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Metabolomic differences of exhaled breath condensate among children with and without asthma

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Abstract

Background: There remains an unmet need in objective tests for diagnosing asthma in children. The objective of this study was to investigate the potential of metabolomic profiles of exhaled breath condensate (EBC) to discriminate stable asthma in Asian children in the community.

Methods: One hundred and sixty-five Asian children (92 stable asthma and 73 non-asthmatic controls) participating in a population-based cohort were enrolled and divided into training and validation sets. Nuclear magnetic resonance-based metabolomic profiles of EBC samples were analyzed by using orthogonal partial least squares discriminant analysis.

Results: EBC metabolomic signature (lactate, formate, butyrate, and isobutyrate) had an area under the receiver operator characteristic curve (AUC) of 0.826 in discriminating children with and without asthma in the training set, which significantly outperformed FeNO (AUC = 0.574; $P < .001$) and FEV₁/FVC % predicted (AUC = 0.569; $P < .001$). The AUC for EBC metabolomic signature was 0.745 in the validation set, which was slightly but not significantly lower than in the testing set ($P = .282$). We further extrapolated two potentially involved metabolic pathways, including pyruvate ($P = 1.67 \times 10^{-3}$; impact: 0.14) and methane ($P = 1.89 \times 10^{-3}$; impact: 0.15), as the most likely divergent metabolisms between children with and without asthma.

Abbreviations: ATS, American Thoracic Society; AUC, area under the receiver operating characteristic curve; BMI, body mass index; EBC, exhaled breath condensate; ERS, European Respiratory Society; FeNO, fraction of exhaled nitric oxide; FEV₁/FVC, forced expiratory volume in 1 nonbreathing space/forced vital capacity; GINA, Global Initiative for Asthma; ICS, inhaled corticosteroids; ISAAC, the International Study of Asthma and Allergies in Childhood; KEGG, Kyoto Encyclopedia of Genes and Genomes; LABA, long-acting β_2 sympathomimetic agonists; LIGHTS, Longitudinal Investigation of Global Healthy in Taiwanese Schoolchildren; NMR, nuclear magnetic resonance; OPLS-DA, orthogonal partial least squares discriminant analysis; PCA, principal component analysis; ROC, receiver operating characteristic; SCFAs, short-chain fatty acids; SD, standard deviation.

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Conclusion: This study provided evidence supporting the role of EBC metabolomic signature to discriminate stable asthma in Asian children in the community, with a discriminative property outperforming conventional clinical tests such as FeNO or spirometry.

KEYWORDS

asthma, butyrate, diagnosis, exhaled breath condensate, formate, isobutyrate, lactate, metabolomics

1 | INTRODUCTION

Asthma is one of common chronic diseases in children, imposing a global health and economic burden around the world.¹ There remains an unmet need in accurate objective tests for diagnosing asthma in children in clinical practice. The sensitivity of spirometry is low for diagnosing asthma in children.² The invasiveness of tissue-specific methods such as bronchoalveolar lavage, bronchoscopy, or bronchial biopsy

precludes their routine clinical use in children.³ Although induced sputum is a valid measurement of airway inflammation,⁴ its application into clinical practice is largely hampered by difficulty in obtaining a proper sputum specimen in children. Fractional exhaled nitric oxide (FeNO) has been shown to discriminate childhood asthma from controls in case-control studies reported by other groups.^{5,6} However, our previous work in a large population-based study has demonstrated that FeNO is robust in discriminating allergic sensitization, but not a reliable diagnostic marker of childhood asthma in clinical practice.⁷

Asthma is characterized by chronic airway inflammation caused by complex cellular and molecular mechanisms, which can hardly be investigated by focusing on a single biomarker. Recently, metabolomics has provided promising opportunities for comprehensive analysis of endogenous metabolites in biological samples from subjects with asthma. Previous case-control studies have suggested the potential diagnostic application of metabolomic profiles in blood, urine, and exhaled breath condensate (EBC).⁸⁻¹²

