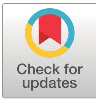
A-to-I editing of miR-200b-3p in airway cells is associated with moderate-to-severe asthma

Kevin M. Magnaye [1,3], Katherine A. Naughton [1], Janel Huffman [1], D. Kyle Hogarth [2], Edward T. Naureckas [2], Steven R. White [2] and Carole Ober [1,3]

[1]Dept of Human Genetics, University of Chicago, Chicago, IL, USA. [2]Dept of Medicine, University of Chicago, Chicago, IL, USA. [3]These two authors contributed equally to this article as lead authors and supervised the work.

Corresponding author: Carole Ober (c-ober@genetics.uchicago.edu)



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A genome-wide study of endogenous ADAR-mediated miRNA editing in airway cells revealed associations between A-to-I editing of miR-200b-3p and a target gene, *SOCS1*, with asthma severity, suggesting a novel mechanism and therapeutic target for severe asthma https://bit.ly/2JWsifY

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Abstract

Background Asthma is a chronic lung disease characterised by persistent airway inflammation. Altered microRNA (miRNA)-mediated gene silencing in bronchial epithelial cells (BECs) has been reported in asthma, yet adenosine deaminase acting on RNA (ADAR)-mediated miRNA editing in asthma remains unexplored.

Methods We first identified adenosine to inosine (A-to-I) edited sites in miRNAs in BECs from 142 adult asthma cases and controls. A-to-I edited sites were tested for associations with asthma severity and clinical measures of asthma. Paired RNA sequencing data were used to perform pathway enrichments and test for associations with bioinformatically predicted target genes of the unedited and edited miRNAs.

Results Of 19 A-to-I edited sites detected in these miRNAs, one site at position 5 of miR-200b-3p was edited less frequently in cases compared with controls ($p_{corrected}$=0.013), and especially compared with cases with moderate ($p_{corrected}$=0.029) and severe ($p_{corrected}=3.9\times10^{-4}$), but not mild ($p_{corrected}$=0.38), asthma. Bioinformatic prediction revealed 232 target genes of the edited miR-200b-3p, which were enriched for both interleukin-4 and interferon-γ signalling pathways, and included the *SOCS1* (suppressor of cytokine signalling 1) gene. *SOCS1* was more highly expressed in moderate ($p_{corrected}$=0.017) and severe ($p_{corrected}=5.4\times10^{-3}$) asthma cases compared with controls. Moreover, both miR-200b-3p editing and *SOCS1* were associated with bronchoalveolar lavage eosinophil levels.

Conclusions Reduced A-to-I editing of position 5 of miR-200b-3p in lower airway cells from moderate-to-severe asthmatic subjects may lead to overexpression of *SOCS1* and impaired cytokine signalling. We propose ADAR-mediated editing as an epigenetic mechanism contributing to features of moderate-to-severe asthma in adulthood.

Introduction

Asthma is a common chronic inflammatory disease of the airways, affecting as many as 300 million people worldwide [1]. Although symptoms in most asthmatic subjects can be controlled with standard therapies, ∼15% of cases respond poorly to treatment and account for a disproportionate burden on healthcare costs and quality of life [2]. Genetic studies of response to the most common therapies, *i.e.* corticosteroids and β-agonists, have revealed few clues to genetic mechanisms underlying interindividual variation in response [3, 4] and a recent genome-wide association study of moderate-to-severe asthma generally mirrored the associations with asthma [5, 6]. These combined findings suggest that other mechanisms of gene regulation contribute to moderate-to-severe asthma.

MicroRNA (miRNA)-mediated gene silencing has been implicated in the pathogenesis of asthma [7, 8]. miRNAs are approximately 22-nucleotide RNAs that bind to 3′-untranslated regions of target genes and

downregulate their expression. Using array-based studies of 876 miRNAs, Solberg *et al.* [7] showed that the percentage of differentially expressed miRNAs far exceeded the percentage of differentially expressed mRNAs in bronchial epithelial cells (BECs) from 47 subjects. In a study of 190 selected miRNAs measured by quantitative PCR in airway T-cells from 43 asthmatic subjects, Simpson *et al.* [8] reported altered expression of miR-19a and its gene targets that promoted type 2 (T2) cytokine production. These findings highlighted miRNA abundances as potentially important gene regulatory mechanisms in the lower airways of individuals with asthma.

miRNA-mediated gene silencing is often altered by adenosine deaminase acting on RNA (ADAR)-mediated editing, a novel epigenetic mechanism that involves the deamination of adenosine to inosine (A-to-I). The ADAR family of enzymes contributes to one of the most prevalent forms of post-transcriptional modifications of miRNAs [9]. Because most editing events in miRNAs occur in the seed regions [10], the critical determinants of target complementarity, A-to-I edited sites are expected to downregulate the expression of gene targets that differ from the nonedited forms. ADAR expression can be induced by interferons (IFNs) [11], suggesting an important role for ADAR-mediated editing in innate inflammatory responses. In fact, elevated ADAR expression and subsequent A-to-I editing activity have significant effects on the transcriptomes of cancer cells and may even correlate with patient survival [12, 13]. Furthermore, widespread A-to-I editing by ADAR is present in immune tissues and cells, such as spleen, thymus and peripheral lymphocytes, in response to inflammation in endotoxin-treated mice [14]. Yet, the role of ADAR-mediated editing of miRNAs in asthma has not previously been explored.

