

ORIGINAL ARTICLE

Detecting gene expression in buccal mucosa in subjects with asthma versus subjects without asthma

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Abstract

Background: Differences in mRNA expression for inflammatory markers have been observed between subjects with asthma vs. controls and in relation to corticosteroid response. However, these studies utilized methods (e.g., bronchoscopy) that are too invasive to be used routinely in children and in the clinic. The primary purpose of this study was to determine the feasibility of obtaining RNA of adequate quantity and quality from buccal mucosa of children and adults for gene expression studies. Secondly, this study aimed to determine whether gene expression patterns in buccal mucosa are similar to those that have been observed in respiratory epithelium.

Methods: We enrolled 94 subjects with and without asthma between 5 and 54 years of age. Relative gene expression in buccal mucosa was determined with quantitative RT-PCR for the following genes: *CCL2*, *EDN1*, *FKBP5*, *IL8*, *IFNAR2*, *NFKB1*, *RELA*, *SERPINB2*, *DENND1B*, *HRH1*, *ICAM1*, *ORMDL3*, *NR3C1*, *CLCA1*, *CRHR1*, *MUC5B*, *FCER2*, *POSTN*, *GAPDH*, *PPIA*.

Results: mRNA Expression of the following genes was detected in buccal mucosa: *CCL2*, *EDN1*, *FKBP5*, *IL8*, *IFNAR2*, *NFKB1*, *RELA*, *SERPINB2*, *DENND1B*, *HRH1*, *ICAM1*, *ORMDL3*, *NR3C1*, *GAPDH*, *PPIA*. *HRH1* was differentially expressed in adults with asthma vs. controls ($p = 0.04$), and *EDN1* was differentially expressed in children with asthma vs. controls 12–18 years old ($p = 0.03$). A similar trend for *HRH1* was observed in children 12–18 years old.

Conclusions: Buccal mucosa sampling is a reliable method for detecting changes in gene expression in patients with asthma. This non-invasive technique may serve as a valuable tool for diagnosing asthma and evaluating therapeutic response.

According to the Centers for Disease Control and Prevention, approximately 20 million Americans have asthma (1). Despite advances in the diagnosis of and treatment for asthma, a sizeable portion of patients do not respond to 'core' treatments. The usefulness of available surrogate end-points, including exhaled nitric oxide and sputum eosinophils, in predicting response to asthma therapies has been limited. Exhaled nitric oxide and sputum eosinophils as clinically useful markers of response are hindered by cumbersome sampling techniques that are difficult to perform in children (inability to exhale adequately or to induce sputum). Both markers may be most useful in asthma patients with an eosinophil predominant phenotype, exercise induced bronchospasm associated with atopy, and atopic asthma but may not be able to predict

response in patients without an eosinophil predominance (e.g., children with viral induced asthma) (2–5).

Microarrays have been used to identify gene expression profiles in airway epithelium of patients with asthma that are altered compared with those observed in controls (6–8). However, these studies have been limited to adults and require sampling of airway epithelial cells during bronchoscopy. Other studies have used RNA isolated from peripheral blood mononuclear cells (PBMCs) from adult patients with asthma (6, 9, 10). As systemic exposure to corticosteroids is limited with standard doses of inhaled corticosteroids (ICS), changes in gene expression in PBMCs following ICS therapy may under-represent actual changes that occur in the lung.

Recently, Guajardo et al. (11) identified gene clusters that distinguish children with stable asthma from healthy children as well as from those experiencing an exacerbation due to their asthma in RNA extracted from nasal epithelium. Therefore, easily accessible tissues, such as nasal epithelium, may serve as appropriate alternatives to the lower respiratory tract to identify informative biomarkers for the characterization of asthma.

Recently, several studies have reported isolation of RNA from buccal mucosa cells for gene expression studies to identify markers of disease susceptibility, drug efficacy, and environmental exposure (12–15). Two of these studies reported concordance in relative gene expression levels between buccal epithelial cells and micro-dissected lung samples derived from the same donor (12, 15). In addition, changes in gene expression in bronchial tissues are reflected by changes in gene expression in buccal and nasal epithelial cells (16). These data suggest that readily accessible cells of the buccal mucosa can serve as surrogate markers for tissue responses at distal sites.

The purpose of this study was to investigate the feasibility of using RNA extracted from cells of the buccal mucosa as a source of gene expression biomarkers in children and adults through assessing mRNA expression of genes associated with asthma.

Materials and methods

Subjects

Adult subjects without asthma and with a diagnosis of asthma on Step 2 or 3 asthma therapy were enrolled after informed consent during visits to Allergy, Asthma, and Pulmonary clinics at Truman Medical Center, Kansas City, MO, USA. Pediatric subjects with asthma on Step 1 or Step 2/3 therapy and those without asthma were enrolled after parent permission/subject assent during visits to the Allergy and Asthma Clinics at Children's Mercy Hospital, Kansas City, MO, USA. Pediatric subjects were stratified into two age groups (aged 5–11 and 12–18), and step therapy was defined per 2007 National Institutes of Health (NIH) asthma guidelines (17). Classification by current step therapy, defined by the medications and doses required to achieve control of asthma symptoms, was used as a surrogate marker of disease severity in this study. As suggested by NIH guidelines (17), this approach provides a dynamic assessment of asthma impairment and risk.