Exhaled breath condensate is a noninvasively collected biofluid containing volatile and non-volatile compounds that may be altered in lung diseases. A few studies have shown that metabolomic analyses of EBC have revealed different metabolomic clusters associated with asthma in adults.^{12,13} However, limited studies investigated metabolomic profiles of EBC in childhood asthma.^{11,14,15}

To date, there are insufficient data regarding the role of EBC metabolomic signature in differentiating subjects with and without asthma. Particularly, no studies have evaluated whether EBC metabolomics can differentiate children with stable asthma in the community. Metabolomic profiles are correlated with dietary patterns,¹⁶ which might be different between Western and Asian dietary patterns. Several previous studies on metabolomic profiling of asthma were conducted in Western countries, and little is known

Key Message

Nuclear magnetic resonance-based metabolomic signature of exhaled breath condensate discriminates children with stable asthma, with a discriminative property outperforming conventional tests including exhaled nitric oxide and spirometry. Our results suggest pyruvate and methane as the most likely divergent metabolic pathways between children with and without asthma.

about the role of metabolomic profiles in discriminating asthma in Asian children, whose dietary patterns differ from that of Caucasian children. This study aimed to investigate the potential of EBC metabolomic profiles to discriminate stable asthma in Asian children in the community.

2 | MATERIALS AND METHODS

2.1 | Subjects and study design

This study recruited 165 Asian children (92 stable asthmatic children and 73 non-asthmatic controls) among participants aged 5-7 years participating in the Longitudinal Investigation of Global Health in Taiwanese Schoolchildren (LIGHTS) cohort.¹⁷ Among those, 165 study subjects were divided into two independent sets: a training set of 60 asthmatic children and 41 non-asthmatic controls enrolled in 2016, and a validation set of 32 asthmatic children and 32 non-asthmatic controls enrolled in 2017.

Parents answered a modified International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire regarding symptoms of allergic diseases and physician-diagnosed allergic diseases. An interview conducted by physicians was applied to all study subjects. The diagnosis of asthma was confirmed by physicians using the Global Initiative for Asthma (GINA) guidelines.¹ Stable asthma was defined as follows: (a) no asthmatic exacerbation for the preceding 4 weeks necessitating systemic corticosteroids or an increased use of inhaled corticosteroid; (b) the use of

rescue treatment no more than three times a week; and (c) with no clinical indication for change in treatment medication.¹⁸ Children without history of asthma or any known underlying lung disease were enrolled in the control group.

FeNO measurements were performed by a chemiluminescence analyzer (CLD 88sp NO analyzer®, Eco Medics) according to the 2005 American Thoracic Society (ATS)/ European Respiratory Society (ERS) recommendations for standardized online measurement, as described elsewhere.¹⁹ Lung function was measured using spirometry (Spirolab II; Medical International Research) in accordance with the ATS/ERS recommendations.²⁰ This study was approved by the Institutional Review Board of Chang Gung Medical Foundation (No. 201600334A3), and parents of all subjects provided written informed consent.

2.2 | Exhaled breath condensate collection

Exhaled breath condensate was collected using a Turbo-Deccs system (Medivac). While wearing a nose clip, children were asked to breathe tidally for 10 minutes through a mouthpiece, which was used for preventing contamination during measurement. Approximately 1 mL of breath condensate was collected and stored at -80°C for subsequent nuclear magnetic resonance (NMR) analysis.

2.3 | ^1H nuclear magnetic resonance spectroscopy measurement

All proton spectra were collected using a Burker Avance III HD 600 MHz spectrometer (Burker BioSpin GmbH) at the Metabolomics Core Laboratory of Chang Gung University Healthy Aging Research Center. For NMR spectra processing, the line broadening was set to 0.6 Hz, and a zero-filling by a factor of 2 was applied to produce 128k Fourier data points. All spectral data were then Fourier-transformed, phase- and baseline-corrected, and were calibrated to trimethylsilylpropionic acid (0.0 ppm) using the TopSpin software (version 3.2; Burker BioSpin). Spectra were acquired using IconNMR automation program.²¹