Here, we present the first genome-wide analysis of ADAR-mediated editing of miRNAs using small RNA sequencing in primary BECs from 142 asthma cases and controls. Our study revealed that A-to-I editing of position 5 of miR-200b-3p was less frequent in BECs from cases with moderate-to-severe asthma. Using paired RNA sequencing data, we identified a predicted target of the edited miR-200b-3p, *SOCS1* (suppressor of cytokine signalling 1), which was upregulated in moderate-to-severe asthma cases. Overall, our study identified ADAR-mediated editing of miR-200b-3p in the lower airway as a novel contributor to moderate-to-severe asthma.

Methods

The primary goal of this study was to investigate an epigenetic mechanism, *i.e.* ADAR-mediated miRNA editing, and its impact on gene expression in BECs of asthma cases and controls.

Subject recruitment and evaluation

The subjects in this study participated in Institutional Review Board-approved, National Institutes of Health-funded research studies on the genetics of asthma, as described elsewhere [15–20]. Adults (>18 years of age) were recruited through asthma clinics and recruitment postings at the University of Chicago Medical Center (Chicago, IL, USA) between 2010 and 2014.

All subjects were clinically evaluated at a first visit when their case or control status was confirmed by pulmonary function and methacholine challenge testing. Spirometry was performed, followed either by methacholine challenge testing when baseline forced expiratory volume in 1 s (FEV_1) was ⩾60% predicted or by reversibility studies when baseline FEV_1 was <60% predicted, following guidelines recommended by the American Thoracic Society [21]. Exhaled nitric oxide fraction (F_{ENO}) was measured on a NIOX MINO device (Aerocrine, Morrisville, NC, USA) during exhalations at $50\ mL \cdot s^{-1}$, following American Thoracic Society/European Respiratory Society guidelines [22]. Asthma diagnosis included the following criteria: 1) a physician's diagnosis of asthma; 2) either a fall in baseline FEV_1 ⩾20% at $<16\ mg \cdot mL^{-1}$ methacholine in subjects whose FEV_1 predicted was ⩾70%, or a ⩾15% increase in baseline FEV_1 after inhalation of a bronchodilator (albuterol) or over time with treatment in subjects whose FEV_1 predicted was <70%; 3) at least two symptoms (cough, wheeze and dyspnoea); 4) <3 pack-years of cigarette exposure; and 5) no medical contraindications or conflicting pulmonary diagnoses. None of the asthma patients were receiving therapy with anti-IgE, anti-interleukin (IL)-5 or anti-IL-4/13 monoclonal antibodies at the time of our studies. Controls were adult subjects who had no current or previous diagnosis of asthma, normal spirometry and methacholine challenge tests, and no medical contraindications for bronchoscopy. Demographic variables and blood samples were collected for DNA, and measurements of total serum IgE, and a complete blood count with differential. During a second visit, bronchoscopy was performed. BECs were obtained *via* endobronchial brushings for small and total RNA studies, and bronchoalveolar lavage (BAL) fluid was collected to measure eosinophil and neutrophil counts.

A total of 161 subjects had either available genotypes for estimating ancestry principal components (n=158), small RNA sequences (n=142) or total RNA sequences (n=128) (supplementary figure S1). The

supplementary material contains a description of our pipeline for processing and quality control of the genotyping, small RNA sequencing and total RNA sequencing.

These studies were approved by the University of Chicago's Institutional Review Board. Written informed consent was obtained from all subjects.

Analysis of A-to-I edited sites

A-to-I edited sites within miRNAs were identified using the A-to-I analysis module in miRge2.0 (supplementary material) [23]. Differential A-to-I editing was examined using a linear model, as implemented in limma [24]. The four A-to-I edited sites that were present in at least 10 subjects were tested for differential editing between asthma cases and controls, adjusting for age, sex, current smoking status and the first three ancestry principal components, using a Bonferroni correction for four tests. Pairwise comparisons between control and asthma severity groups using STEP classification [25] were performed using the makeContrasts function in limma, using a Bonferroni correction for three tests.

Analyses of target gene expression

Target genes for the unedited and edited miR-200b-3p were bioinformatically predicted using TargetScanHuman5.2 and TargetScanHuman5.2 Custom, respectively (supplementary material) [26]. Pathway analysis was performed for expressed target genes (counts per million >1 in at least 25% of 124 subjects with processed total RNA expression data) of the unedited and edited miR-200b-3p using the TopFunn function within the ToppGene Suite for gene list enrichment analysis [27]. Significant pathways ($p<0.05$ after Bonferroni correction) are reported.

The average normalised expression of gene targets of the four enriched pathways of the edited miR-200b-3p was tested for differential expression between 83 asthma cases and 41 controls. For pathways reported from multiple databases, the pathway enrichment with the greatest enrichment of genes was selected. Average expression was measured as the mean over the standard deviation of normalised gene expression. Differential expression analysis was performed using a linear model in limma, adjusted for age, sex, current smoking status, sequencing pool, the first three ancestry principal components and 15 surrogate variables, and Bonferroni-corrected for four tests. Average expression and individual gene expression of the 10 predicted targets of the edited miR-200b-3p that were enriched for IL-4 signalling or IFN-γ pathways were also tested for differential expression between cases and controls and between STEP classification groups using the makeContrasts function in limma; p-values were Bonferroni-corrected for 10 tests.