Asthma was defined as episodic airflow obstruction which is reversible (improvement in FEV₁ >12% via spirometry) after administration of a short acting beta-agonist (SABA). In children unable to perform spirometry, the diagnosis of asthma was clinically determined by an allergy/asthma/immunology specialist based on history of persistent, recurrent cough, wheeze, or shortness of breath with relief of symptoms in response to SABA, ICS, and/or systemic steroids. Subjects who were current smokers had been smokers in the past 6 months or had a diagnosis of COPD, bronchiectasis, allergic bronchopulmonary aspergillosis, bacterial, fungal, or viral pneumonia in the past 6 months, or other chronic lung diseases were excluded to ensure exclusion of subjects with symptomatology

suggestive of asthma but do not actually have the disease. Subjects treated with intranasal steroids in a 2-week period preceding the study, an oral corticosteroid in the past 6 weeks, or with immune modulator medications were excluded. These studies were approved by the Institutional Review Boards at the University of Missouri-Kansas City and Children's Mercy Hospital.

Sample collection and processing

After subjects' mouths were rinsed (Listerine® [adults] or water [children]), samples were obtained by scraping the inside of each cheek with sterilized soft bristled toothbrushes. Cells were immersed in Saliva Protect (Qiagen, Valencia, CA, USA) for at least 24 h at 4°C prior to RNA extraction. Total RNA extractions, RNA quality determinations, and quantitative RT-PCR were performed by routine methods as described in the online Supplementary Information.

Data analysis

Molecule numbers were normalized with the geometric mean of *GAPDH* and *PPIA* and transformed by the logarithmic function at base 2. The effects on normalized gene expression of asthma, asthma severity, and their interaction with age were assessed with gender and race effects as covariates using the general linear model (GLM), a method that takes covariates into account. Demographics were compared by chi-square, Fisher's exact, and *t*-tests. Non-parametric tests were performed to compare gene expression among different groups and were consistent with parametric GLM analysis. All analyses were performed using SAS 9.2 (SAS Institute, Inc., Cary, NC) and SPSS 18 (IBM Corp., Armonk, NY) with statistical significance at $p < 0.05$.

Results

Demographics

Thirty adults, aged 19–54, and 64 children, aged 5–18, were enrolled and stratified as described in the Methods. Samples from six subjects (three adults and three children) were removed from analysis due to RNA expression levels below limits of quantification for all genes. Patient demographics of samples included in the data analysis are described in Table 1. The distributions of individuals with or without asthma were not significantly different by gender, race, or age ($p > 0.05$) in either children or adults.

RNA quality

Average RNA quality indicator (RQI) across all samples was 3.1 (range: 1.8–7.6). RQI values >8 are associated with high-quality RNA, and values >5 are associated with good-quality RNA (18). However, RQI scores are highly dependent upon ribosomal RNA integrity; therefore, to determine whether RNA from buccal mucosa might be suitable for quantitation of mRNA, *GAPDH* 3':5' assays were performed. RNA integrity is

Table 1 Patient demographics

	Healthy controls	Step 1 level therapy	Step 2 or 3 level therapy
Adults			
n	10		17
Males (%)*	3 (33%)		5 (29%)
Caucasian (%)	5 (50%)		4 (24%)
African American (%)	2 (20%)		12 (71%)
Median Age (range)	38 (27–54)		44 (21–54)
Children, aged 5–11			
n	11	13	9
Males (%)	7 (64%)	9 (69%)	7 (78%)
Caucasian (%)	2 (18%)	5 (38%)	3 (33%)
African American (%)	6 (55%)	7 (54%)	5 (55%)
Median Age (range) [†]	9 (5–11)	9 (6–11)	8 (6–10)
Children, aged 12–18			
n	10	10	8
Males (%)	5 (50%)	7 (70%)	6 (75%)
Caucasian (%)	5 (50%)	5 (50%)	4 (50%)
African American (%)	4 (40%)	5 (50%)	4 (50%)
Median Age (range) [†]	14 (12–18)	14 (12–17)	13 (12–18)

*The gender or race did not differ between healthy subjects and asthmatic subjects in any subgroup ($p > 0.05$).

[†]Within each age group (adults, children, 5–11, or children aged 12–18), the actual age did not differ statistically between among control, step 1, and step 2/3 subjects ($p > 0.05$).

inferred from the ratio of quantities of 2 PCR products targeting the 3' and 5' ends of *GAPDH* mRNA, which reflects successful reverse transcription across *GAPDH*. Ratios >5 suggest degradation. Average *GAPDH* 3':5' ratio was 2.4 (range: 0.47–4.9), and no correlation was observed with RQI. In children 5–11 years of age, RQI scores suggested lower-quality RNA isolated from buccal samples from subjects with asthma versus those without asthma (control = 4.06 ± 1.97 vs. asthma = 2.44 ± 0.49 , $p = 0.001$). *GAPDH* 3':5' ratios were increased in children (children = 2.57 ± 0.77 vs. adults = 2.01 ± 0.46 ; $p = 0.001$). No other differences in RQI scores or *GAPDH* ratios were observed. These results suggest that RNA extracted from buccal mucosa is suitable for quantitative RT-PCR. However, quality indicators imply that RNA isolated from buccal mucosa from children may be of lower quality.