2.4 | NMR data processing and analysis

The ^1H -NMR spectra from EBC were binned with a bin width of 0.01 ppm using the Bucker AMIX software (version 3.9.14; BurkerBioSpin). The spectral data were normalized to the total sum of the spectral intensity, and then, the normalized spectral bucket values were imported into the SIMCA-P software (version 13.0; Umetrics) for analyses using unsupervised principal component analysis (PCA) and the orthogonal partial least squares discriminant analysis (OPLS-DA). We further used 10-fold internal cross-validation to evaluate the quality of the multivariate models using both goodness-of-fit (R^2) and goodness-of-prediction (Q^2) parameters.

The chemical shift of each metabolite was identified by the library of in-house, Chenomx NMR Suite (version 7.1), or the Human Metabolome Database (<http://www.hmdb.ca/>).

2.5 | Statistical analysis

Demographic and clinical data are expressed as the mean $\pm$ standard deviation (SD) for continuous variables and as the number and corresponding percentage for categorical variables. We performed Student's *t* test for continuous variables and chi-squared test for categorical variables, respectively, to test the differences between children with and without asthma. We applied stepwise model selection under a logistic regression framework to determine the set of discriminative metabolites related to asthma diagnosis in the training set. The receiver operating characteristic (ROC) curves and the area under the curves (AUC) were constructed to assess the performance of the predictive models in training and validation sets, respectively. We employed metabolomic pathway analysis for the identified metabolites using MetaboAnalyst 4.0 (<https://www.metaboanalyst.ca/>)²² and mapped those metabolites based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. *P*-values less than .05 were considered statistically significant. Statistical analysis was performed using SPSS v.22.0 (SPSS).

3 | RESULTS

3.1 | Subject characteristics

The characteristics of the 165 study subjects were presented in Table 1. Asthmatic children had higher FeNO levels and lower forced expiratory volume in 1 second/ forced vital capacity (FEV_1/FVC) ratio, compared to the non-asthmatic controls. Among the 73 non-asthmatic controls, 24 and nine children had physician-diagnosed rhinitis and eczema, respectively. The characteristics of 101 subjects in the training set were similar to 64 subjects in the validation set (Table S1).

3.2 | EBC metabolomic profiles discriminate between children with stable asthma and controls

Principal component analysis was first applied to analyze 101 EBC samples from 60 children with stable asthma and 41 controls in the training set. No outliers within the two groups were highlighted. All 101 subjects were therefore included in subsequent analyses. The OPLS-DA model demonstrated a discriminative separation between asthmatic children and controls ($R^2\text{X} = 0.932$, $R^2\text{Y} = 0.886$, and $Q^2 = 0.441$; Figure 1A). The OPLS-DA loading plot showed the differentially abundant metabolites responsible for the separation between the two groups (Figure 1B). Specifically, four metabolites including lactate, formate, butyric acid, and isobutyrate were found

TABLE 1 Demographic and clinical characteristics of study children

Variable	Asthmatics (N = 92)	Non-asthmatics (N = 73)	P-value ^a
Age, years, mean ± SD	6.3 ± 0.3	6.4 ± 0.4	.172
Gender, male, n (%)	49 (53.3)	37 (50.7)	.742
BMI, kg/m ² , mean ± SD	15.8 ± 2.3	15.7 ± 2.1	.940
Rhinitis, n (%)	65 (70.7)	24 (32.9)	<.001
Eczema, n (%)	41 (44.6)	9 (12.3)	<.001
FeNO, ppb, mean ± SD	19.6 ± 17.3	12.8 ± 12.9	.011
FEV ₁ /FVC % predicted, %, mean ± SD	99.5 ± 6.5	101.5 ± 4.6	.027
Concomitant medication, n (%)			
Leukotriene receptor antagonist	14 (15.2)	0 (0.0)	<.001
ICS	6 (6.5)	0 (0.0)	.026
ICS-LABA	3 (3.3)	0 (0.0)	.119
Intranasal corticosteroids	3 (3.3)	0 (0.0)	.119
Antihistamines	8 (8.7)	2 (2.7)	.111

Note: Values shown are mean ± SD or number (%) of subjects where appropriate. Values in bold are statistically significant at $P < .05$

Abbreviations: BMI, body mass index; FEV₁/FVC, forced expiratory volume in 1 second/ forced vital capacity; ICS, inhaled corticosteroids; LABA, long-acting β_2 sympathomimetic agonists.

