133–e.
- Kanehisa M, Sato Y, Kawashima M, Furumichi M, Tanabe M. KEGG as a reference resource for gene and protein annotation. Nucleic Acids Res. 2016;44(D1):D457–62.
- 31. Yu G, Wang L-G, Han Y, He Q-Y. clusterProfiler: an R package for comparing biological themes among gene clusters. OMICS. 2012;16(5):284–7.
- Chang L, Zhou G, Soufan O, Xia J. miRNet 2.0: network-based visual analytics for miRNA functional analysis and systems biology. Nucleic Acids Res. 2020;48(W1):W244–51.
- Han H, Cho JW, Lee S, Yun A, Kim H, Bae D, et al. TRRUST v2: an expanded reference database of human and mouse transcriptional regulatory interactions. Nucleic Acids Res. 2018;46(D1):D380–6.
- Tong Z, Cui Q, Wang J, Zhou Y. TransmiR v2.0: an updated transcription factormicroRNA regulation database. Nucleic Acids Res. 2019;47(D1):D253–8.
- Chou CH, Shrestha S, Yang CD, Chang NW, Lin YL, Liao KW, et al. miRTarBase update 2018: a resource for experimentally validated microRNA-target interactions. Nucleic Acids Res. 2018;46(D1):D296–302.
- Silva GE, Sherrill DL, Guerra S, Barbee RA. Asthma as a risk factor for COPD in a longitudinal study. Chest. 2004;126(1):59–65.
- McGeachie MJ, Yates KP, Zhou X, Guo F, Sternberg AL, Van Natta ML, et al. Patterns of growth and decline in lung function in Persistent Childhood Asthma. N Engl J Med. 2016;374(19):1842–52.
- Kho AT, Sharma S, Davis JS, Spina J, Howard D, McEnroy K, et al. Circulating MicroRNAs: Association with lung function in Asthma. PLoS ONE. 2016;11(6):e0157998–e.
- 39. Huang Z, Fu B, Qi X, Xu Y, Mou Y, Zhou M, et al. Diagnostic and therapeutic value of Hsa_circ_0002594 for T helper 2-Mediated allergic asthma. Int Arch Allergy Immunol. 2021;182(5):388–98.
- Li J, Tiwari A, Mirzakhani H, Wang AL, Kho AT, McGeachie MJ et al. Circulating MicroRNA: Incident Asthma prediction and vitamin D effect modification. J Pers Med. 2021;11(4).
- Davis JS, Sun M, Kho AT, Moore KG, Sylvia JM, Weiss ST, et al. Circulating microRNAs and association with methacholine PC20 in the Childhood Asthma Management Program (CAMP) cohort. PLoS ONE. 2017;12(7):e0180329.