Reproducibility of quantitative RT-PCR results with RNA from buccal mucosa was determined via comparison of RNA expression levels of *GAPDH* determined with 3 separate assays amplifying exon 3, exons 6–7, and exon 9. RNA quantities between the assays that targeted exon 3 and exon 9 were highly correlated (Fig. 1, $r^2 = 0.86$, $p < 0.0001$). Statistically significant correlations were also observed between molecule numbers for exon 3 and exon 6–7 ($r^2 = 0.81$, $p < 0.0001$) and between exons 6–7 and exon 9 ($r^2 = 0.95$, $p < 0.0001$) (Figs S1 and S2). These data suggest that relative levels of gene expression between individuals can be reproducibly measured in RNA extracted from buccal mucosa.

RNA expression

mRNA expression levels for 20 genes (Table S1) were determined in RNA extracted from buccal mucosa. These

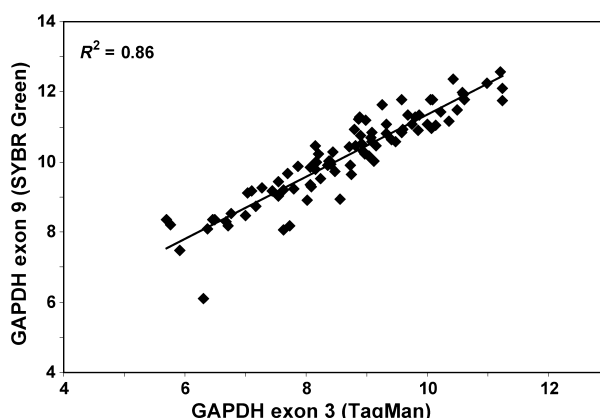


Figure 1 Significant correlation between two separate RT-PCR assays for *GAPDH* mRNA suggests that changes in gene expression can reproducibly detected in buccal mucosa. Quantitative, two-step RT-PCR was performed as described in the Methods with two primer sets for *GAPDH*, which target exon 3 (TaqMan hydrolysis probe) and exon 9 (SYBR green dsDNA-binding dye).

genes represent a subset that are differentially expressed in respiratory epithelium of adults and children with asthma, as well as exhibit altered gene expression patterns relative to ICS exposure (7, 11). Five genes had levels below the limits of quantification for $>70\%$ of samples (*CLCA1*, *CRHR1*, *MUC5B*, *POSTN*, and *FCER2*) and were removed from analysis.

PPIA and *GAPDH* were detected at lower levels in RNA isolated from patients with asthma relative to controls, after

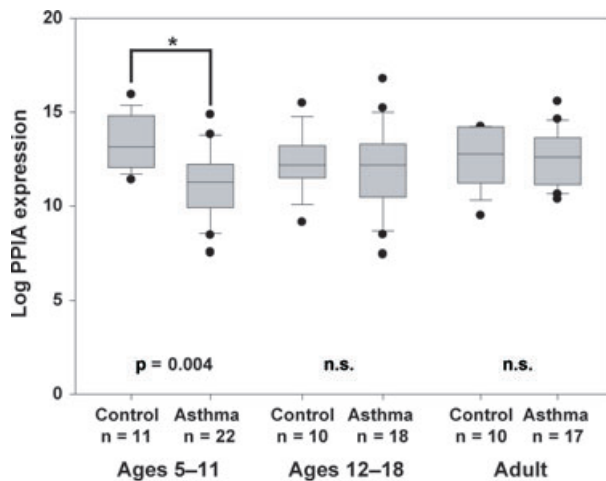


Figure 2 Gene expression of *PPIA* mRNA in buccal mucosa is lower in patients with asthma relative to subjects without asthma. Quantitative, two-step RT-PCR was performed as described in the Methods. No difference in mRNA expression was observed between children and adults for either gene. In the combined population of adults and children, *PPIA* ($p = 0.036$) was expressed at statistically significantly lower values in subjects with asthma. However, when separated into three cohorts by age, statistically significant lower *PPIA* gene expression with respect to asthma was only observed in children aged 5–11 ($p = 0.004$). No differences were observed between controls and subjects with asthma for both adults and children, aged 12–18. *indicates $p < 0.05$.

adjusting by race and gender. However, upon further analysis, differential gene expression between controls and subjects with asthma was only observed in the cohort of younger children, aged 5–11 (*PPIA* (Fig. 2), $p = 0.004$; *GAPDH* (Fig. S3), $p = 0.006$) but not for older children (aged 12–18; *PPIA* (Fig. 2), $p = 0.36$; *GAPDH* (Fig. S3, $p = 0.469$) or adults *PPIA* (Fig. 2), $p = 0.99$; *GAPDH* (Fig. S3), $p = 0.788$). The observed differential gene expression in younger children may be the result of developmental changes in RNA expression, issues with sampling, and/or differences in asthma between young children and older patients.

After normalization, gene expression for the gene encoding the histamine H1 receptor, *HRH1*, was increased in subjects with asthma relative to those without asthma in adults (Fig. 3a, $p = 0.04$). Furthermore, mRNA expression of *HRH1* increased in children, aged 12–18 years of age, with asthma relative to controls; however, this difference did not reach statistical significance ($p = 0.07$). There was no statistically significant difference in normalized *HRH1* gene expression between controls and subjects with asthma in children aged 5–11 (control = 11.2 ± 1.8 vs. asthma = 11.8 ± 1.9 , $p = 0.46$). However, this result may not reliably reflect changes in *HRH1* mRNA expression in younger children with respect to asthma due to differences in mRNA expression of the normalization genes (Fig. 2).