^aChi-squared tests were used for categorical variables, and Student's *t* tests for continuous variables.

at significantly higher relative abundance in the EBC samples of asthmatic children than those in the samples of non-asthmatic children. The fold changes of the identified metabolites are shown in Table S2. The OPLS-DA model among the control group showed that non-asthmatic allergic subjects were not distinguishable from healthy control subjects.

3.3 | Comparison of EBC metabolomic signature with conventional tests in discriminating stable asthma

The EBC metabolomic signature composed of four metabolites (lactate, formate, butyric acid, and isobutyrate) discriminated asthmatic children from non-asthmatic controls. The logistic regression equation is expressed as: EBC metabolomic signature = $-18.183 - 791.562 \times \text{formate} + 2442.914 \times \text{butyric acid} + 47.808 \times \text{lactate} + 534.753 \times \text{isobutyrate}$. The results of the discriminative properties of EBC metabolomic signature, FeNO, and FEV₁/FVC % predicted are shown in Figure 2. EBC metabolomic signature provided the highest discriminative capacity (AUC = 0.826),

which significantly outperformed the models with FeNO (AUC = 0.574; $P < .001$) or FEV₁/FVC % predicted (AUC = 0.569; $P < .001$). The optimal cutoff values, sensitivity, specificity, and positive and negative predictive values, respectively, for EBC metabolomic signature, FeNO, and FEV₁/FVC % predicted are shown in Table 2.

When subjects with physician-diagnosed rhinitis or eczema were excluded from the control group, the EBC metabolomic signature differentiated asthmatic children from non-allergic healthy controls (AUC = 0.838), which significantly outperformed FeNO (AUC = 0.599; $P = .002$) or FEV₁/FVC % predicted (AUC = 0.587; $P = .002$).

3.4 | Validity assessment in independent samples

The validity of EBC metabolomic signature in discriminating asthma was assessed in an independent validation set of 32 asthmatic children and 32 non-asthmatic controls. The EBC metabolomic signature correctly predicted 22 of 32 asthmatic children and 30 of 32 non-asthmatic controls, showing an overall accuracy of 81.3%. The AUC for EBC metabolomic signature was 0.745 in the validation set, which was slightly but not significantly lower than in the testing set ($P = .282$).

3.5 | Metabolic pathway analysis

To understand the biologic context of EBC metabolites in relation to childhood asthma, we conducted a metabolic pathway analysis using the online MetaboAnalyst 4.0 software as shown in Figure 3. Using the discriminating metabolites, we unveiled two potentially metabolic pathways involved in childhood asthma, which appeared to be significantly dysregulated. Specifically, pyruvate ($P = 1.67 \times 10^{-3}$; impact: 0.14) and methane ($P = 1.89 \times 10^{-3}$; impact: 0.15) metabolisms are the most likely divergent metabolic pathways between children with and without asthma. A summary of the alterations of metabolic pathways and appearance of specific metabolites in the EBC of children with asthma is shown in Figure 4.

4 | DISCUSSION

This study investigated potential application of EBC metabolomics in discriminating stable asthma in Asian children in the community. Our results demonstrated that NMR-based EBC metabolomic signature composed of four metabolites had a discriminative property of diagnosing asthma among Asian children in a population-based cohort, outperforming conventional tests such as FeNO or spirometry. Four EBC metabolites, including lactate, formate, butyric acid, and isobutyrate, were identified as potential biomarkers of asthma in children, which may partly explain the pathophysiology of asthma. Metabolic pathway analysis revealed altered pyruvate and methane