Correlations with measures of asthma

Asthma-related measures were tested for associations with both asthma and asthma severity [25]. Continuous and categorical variables were evaluated between cases and controls with the Wilcoxon rank-sum test or Fisher's exact test, respectively. Associations with asthma severity were tested with an ordinal logistic regression using the MASS package in R; p-values were Bonferroni-corrected for nine tests (clinical measures).

Spearman's rank correlation coefficients were used to assess correlations of A-to-I editing at position 5 of miR-200b-3p and *SOCS1* expression with six clinical measures that were at least nominally associated with asthma or asthma severity: total serum IgE, F_{ENO}, BAL eosinophilia, blood eosinophilia, body mass index (BMI) and an epithelial cell signature of T2 inflammation that is a marker for T2-high asthma [28]. The latter was measured as the sum of the expression levels of *POSTN*, *CLCA1* and *SERPINB2* transcripts (T2 gene signature) [29] using processed RNA sequencing data in the same BECs described earlier. Because asthma severity (STEP classification) was based on steroid use and lung function measures, we excluded inhaled corticosteroid, oral corticosteroid, $\mathrm{FEV_1}$ % pred and $\mathrm{FEV_1}$/forced vital capacity (FVC) from correlations with asthma severity. The same covariates, as in the associations with asthma described earlier, were regressed out from the percentage of A-to-I editing; the A-to-I editing residuals and *SOCS1* expression were tested for correlations with each of six measures; p-values were Bonferroni-corrected for six tests.

Results

Identifying A-to-I edited sites in BECs

Of the 142 adults in our study, 138 (94 asthma cases and 44 nonasthma controls) had more than 2 million small RNA reads in BECs and were included in downstream analyses. We identified 19 A-to-I edited sites in 17 miRNAs (supplementary table S1). The mean A-to-I editing at the 19 sites ranged from 0.14% to 34.09% across individuals with detectable levels of editing (see Methods); 16 of the 19 A-to-I edited sites were in miRNA seed regions (positions 2–8). Nearly all of the A-to-I edited sites (18 out of 19) were

TABLE 1 A-to-I edited sites detected in bronchial epithelial cells from at least 10 out of 138 subjects

miRNA	Position	Sequence	Average mapped counts n	Average A-to-I reads %	Subjects with edited miRNA n
miR-200b-3p	5	TAATACTGCCTGGTAATGATGA	75 358	0.52	135
miR-191-5p	3	CAACGGAATCCCAAAAGCAGCTG	58 512	0.17	27
miR-200b-3p	3	TAATACTGCCTGGTAATGATGA	91 380	0.15	22
miR-186-5p	3	CAAAGAATTCTCCTTTTGGGCT	24 646	0.18	13

miRNA: microRNA. The edited adenosine within the mature miRNA sequence is in bold font; the seed sequence is underlined. All 19 A-to-I edited sites are shown in supplementary table S1.

detected in <20% of individuals (range 1–27). Position 5 of miR-200b-3p was the most frequently edited site in the sample: 97.10% of individuals had edited sites at this position.

Differential A-to-I editing of miR-200b-3p between cases and controls

Four edited sites were observed in more than 10 subjects (table 1), consistent with the number of edited sites in previous studies of glioblastoma that included healthy lung tissue [10] and of tumour cells from lung adenocarcinoma patients [13]. In the 135 subjects with available genotypes for estimating ancestry principal components, differential editing (% A-to-I) was tested between 91 asthma cases and 44 nonasthma controls. The most edited site, position 5 of miR-200b-3p, was significantly less frequently edited in cases compared with controls ($p_{corrected}$=0.013) (figure 1a). This significant decrease in A-to-I editing remained after excluding one outlier among the controls ($p_{corrected}$=0.023) (supplementary figure S2). The other three sites were not differentially edited between asthma cases and controls (supplementary figure S3).

We next assessed whether A-to-I editing of position 5 of miR-200b-3p was associated with asthma severity. A-to-I editing was less frequent in the moderate (n=22) and severe (n=46) asthma groups compared with controls (n=44) ($p_{corrected}$=0.029 and 3.9×10^{-4}, respectively) (figure 1b and supplementary figure S2). The frequency of editing did not differ between mild asthma cases (n=23) and controls ($p_{corrected}$=0.38).