EDN1 mRNA expression was higher in buccal mucosa in 12- to 18-year-old children with asthma relative to control

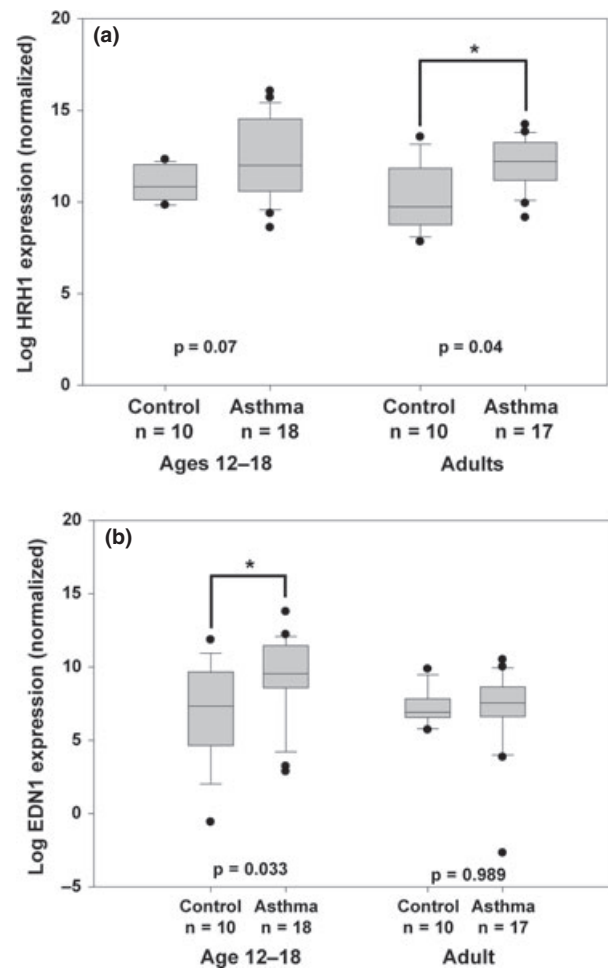


Figure 3 *HRH1* and *EDN1* mRNA expression in buccal mucosa in adults and children with asthma. Quantitative, two-step RT-PCR was performed as described in the Methods. (a) *HRH1* was increased in the buccal mucosa in subjects with asthma in adults ($p = 0.04$), and a similar increase in gene expression was observed in children aged 12–18 years of age, but was not statistically significant. (b) *EDN1* was increased in the buccal mucosa in subjects with asthma in children 12–18 years of age ($p = 0.033$). No increase in *EDN1* mRNA expression was observed in adults with asthma ($p = 0.989$). *denotes $p < 0.05$.

subjects (Fig. 3b, $p = 0.033$). However, in adults, no difference was observed ($p = 0.989$).

The gene encoding the cytokine IL8 was expressed at higher levels in males versus females in adults (Fig. 4, $p = 0.043$). In children 12–18 years of age, males also exhibited a trend toward higher gene expression ($p = 0.55$). Similar to adults, males, aged 5–11, had statistically higher *IL8* gene expression than females (females = 10.1 ± 2.0 vs. males = 12.1 ± 1.7 , $p = 0.016$). Although no statistically significant differences in the mRNA expression of the normalizer genes were observed with respect to gender, data from children, aged 5–11, should be interpreted with caution.

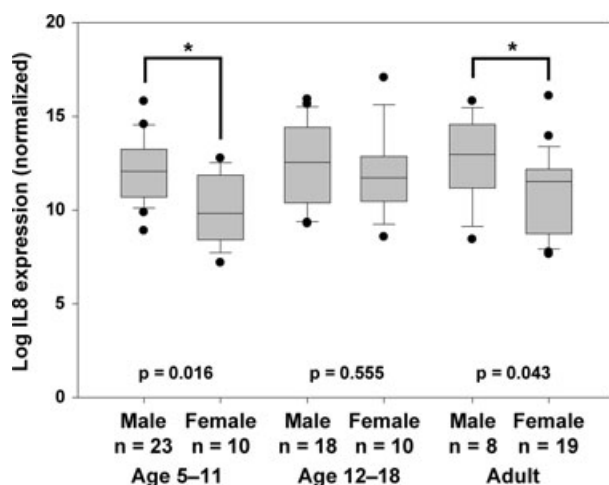


Figure 4 Gene expression of *IL8* mRNA in buccal mucosa. Quantitative, two-step RT-PCR was performed as described in the Methods. *IL8* mRNA expression was reduced in females relative to males in adults and children 5–11 years of age ($p = 0.043$, adults; and $p = 0.016$, children 5–11). A similar trend was observed in children aged 12–18 years of age but did not meet statistical significance. *indicates $p < 0.05$.

Discussion

Here, we describe the use of cells obtained from buccal mucosa as a source of RNA to study gene expression biomarkers in subjects with asthma. These cells represent a non-invasive source of tissue that may be utilized to diagnose asthma and to predict response to therapy. In general, RNA quality and quantity were modest; however, RNA from buccal mucosa is suitable for gene expression analysis using RT-PCR with appropriate considerations for experimental design.