- Vinas JL, Ventayol M, Brune B, Jung M, Sola A, Pi F, et al. miRNA let-7e modulates the wnt pathway and early nephrogenic markers in mouse embryonic stem cell differentiation. PLoS ONE. 2013;8(4):e60937.
- Dong P, Konno Y, Watari H, Hosaka M, Noguchi M, Sakuragi N. The impact of microRNA-mediated PI3K/AKT signaling on epithelial-mesenchymal transition and cancer stemness in endometrial cancer. J Transl Med. 2014;12:231.
- Wagner S, Ngezahayo A, Murua Escobar H, Nolte I. Role of miRNA let-7 and its major targets in prostate cancer. Biomed Res Int. 2014;2014:376326.
- Rutledge H, Baran-Gale J, de Villena FP-M, Chesler EJ, Churchill GA, Sethupathy P, et al. Identification of microRNAs associated with allergic airway disease using a genetically diverse mouse population. BMC Genomics. 2015;16(1):633.
- 46. Bakakos A, Vogli S, Dimakou K, Hillas G. Asthma with fixed airflow obstruction: from fixed to Personalized Approach. J Pers Med. 2022;12(3).
- Romero-Cordoba SL, Rodriguez-Cuevas S, Bautista-Pina V, Maffuz-Aziz A, D'Ippolito E, Cosentino G, et al. Loss of function of mir-342-3p results in MCT1 over-expression and contributes to oncogenic metabolic reprogramming in triple negative breast cancer. Sci Rep. 2018;8(1):12252.
- Molina-Pinelo S, Pastor MD, Suarez R, Romero-Romero B, De la Gonzalez M, Salinas A, et al. MicroRNA clusters: dysregulation in lung adenocarcinoma and COPD. Eur Respir J. 2014;43(6):1740–9.
- Fodor LE, Gézsi A, Ungvári L, Semsei AF, Gál Z, Nagy A, et al. Investigation of the possible role of the Hippo/YAP1 Pathway in Asthma and Allergy. Allergy Asthma Immunol Res. 2017;9(3):247–56.
- Huang J, Wu S, Barrera J, Matthews K, Pan D. The Hippo Signaling Pathway Coordinately regulates cell proliferation and apoptosis by inactivating Yorkie, the Drosophila Homolog of YAP. Cell. 2005;122(3):421–34.
- Alarcón C, Zaromytidou A-I, Xi Q, Gao S, Yu J, Fujisawa S, et al. Nuclear CDKs drive smad transcriptional activation and turnover in BMP and TGF-beta pathways. Cell. 2009;139(4):757–69.
- Strano S, Munarriz E, Rossi M, Castagnoli L, Shaul Y, Sacchi A, et al. Physical Interaction with Yes-associated protein enhances p73 transcriptional activity. J Biol Chem. 2001;276(18):15164–73.
- Vassilev A, Kaneko KJ, Shu H, Zhao Y, DePamphilis ML. TEAD/TEF transcription factors utilize the activation domain of YAP65, a Src/Yes-associated protein localized in the cytoplasm. Genes Dev. 2001;15(10):1229–41.
- Mahoney JE, Mori M, Szymaniak AD, Varelas X, Cardoso WV. The hippo pathway effector Yap controls patterning and differentiation of airway epithelial progenitors. Dev Cell. 2014;30(2):137–50.
- Lange AW, Sridharan A, Xu Y, Stripp BR, Perl A-K, Whitsett JA. Hippo/Yap signaling controls epithelial progenitor cell proliferation and differentiation in the embryonic and adult lung. J Mol Cell Biol. 2015;7(1):35–47.
- Chaulk SG, Lattanzi VJ, Hiemer SE, Fahlman RP, Varelas X. The Hippo pathway effectors TAZ/YAP regulate dicer expression and microRNA biogenesis through Let-7. J Biol Chem. 2014;289(4):1886–91.
- Johnson CD, Esquela-Kerscher A, Stefani G, Byrom M, Kelnar K, Ovcharenko D, et al. The let-7 MicroRNA represses cell proliferation pathways in human cells. Cancer Res. 2007;67(16):7713–22.
- Ungvari I, Hullam G, Antal P, Kiszel PS, Gezsi A, Hadadi E, et al. Evaluation of a partial genome screening of two asthma susceptibility regions using bayesian network based bayesian multilevel analysis of relevance. PLoS ONE. 2012;7(3):e33573.
- Ungvari I, Hadadi E, Virag V, Bikov A, Nagy A, Semsei AF, et al. Implication of BIRC5 in asthma pathogenesis. Int Immunol. 2012;24(5):293–301.
- 60. Wenzel SE. Asthma: defining of the persistent adult phenotypes. Lancet. 2006;368(9537):804–13.
- Brett J, Schmidt AM, Yan SD, Zou YS, Weidman E, Pinsky D, et al. Survey of the distribution of a newly characterized receptor for advanced glycation end products in tissues. Am J Pathol. 1993;143(6):1699–712.
- Sukkar MB, Ullah MA, Gan WJ, Wark PA, Chung KF, Hughes JM, et al. RAGE: a new frontier in chronic airways disease. Br J Pharmacol. 2012;167(6):1161–76.
- Oczypok EA, Perkins TN, Oury TD. All the RAGE in lung disease: the receptor for advanced glycation endproducts (RAGE) is a major mediator of pulmonary inflammatory responses. Paediatr Respir Rev. 2017;23:40–9.
- 64. Perkins TN, Donnell ML, Oury TD. The axis of the receptor for advanced glycation endproducts in asthma and allergic airway disease. Allergy. 2021;76(5):1350–66.
- Pelaia C, Vatrella A, Sciacqua A, Terracciano R, Pelaia G. Role of p38-mitogenactivated protein kinase in COPD: pathobiological implications and therapeutic perspectives. Expert Rev Respir Med. 2020;14(5):485–91.
- 66. DiMango E, Rogers L, Reibman J, Gerald LB, Brown M, Sugar EA, et al. Risk factors for Asthma Exacerbation and Treatment failure in adults and adolescents