Downstream targets of unedited and edited miR-200b-3p

The A-to-I edited site at position 5 is in the seed region of miR-200b-3p and predicted to downregulate the expression of novel target genes. We used TargetScanHuman to bioinformatically predict the target genes

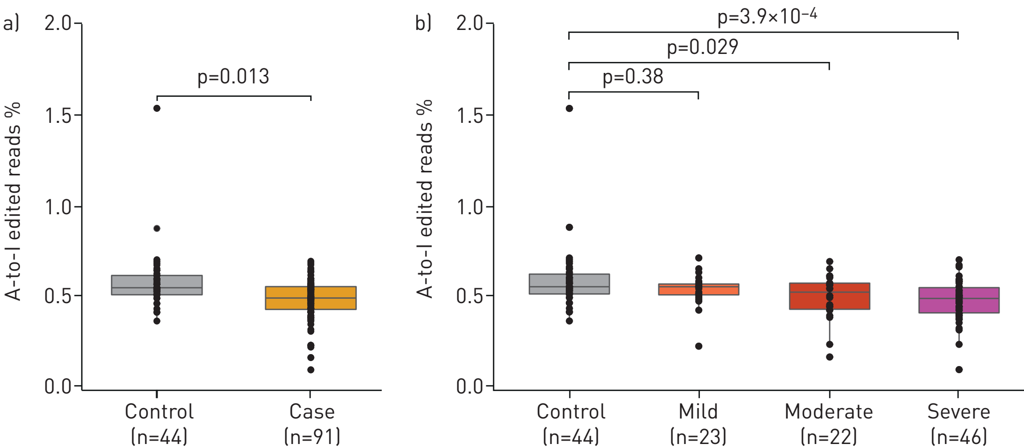
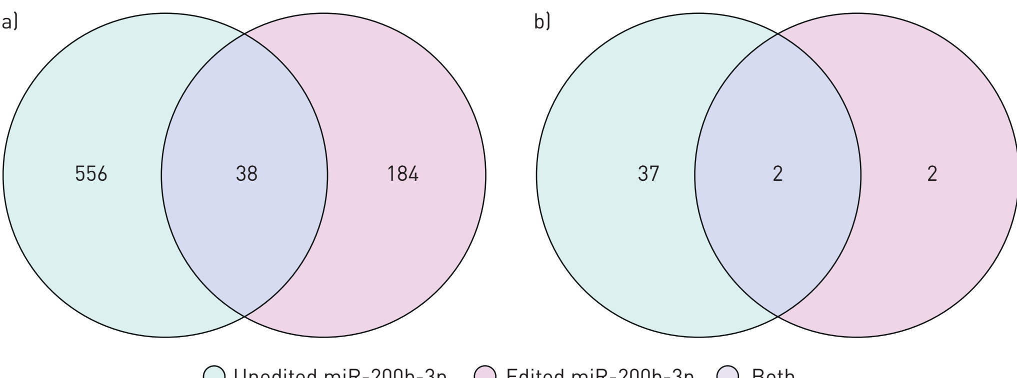


FIGURE 1 A-to-I editing of position 5 of miR-200b-3p in asthma cases and controls. Associations of percentage of A-to-I editing of position 5 of miR-200b-3p in bronchial epithelial cells with a) asthma and b) asthma severity groups based on STEP classification scores. Box-and-whisker plots show median (interquartile range) and minimum–maximum; outliers are indicated. One outlier with the highest percentage of edited reads in the control group (>3SD) was included here (see supplementary figure S2 with the outlier excluded). The number of subjects is shown below each risk group. Adjusted p-values are shown; the p-values for a) and b) were corrected for four and three tests, respectively, using a Bonferroni correction (see main text).



c)

ID	Pathway name	Database	Adjusted p-value	Genes from input n	Genes in annotation n	Genes from input
M195	C-MYB transcription factor network	MSigDB C2 BIOCARTA (version 7.1)	1.85E-03	9	84	*CEBPB, SPI1, ETS1, KRAS, SKI, CSF1R, ATP2B1, MYB, CDK6*
138073	C-MYB transcription factor network	BioSystems: Pathway Interaction Database	1.02E-02	8	78	*CEBPB, SPI1, ETS1, SKI, CSF1R, ATP2B1, MYB, CDK6*
138036	FoxO family signalling	BioSystems: Pathway Interaction Database	4.81E-02	6	48	*ZFAND5, FOXO1, YWHAE, YWHAZ, SIRT1, CSNK1G2*

d)

ID	Pathway name	Database	Adjusted p-value	Genes from input n	Genes in annotation n	Genes from input
137933	IL-4-mediated signalling events	BioSystems: Pathway Interaction Database	1.99E-03	8	63	*CEBPB, SPI1, ETS1, PIK3CA, CBL, SOCS5, SOCS1, MYB*
M28	IL-4-mediated signalling events	MSigDB C2 BIOCARTA (version 7.1)	2.25E-03	8	64	*CEBPB, SPI1, ETS1, PIK3CA, CBL, SOCS5, SOCS1, MYB*
M161	IFN-γ pathway	MSigDB C2 BIOCARTA (version 7.1)	1.64E-02	6	40	*CEBPB, PIK3CA, RAP1A, RAP1B, CBL, SOCS1*