Using RNA extracted from buccal mucosa, we investigated RNA levels of 18 genes that have been associated with asthma and/or treatment response. The results presented here suggest that gene expression patterns obtained from buccal mucosa may be a useful surrogate marker in asthma diagnosis. Our findings are especially important in children where obtaining objective measures of asthma impairment and risk are often difficult to obtain and may lack reliability. However, data in this study suggest a systematic difference in the collection and/or stability of buccal mucosa RNA from the cohort of younger children. Additional studies are required to determine the source of the observed differences and to optimize procedures for storage and extraction techniques for use in small children.

A limitation of the use of RNA isolated from buccal mucosa in this study was modest quality of the RNA. However, RNA from buccal mucosa had RQI scores better than those considered suitable for downstream applications for RNA isolated from FFPE specimens with appropriate experimental controls (19, 20). Furthermore, as illustrated by the results from the 3':5' *GAPDH* assays, the range in quality observed in the RNA isolated from buccal mucosa is adequate for RT-PCR applications. Current studies are underway to optimize

sample collection, stabilization, and isolation methods to improve the quality of RNA isolated from buccal mucosa.

Although the purpose of this pilot study was to explore the feasibility of using RNA from buccal mucosa to measure gene expression and was not designed to detect differences in mRNA expression between subjects with and without asthma, differences were observed. *HRH1* mRNA expression was significantly up-regulated in adults with asthma, and a trend also observed in children 12–18 years old. *HRH1* encodes the histamine H1 receptor that mediates the effects of histamine released from mast cells and other inflammatory cells. Histamine is important in the allergic/inflammatory cascade and has been shown to induce bronchial hyper-responsiveness. The association of increased mRNA expression in buccal mucosa with respect to asthma is consistent with reports that *HRH1* is expressed at higher levels in severe asthma (21). Previous studies have revealed mixed findings regarding the benefit of antihistamines in the treatment for asthma; however, previous studies have not been conducted with well-defined asthma phenotypes (22). *HRH1* gene expression levels may differentiate an asthma phenotype that would benefit from antihistamine treatment.

In this study, we observed higher levels of *IL8* mRNA in males relative to females; however, the basis for this difference is not clear. Genetic variation in the gene encoding *IL8*, a potent inflammatory cytokine, has been associated with asthma. A promoter polymorphism, -251T which confers increased activity to the *IL8* gene promoter, has been linked to asthma (23) and increased blood eosinophil counts (24). Furthermore, polymorphisms in *IL8* are strongly linked to disease severity in males with cystic fibrosis but are only weakly linked to severity in females (25), which may be consistent with gender-specific regulation. The basis and clinical consequences for gender differences in *IL8* require further testing.

The diagnosis of asthma in children is limited by inability to obtain valid results in objective testing such as spirometry. This is a problem especially among children <5 years of age. In clinical practice, the diagnosis of asthma in children who are unable to reliably perform spirometry is often made by clinical history (persistent/recurrent cough, wheeze, shortness of breath; response to SABAs; improvement in symptoms with ICS or oral steroid treatment). In our study, we utilized an allergy/asthma/immunology specialist diagnosis in children who were unable to perform spirometry based on standard clinical criteria.

Genes for which mRNA expression levels in buccal mucosa were determined included those whose mRNA expression is altered in lung epithelium of patients with asthma. Direct comparisons between buccal mucosa and lung epithelium are difficult, especially in children, due to the invasive nature and risks associated with bronchial brushings. While gene expression patterns in buccal mucosa most likely do not perfectly reflect those found in distal airway, our data suggest that cells of the buccal mucosa are a promising source of biomarkers to assess disease. As these tissues are exposed to treatments used in asthma (e.g., inhaled steroids), they may be useful in assessing treatment response to such medications.

In conclusion, we demonstrate the feasibility of RNA isolated from cells of the buccal mucosa to investigate mRNA

expression of genes associated with asthma in adults and children. Studies are underway to optimize sample collection and stabilization methods to improve yield and quality of RNA. Further studies specifically designed to evaluate gene expression differences between those with and without asthma in addition to determining the applicability of this technique in predicting therapeutic response are also being undertaken. This method holds promise as a non-invasive surrogate marker for

identifying changes in gene expression associated with disease, disease severity and phenotypes, and asthma treatment.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Gene Expression Assays.

Figure S1. Significant correlation between two separate RT-PCR assays for *GAPDH* mRNA suggest that changes in gene expression can be reproducibly detected in buccal mucosa.

Figure S2. Significant correlation between two separate RT-PCR assays for *GAPDH* mRNA suggest that changes in gene expression can be reproducibly detected in buccal mucosa.

Figure S3. Expression of *GAPDH* mRNA in buccal mucosa is lower in patients with asthma relative to subjects without asthma.