with Well-controlled asthma during continuation and step-down therapy. Ann Am Thorac Soc. 2018;15(8):955–61.

- 67. Athari SS. Targeting cell signaling in allergic asthma. Signal Transduct Target Ther. 2019;4:45.
- Guo Q, Jin Y, Chen X, Ye X, Shen X, Lin M, et al. NF-kappaB in biology and targeted therapy: new insights and translational implications. Signal Transduct Target Ther. 2024;9(1):53.
- 69. Bozinovski S, Vlahos R, Hansen M, Liu K, Anderson GP. Akt in the pathogenesis of COPD. Int J Chron Obstruct Pulmon Dis. 2006;1(1):31–8.
- Theodorou J, Nowak E, Bock A, Salvermoser M, Beerweiler C, Zeber K, et al. Mitogen-activated protein kinase signaling in childhood asthma development and environment-mediated protection. Pediatr Allergy Immunol. 2022;33(1):e13657.
- 71. Moosavi SM, Prabhala P, Ammit AJ. Role and regulation of MKP-1 in airway inflammation. Respir Res. 2017;18(1):154.
- Yap HM, Israf DA, Harith HH, Tham CL, Sulaiman MR. Crosstalk between signaling pathways involved in the regulation of Airway smooth muscle cell hyperplasia. Front Pharmacol. 2019;10:1148.
- Burgess JK, Lee JH, Ge Q, Ramsay EE, Poniris MH, Parmentier J, et al. Dual ERK and phosphatidylinositol 3-kinase pathways control airway smooth muscle proliferation: differences in asthma. J Cell Physiol. 2008;216(3):673–9.
- Wu Y, Lu Y, Zou F, Fan X, Li X, Zhang H, et al. PTEN participates in airway remodeling of asthma by regulating CD38/Ca(2+)/CREB signaling. Aging. 2020;12(16):16326–40.

- Luo L, Gong YQ, Qi X, Lai W, Lan H, Luo Y. Effect of tumor suppressor PTEN gene on apoptosis and cell cycle of human airway smooth muscle cells. Mol Cell Biochem. 2013;375(1–2):1–9.
- 76. Alexandrova E, Miglino N, Hashim A, Nassa G, Stellato C, Tamm M, et al. Small RNA profiling reveals deregulated phosphatase and tensin homolog (PTEN)/ phosphoinositide 3-kinase (PI3K)/Akt pathway in bronchial smooth muscle cells from asthmatic patients. J Allergy Clin Immunol. 2016;137(1):58–67.
- 77. Sharma R, Tiwari A, Kho AT, Wang AL, Srivastava U, Piparia S, et al. Circulating microRNAs associated with bronchodilator response in childhood asthma. BMC Pulm Med. 2024;24(1):553.
- Wong R, Desai B, Sharma R, Srivastava U, Kho AT, Weiss ST, et al. Identification of MicroRNAs in children with increased asthma bronchodilator usage in genetics of asthma in Costa Rica Study. Pediatr Pulmonol. 2024;59(12):3491–8.

Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.