FIGURE 2 Significant pathways for the target genes of the unedited and edited miR-200b-3p. a) Gene targets. The 604 gene targets of the unedited miR-200b-3p and the 232 gene targets of the edited miR-200b-3p that were expressed in bronchial epithelial cells (counts per million >1) were included as input for pathway analysis, as implemented in TopFunn. p-values were adjusted for using a Bonferroni correction. b) Pathways. The 604 gene targets of the unedited miR-200b-3p were enriched for at least 39 pathways (supplementary table S2) and the 232 gene targets of the edited miR-200b-3p were enriched for four pathways. c) Pathways shared by the target genes of the edited and unedited miR-200b-3p. Of the four significant pathways enriched for gene targets of the edited miR-200b-3p, two pathways that included "C-MYB transcription factor network" from two databases and "FoxO family signalling" were also enriched for the target genes of both the unedited and edited miR-200b-3p. d) Pathways specific to the target genes of the edited miR-200b-3p. The two pathways that were only enriched for the target genes of the edited miR-200b-3p were "IL-4-mediated signalling events" from two databases and "IFN-γ pathway". MSigDB: www.gsea-msigdb.org/gsea/msigdb; BioSystems: www.ncbi.nlm.nih.gov/biosystems.

of the unedited and edited miR-200b-3p. Of the 798 *in silico* target genes of the unedited miR-200b-3p, 604 (75.7%) were detected as expressed in BECs; of the 320 *in silico* target genes for the edited miR-200b-3p, 232 (72.5%) were detected as expressed in BECs (figure 2a). Only 48 expressed genes overlapped between the two sets of target genes. Pathway analysis (TopFunn) of the 604 expressed target genes of the unedited miR-200b-3p, which was more frequent in the cases, resulted in 39 significant pathways, most of which were involved in kinase signalling, proliferation and differentiation (supplementary table S2). In contrast, the 232 expressed target genes of the edited miR-200b-3p, which was more frequent in the controls, were significantly enriched for four pathways (figure 2b). Two pathways were the same for the edited and unedited miR-200b-3p gene targets: "C-MYB transcription factor network" and "FoxO family signalling" (figure 2c). Two pathways were specific to the edited miR-200b-3p: "IL-4-mediated signalling events" and "IFN-γ pathway" (figure 2d).

For 124 subjects with available RNA sequencing data, we examined the average normalised expression of the expressed target genes in each of the four enriched pathways of the edited miR-200b-3p. The average expression of the eight target genes for "IL-4-mediated signalling events" and the six target genes in the "IFN-γ pathway" were both significantly increased in cases (n=83) compared with controls (n=41) ($p_{corrected}=2.9\times10^{-3}$ and 1.8×10^{-3}, respectively) (figure 3a and b). In contrast, the average expression was not different between cases and controls for the nine target genes in the "C-MYB transcription factor