RNA Extraction and Quantitative RT-PCR: Total RNA was prepared from buccal tissue (9-43mg) using the Illustra RNAspin Mini RNA Isolation Kit (GE Healthcare, Piscataway, NJ) according to manufacturer's protocol. RNA was isolated from a mean of 24 mg of buccal mucosa (range = 7.1-34.8 mg) and resulted in, on average, 270 ng of total RNA (range = 45.9-21560 ng). Quality was determined on an Experion Automated Electrophoresis System (Bio-Rad, Hercules, CA) and quantity was determined on a Nanodrop ND-1000 Spectrophotometer. Total RNA (20ng) was reverse transcribed using the High Capacity RNA to cDNA kit (Applied Biosystems, Foster City, CA). To increase the quantity of specific targets for quantitative PCR, cDNA was subjected to preamplification using the TaqMan PreAmplification kit (Applied Biosystems). Briefly cDNA, pooled TaqMan gene expression assays (Applied Biosystems, Table 1), and preamplification reagent were incubated at 95°C for 10 min followed by 14 cycles of 95°C for 15 sec and 60°C for 4 min. Assays for *GAPDH* and *PPIA* were subjected to 10 cycles of preamplification. After preamplification, reactions were diluted 1:40 (1:10 if ten cycles) with 1X TE. Gene expression assays were performed using 3.2 µl preamplified cDNA, TaqMan Gene Expression assay (Supplementary Table), and PerfeCTa qPCR SuperMix (Quanta Biosciences, Gaithersburg, MD). Standard curves were generated using serial dilutions from 10 to 10⁸ copies/µl of PCR amplicons or cDNA plasmids. Molecule numbers were determined from linear regression of the standard curves.

Supplementary Table 1

Gene	TaqMan Gene Expression Assay ID	Accession Numbers Detected	Assay length	Assay location	LOQ* (CV%)	Assay Efficiency	Splice Variants Detected	Comment	References
CCL2	Hs00234140_m1	NM_002982	101	Ex 1-2	30 (7.1%)	101%	All known	Increased in lung epithelium in asthma, decreased by ICS	(1)
EDN1	Hs01115919_m1	NM_001168319 NM_001955	128	Ex 4-5	80 (7.2%)	93%	Variants 1, 2	Increased in lung epithelium in asthma, decreased by ICS	(1)
FKBP5	Hs01561001_m1	NM_001145775 NM_00114577 NM_004117	81	Ex 10-11	80 (3.3%)	84%	Variants 2, 3, 4	Increased in lung epithelium in asthma	(2)
IFNAR2	Hs00174198_m1	NM_207584, NM_207585, NM_000874	68	Ex 7-8	10 (7.0%)	85%	Variants 1, 2, 3	Decreased in lung epithelium in asthma	(3)
NFKB1	Hs00231653_m1	NM_001165412 NM_003998	73	Ex 19-20	50 (6.8%)	98%	Variants 1, 2	Increased activity in asthma reduced by corticosteroids	(4)
RELA	Hs00153294_m1	NM_001145138 NM_021975	71	Ex 8-9	60 (3.4%)	91%	Variants 1, 2	Increased activity in asthma reduced by corticosteroids	(4)
SERPINB2	Hs00234032_m1	NM_002575 NM_001143818	120	Ex 3-4	100 (5.3%)		Variants 1, 2	Increased in lung epithelium in asthma, decreased by ICS	(2)
GAPDH	4333764F	NM_002046	122	Exon 3	80 (6.2%)	96%		Normalization gene	
PPIA	4333763F	NM_021130	98	Exon 4	20 (8.0%)	100%		Normalization gene	
DENND1B	Hs00376502_m1	NM_001142795 NM_144977	69	Ex 1-2	150 (1.2%)	89%	Variants 1, 2	Genetic variants associated with asthma in children	(5)
HRH1	Hs00911670_s1	NM_001098213 NM_001098212 NM_001098211 NM_000861	80	Ex 2	20 (6.2%)	87%	Variants 1, 2, 3, 4	Induced in bronchial biopsies of brittle asthma	(6)

ICAM1	Hs99999152_m1	NM_000201	99	Ex 5-6	100 (5.6%)	87%	All known	Increased in lung epithelium in asthma, decreased by corticosteroids	(7, 8)
ORMDL3	Hs00918021_m1	NM_139280	114	Ex 2-3	300 (7.2%)	101%	All known	Genetic variants associated with asthma and expression	(9, 10)
NR3C1	Hs00353740_m1	NM_001018077 NM_001018074 NM_001018075 NM_001018076 NM_000176 NM_001020825 NM_001024094	73	Ex 4-5	500 (5.6%)	110%	Variants 1-5 (α), 6 (β), 7 (γ)	Intracellular receptor for corticosteroids	
CLCA1	Hs00154490_m1	NM_001285	69	Ex 9-10	30 (4.9%)	96%	All known	Increased in lung epithelium in asthma, decreased by ICS	(2)
CRHR1	Hs01062290_m1	NM_001145146 NM_004382, NM_001145147 NM_001145148	69	Ex 45	80 (4.2%)	94%	All known	Associated with response to ICS in asthma	(11)
MUC5B	Hs00861588_m1	NM_002458	55	Ex 33-34	400 (5.0%)		All known	Decreased in lung epithelium in asthma	(2)
FCER2	Hs01077044_m1	NM_002002	130	Ex 10-11	100 (5.9%)		All known	Associated with asthma exacerbations in children	(12)
POSTN	Hs01566748_m1	NM_001135934 NM_001135935 NM_001135936 NM_006475	138	Ex 2-3			All known	Increased in lung epithelium in asthma, decreased by ICS	(2)
IL8	Hs99999034_m1	NM_00584	81	Ex 1-2	20 (6.3%)	89%	All known	Increased in lung epithelium in asthma, decreased by ICS	(1)

*Limit of quantitation in molecules of cDNA. LOQ was determined by performing QPCR on serial dilutions of plasmid DNA containing the cloned cDNA amplicon.

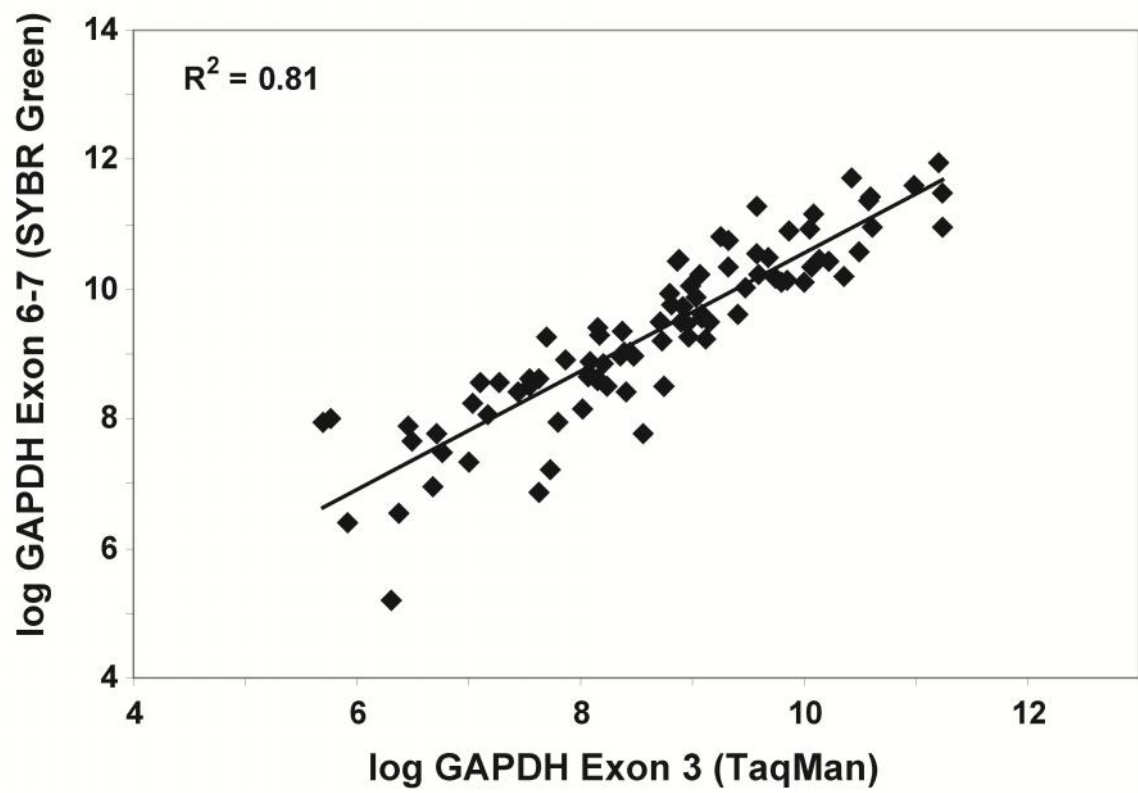
Legends to Supplementary Figures

Supplementary Figure 1. Significant correlation between two separate RT-PCR assays for *GAPDH* mRNA suggest that changes in gene expression can be reproducibly detected in buccal mucosa. Quantitative, two-step RT-PCR was performed as described in the Methods with two primer sets for *GAPDH* which target exon 3 (TaqMan hydrolysis probe) and exon 6-7 (SYBR green dsDNA binding dye). Log transformed molecule numbers are significantly correlated with $r^2=0.81$, $p<0.0001$.