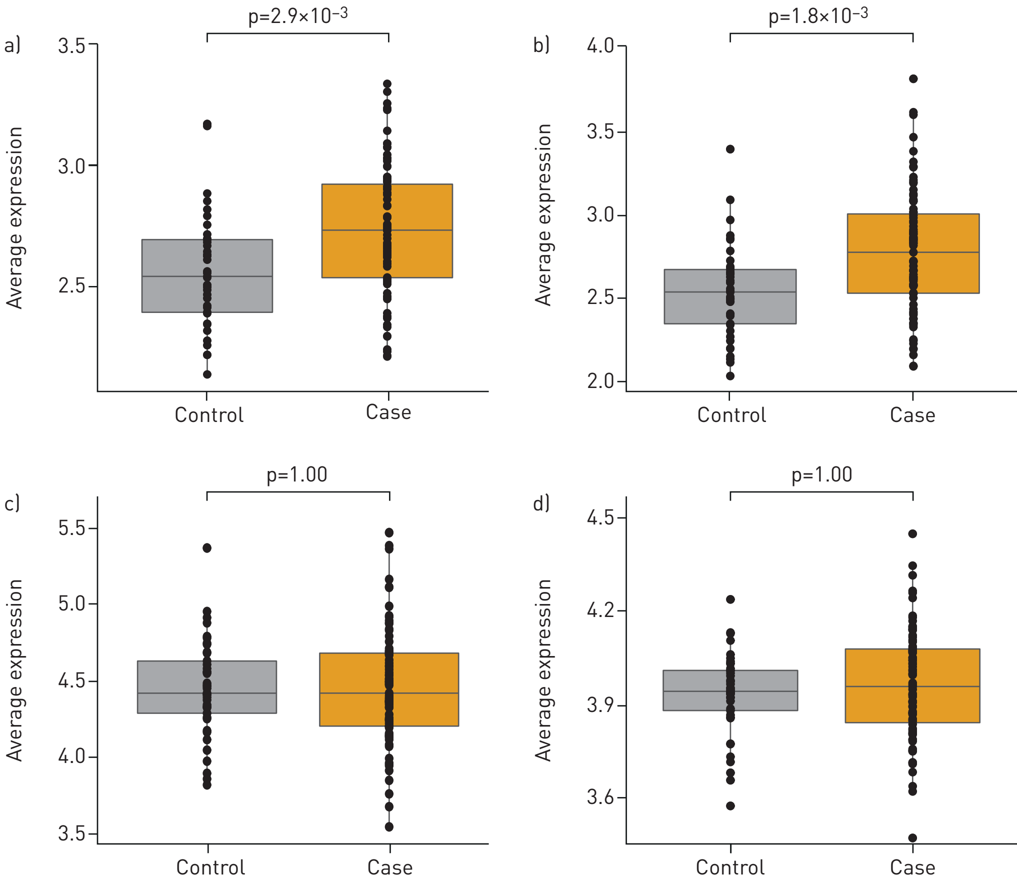


FIGURE 3 Average normalised expression of the genes in each of the four pathways of the edited miR-200b-3p in asthma cases and controls. Only gene targets that were expressed (counts per million >1) in bronchial epithelial cells in at least 25% of the 124 subjects (83 cases and 41 controls) with RNA sequencing data were included. a, b) Average normalised expression of the two pathways that were enriched only for target genes of the edited miR-200b-3p: a) "IL-4-mediated signalling events" (eight genes) and b) "IFN-γ pathway" (six genes). c, d) Average normalised expression of the two pathways that were enriched for target genes of both the unedited and edited forms of miR-200b-3p: c) "C-MYB transcription factor network" (nine genes) and d) "FoxO family signalling" (six genes). Box-and-whisker plots show median (interquartile range) and minimum–maximum; outliers are indicated. Bonferroni-corrected p-values (for four tests) are shown.

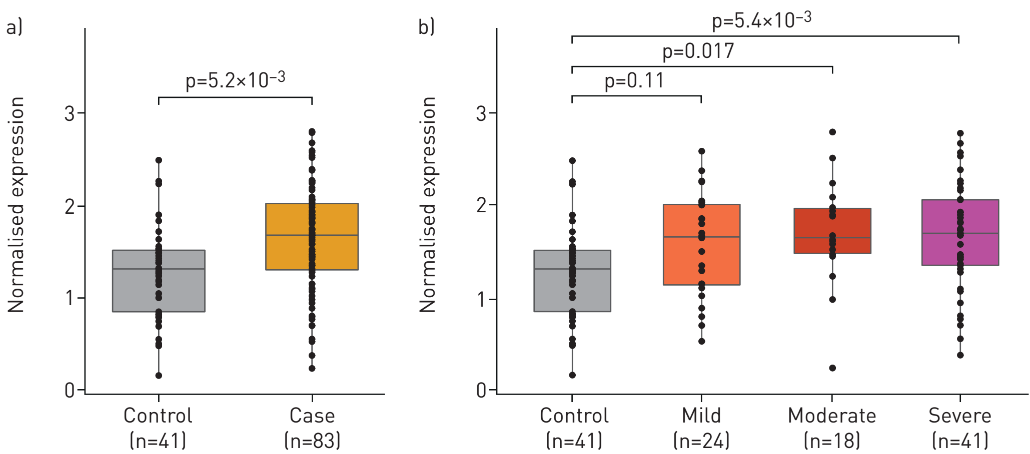


FIGURE 4 Normalised gene expression of *SOCS1*, a target of the edited miR-200b-3p, in bronchial epithelial cells between a) asthma cases and controls and b) asthma severity groups based on STEP classification. Box-and-whisker plots show median (interquartile range) and minimum–maximum; outliers are indicated. The numbers of subjects are shown below the risk groups. The p-values for a) and b) were corrected for 10 and three tests, respectively, using a Bonferroni correction (see main text)

network" or for the six target genes for "FoxO family signalling" ($p_{corrected}$=1.00) (figure 3c and d). The increased expression of the target genes of both the IL-4 signalling and IFN-γ pathways in the cases is consistent with the edited miRNA binding to and downregulating the expression of these genes, and with the observed higher frequency of editing of this miRNA in the controls.

Of the 10 target genes in the IL-4 and IFN-γ pathways, *SOCS1*, a negative regulator of both T2 cytokine signalling [30, 31] and IFN signalling induction [32, 33], was the only individual gene that was significantly overexpressed in cases (n=83) ($p_{corrected}=5.2\times10^{-3}$) (figure 4a), and in moderate (n=18) and severe (n=41), but not in mild (n=24), cases compared with controls (n=41) ($p_{corrected}$=0.017, 5.4×10^{-3} and 0.11, respectively) (figure 4b). None of the other nine target genes were differentially expressed between cases and controls (supplementary figure S4). Thus, the decreased editing of position 5 of miR-200b-3p in BECs from moderate and severe asthma cases is associated with overexpression of an important negative regulator of cytokine signalling, *SOCS1*.

miR-200b-3p editing, SOCS1 expression and measures of asthma

Because both miR-200b-3p editing and *SOCS1* expression were associated with asthma severity (figures 1 and 4), we next examined their effects on clinical measures of asthma. We first assessed their relationships with asthma and asthma severity (table 2). Compared with controls, asthma cases had significantly lower lung function, as measured by FEV_1 % pred and FEV_1/FVC, and higher F_{ENO}, BAL eosinophils, blood eosinophils, BMI and the T2 gene signature (*CLCA1*, *POSTN* and *SERPINB2*) (table 2). We next examined associations between these measures and STEP classification of asthma severity, based on corticosteroid use and lung function (table 2). Three measures, in addition to the lung function measures, were associated with STEP classification. BAL eosinophils were highest in the moderate and severe asthma cases, BMI was highest in the severe asthma cases, and the T2 gene signature was highest in the moderate asthma cases. Severe asthma cases were also older and had higher total serum IgE and lower F_{ENO} compared with moderate asthma cases, although these differences were not significant after multiple testing correction. Overall, these clinical profiles suggest that moderate and severe cases differ with respect to distinct sets of T2 asthma features.

We next tested for correlations between six clinical measures that were at least nominally associated with asthma or asthma severity with A-to-I editing of position 5 of miR-200b-3p and *SOCS1* expression (table 3). Because STEP classification is a composite score based on lung function measures, those measures were not included in this analysis. A-to-I editing of position 5 of miR-200b-3p was negatively correlated with BAL eosinophils and BMI, as well as nominally with the T2 gene signature. *SOCS1* expression was positively correlated with BAL eosinophils and the T2 gene signature, as well as nominally with F_{ENO}, a marker of airway inflammation, although not with BMI. These combined data support a link between

TABLE 2 Clinical characteristics of subjects at the time of bronchoscopy

	Asthma			Asthma severity				
	Control	All cases	p-value[#]	Control	Mild	Moderate	Severe	p-value[¶]
Subjects n	44	94		44	23	23	48	
Covariates								
Age years	37.25±11.54	40.16±12.77	0.21	37.25±11.54	34.83±12.61	37.96±13.83	43.77±11.37	9.48×10^{-3}
Female %	64	73	0.32	64	61	70	81	0.06
Ethnicity n			0.03					0.02
African American	28	54		28	16	16	22	
European American	12	39		12	7	7	25	
Other	4	1		4	0	0	1	
Smoker at bronchoscopy %	16	4	0.04	16	9	0	4	0.03
Clinical measures								
ICS use %		74			9	91	98	
OCS use %		37			0	0	73	
FEV_1 % pred	95.18±11.31	73.67±19.20	2.03×10^{-10}*	95.18±11.31	83.61±16.60	79.96±18.84	65.90±17.47	5.34×10^{-10}*
FEV_1/FVC	0.81±0.05	0.76±0.30	1.88×10^{-7}*	0.81±0.05	0.80±0.25	0.74±0.08	0.75±0.37	2.05×10^{-6}*
Total serum IgE IU·mL^{-1}	56.50 (27.25–169.00)	121.50 (37.00–360.25)	0.02	56.50 (27.25–169.00)	95.00 (28.50–171.50)	116.00 (70.50–465.50)	194.50 (24.75–387.00)	0.02
F_{ENO} ppb	14.00 (10.75–17.75)	25.00 (14.00–45.00)	9.75×10^{-5}*	14.00 (10.75–17.75)	25.00 (17.00–54.00)	31.00 (19.00–47.50)	20.00 (13.00–34.00)	0.04
BAL eosinophilia %	0.2 (0.0–0.5)	2.9 (1.3–5.8)	3.92×10^{-14}*	0.2 (0.0–0.5)	1.9 (1.0–4.8)	3.3 (1.7–7.2)	3.1 (1.5–6.0)	1.21×10^{-5}*
BAL neutrophilia %	4.8 (3.0–6.2)	4.5 (2.3–6.0)	0.59	4.8 (3.0–6.2)	3.7 (1.9–5.3)	5.0 (2.9–6.9)	4.5 (2.5–6.3)	0.93
Blood eosinophilia cells·μL^{-1}	100 (70–170)	190 (80–318)	1.43×10^{-3}*	100 (70–170)	140 (80–250)	220 (135–335)	200 (78–303)	0.01
BMI kg·m^{-2}	28.00 (24.68–31.70)	32.80 (26.08–40.50)	4.24×10^{-3}*	28.00 (24.68–31.70)	29.60 (24.00–33.25)	28.10 (24.10–33.40)	38.00 (30.95–43.50)	2.29×10^{-6}*
T2 gene signature	1.64 (0.15–5.53)	7.38 (2.12–11.68)	7.55×10^{-6}*	1.64 (0.15–5.53)	5.49 (0.80–12.07)	10.03 (7.13–11.39)	5.45 (1.89–11.48)	4.35×10^{-4}*

Data are presented as mean±SD or median (interquartile range), unless otherwise stated. ICS: inhaled corticosteroid; OCS: oral corticosteroid; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; F_{ENO}: exhaled nitric oxide fraction; BAL: bronchoalveolar lavage; BMI: body mass index; T2: type 2. [#]: continuous variables were evaluated with the Wilcoxon rank-sum test and categorical variables were evaluated with Fisher's exact test; [¶]: all variables were assessed using an ordinal logistic regression. Five severe asthma cases had missing F_{ENO} measurements. One control each was missing BAL and blood eosinophil measurements. Normalised and covariate-adjusted gene expression was available for 41 controls and 83 cases (24 mild, 18 moderate and 41 severe) to assess the T2 gene signature (sum of *POSTN*, *CLCA1* and *SERPINB2*). *: significant correlations after correcting for nine tests (clinical measures) using a Bonferroni correction.