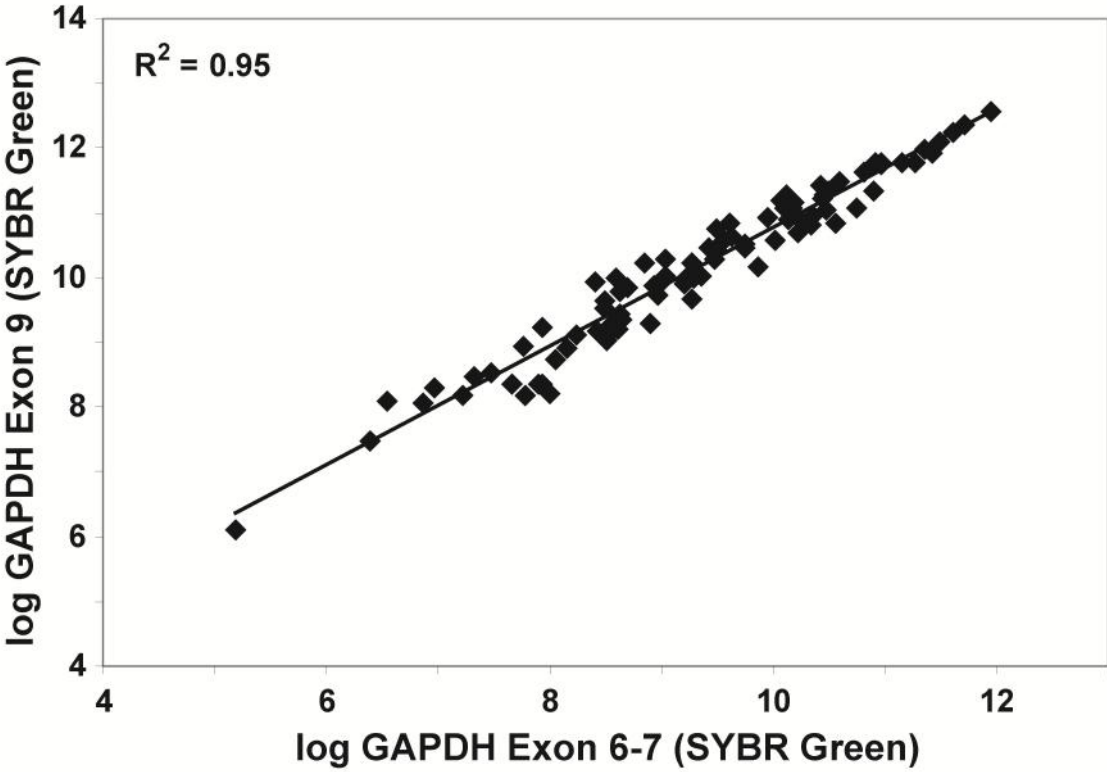
Supplementary Figure 2. Significant correlation between two separate RT-PCR assays for *GAPDH* mRNA suggest that changes in gene expression can be reproducibly detected in buccal mucosa. Quantitative, two-step RT-PCR was performed as described in the Methods with two primer sets for *GAPDH* which target exon 6-7 (SYBR green dsDNA binding dye) and exon 9 (SYBR green dsDNA binding dye). Log transformed molecule numbers are significantly correlated with $r^2=0.95$, $p<0.0001$.

Supplementary Figure 3. Expression of *GAPDH* mRNA in buccal mucosa is lower in patients with asthma relative to subjects without asthma. Quantitative, two-step RT-PCR was performed as described in the Methods. No difference in expression was observed between children and adults for either gene. In the combined population of adults and children, *GAPDH* ($p=0.035$) was expressed at statistically significantly lower values in subjects with asthma (A). However, when separated into three cohorts by age (B), statistically significant lower *GAPDH* expression with respect to asthma was only observed in children ages 5-11 ($p=0.003$). No differences were observed between controls and subjects with asthma for both adults and children, ages 12-18. * indicates $p<0.05$.

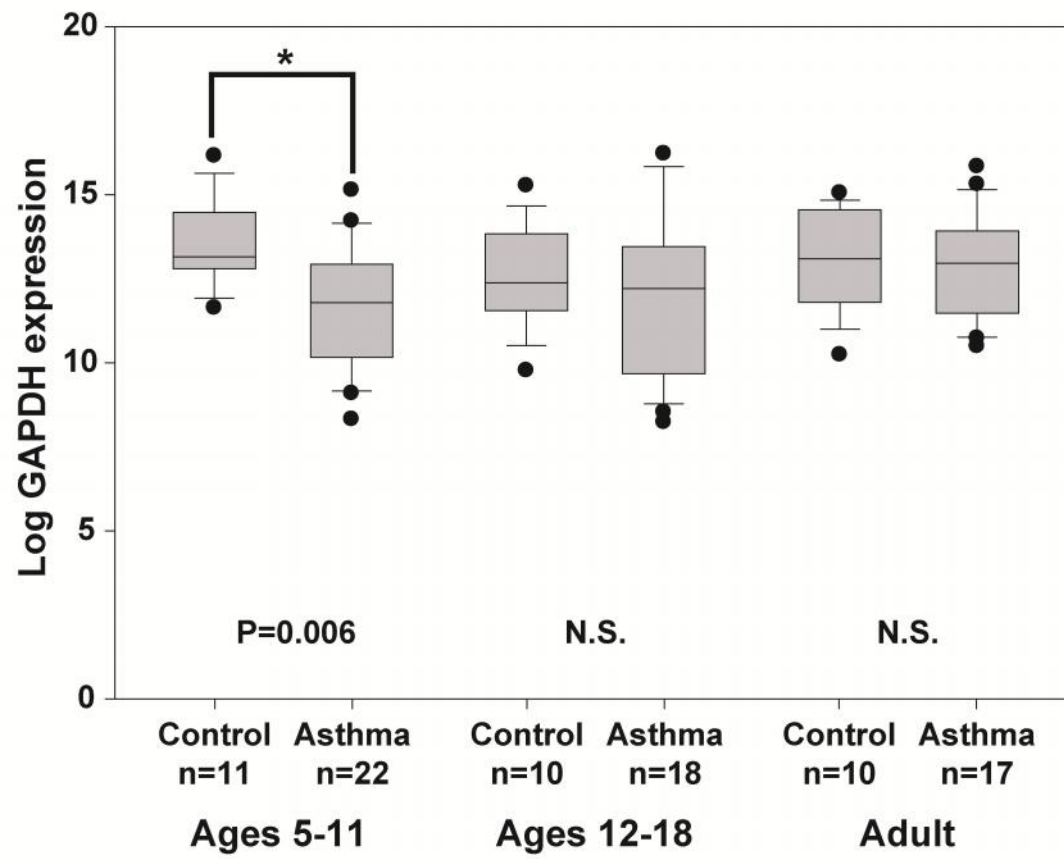
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary References

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