TABLE 3 miR-200b-3p editing, *SOCS1* expression and measures of asthma

	miR-200b-3p editing				*SOCS1* expression			
	Subjects n[#]	ρ	p-value	Adjusted p-value	Subjects n[#]	ρ	p-value	Adjusted p-value
Total serum IgE IU·mL^{-1}	135	−0.07	0.41	1.00	124	0.21	0.017	0.10
F_{ENO} ppb	130	−0.15	0.089	0.53	119	0.24	8.5×10^{-3}	0.05
BAL eosinophilia %	134	−0.24	5.9×10^{-3}*	0.04*	123	0.30	6.2×10^{-3}*	0.04*
Blood eosinophilia cells·µL^{-1}	134	−0.05	0.60	1.00	123	0.08	0.37	1.00
BMI kg·m^{-2}	135	−0.26	2.2×10^{-3}*	0.01*	124	0.11	0.21	1.00
T2 gene signature	109	−0.25	8.4×10^{-3}	0.05	124	0.37	2.8×10^{-5}*	1.7×10^{-4}*

F_{ENO}: exhaled nitric oxide fraction; BAL: bronchoalveolar lavage; BMI: body mass index; T2: type 2. #: number of subjects with measurements for each test of association. *: significant correlations after correcting for six tests using a Bonferroni correction.

decreased A-to-I editing of position 5 of miR-200b-3p and increased expression of *SOCS1* in BECs from asthmatic subjects with different T2 endotypes of moderate-to-severe asthma.

Discussion

We performed the first genome-wide scan of ADAR-mediated miRNA editing in asthma and identified endogenous post-transcriptional A-to-I editing of a miRNA in BECs as an epigenetic mechanism potentially leading to more severe clinical manifestations and poorer response to standard therapies in individuals with asthma. A-to-I editing of one site, position 5 of miR-200b-3p, was increased in BECs from nonasthmatic control subjects compared with cases with moderate-to-severe asthma. This same A-to-I edited site has been reported in various cancer tumours, including glioblastoma [34] and lung adenocarcinoma [35], and is consistently increased in tumour samples compared with normal tissue and inversely correlated with patient survival [13]. Functional characterisation of miR-200b-3p in this context identified its role as a tumour suppressor that becomes an oncogenic miRNA after A-to-I editing of position 5. Together with our study, these results suggest that edited miR-200b-3p is critical to different disease processes, in which editing of the same site can be promoting (cancer) or protective (asthma). Editing at this site may therefore be highly regulated to optimise the expression of key genes of the edited form of miR-200b-3p.

Indeed, the predicted target genes of the edited miR-200b-3p were enriched for IL-4 and IFN-γ signalling pathways, both of which included the *SOCS1* gene. Consistent with decreased A-to-I editing of position 5 of miR-200b-3p, *SOCS1* expression was increased in BECs from cases with moderate and severe asthma. *SOCS1* is a centrally important transcription factor that has dual roles in suppressing T2 cytokine [30, 31] and IFN [32, 33] signalling pathways. Although the evidence is conflicting, most studies have reported increased expression of *SOCS1* in BECs of subjects with severe asthma [36–38], similar to our study, whereas only one study reported decreased expression [39].

Asthma severity, miR-200b-3p editing and/or *SOCS1* expression were also correlated with BAL eosinophils, the T2 gene signature and BMI. Notably, BAL eosinophils, a marker of T2 asthma, were increased in both moderate and severe asthma cases, whereas the T2 gene signature was highest in cases with moderate disease and similar in cases with severe and mild disease. BMI was also significantly increased in cases with severe disease but similar in cases with mild and moderate disease. The severe asthma cases also had the highest levels of IgE, were older and comprised of more females, although these differences were not significant after multiple testing correction. Thus, the moderate and severe asthma cases in our study have distinct clinical profiles that may reflect different endotypes of T2 asthma. We suggest that decreased A-to-I editing of position 5 of miR-200b-3p in the cases leads to increased *SOCS1* expression, whose dual functions contribute to moderate or severe asthma through T2-dependent features that result from either impaired T2 cytokine signalling, impaired IFN responses or both.

Despite the novelties of our study, there are limitations. First, direct measures of T2 cytokines or IFNs were not available in these subjects, so we could not test for associations between cytokine profiles and editing or *SOCS1* expression. Such measurements could provide information that would allow us to better link the moderate and severe asthma phenotypes to increased inflammation *via* IL-4 signalling or to asthma exacerbations mediated by impaired IFN signalling, respectively, and better define the T2 endotypes in each group. Second, our sample sizes were relatively small, which may have prevented us from detecting significant differences in the mean expression of all target genes of the unedited or edited miR-200b-3p. It is possible that subtle changes in the expression of target genes of the edited miR-200b-3p other than

SOCS1 contribute to moderate-to-severe asthma phenotypes. Third, we used a bioinformatic tool (TargetScanHuman) to identify predicted target genes for the unedited and edited miR-200b-3p. We highlighted *SOCS1* using a rigorous stepwise approach that involved keeping all potential target genes, applying pathway analysis to identify related genes and using RNA sequencing data in the same cells to examine differential expression. However, there may be other important downstream target genes of the edited miR-200b-3p that may be identified in studies of miR-200b-3p in larger samples.

In summary, using an unbiased genome-wide approach, we identified associations between asthma and an A-to-I edited site within a miRNA, *i.e.* position 5 of miR-200b-3p. To the best of our knowledge, this is the first study to implicate miRNA editing as an epigenetic mechanism contributing to asthma severity. Additional studies are required to elucidate the direct functional effects of this A-to-I edited site on moderate-to-severe asthma. Nonetheless, we demonstrate an important role for ADAR-mediated miRNA editing on asthma pathogenesis and identify new therapeutic targets for treatment of asthma that is difficult to control with standard therapies.

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