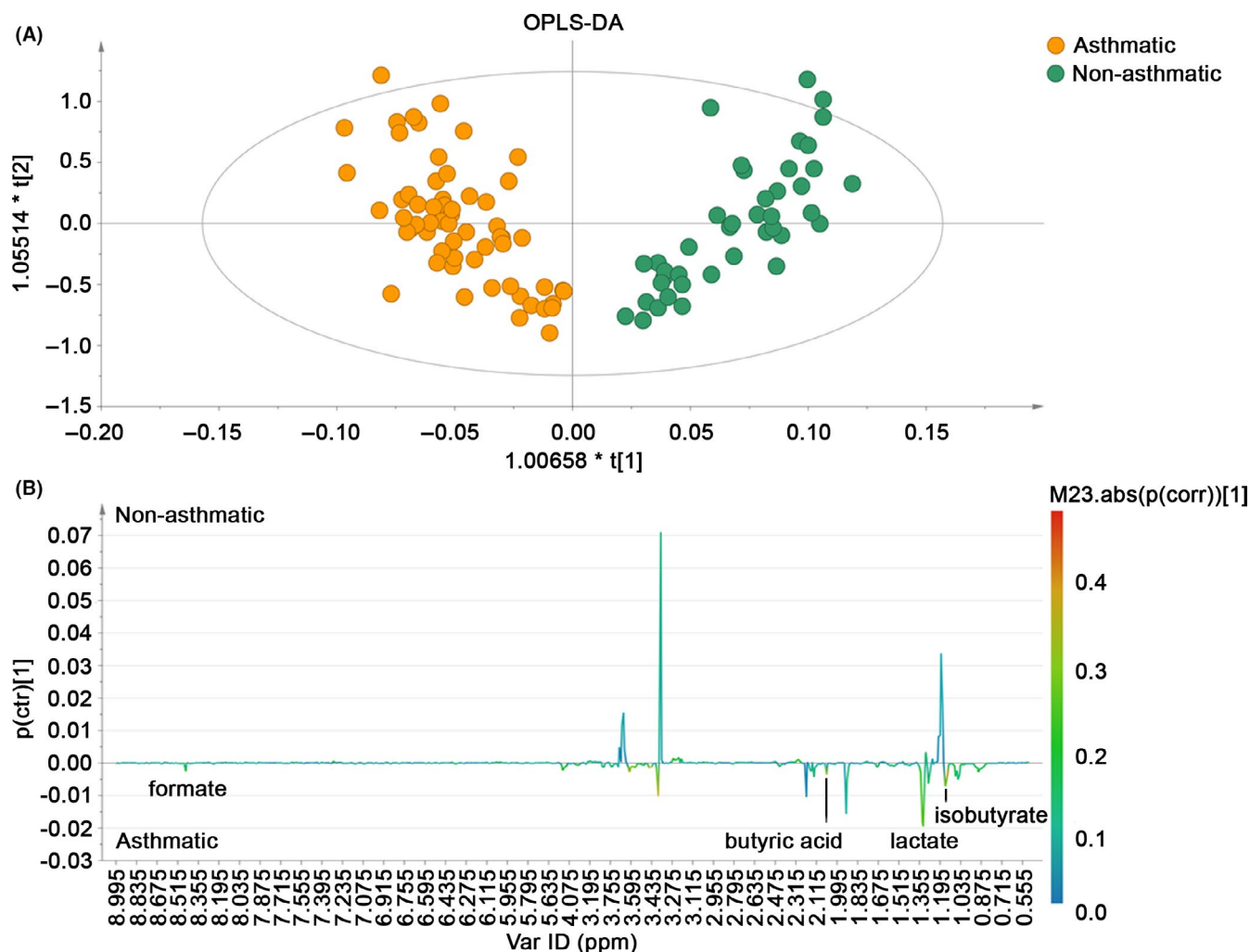


FIGURE 1 Orthogonal partial least squares discriminant analysis (OPLS-DA) of EBC samples between asthmatic children and controls. A, OPLS-DA score plot depicting that asthmatic children (orange) were distinguishable from non-asthmatic controls (green). The ellipse shown in the model represents Hotelling's T^2 statistic with 95% confidence. B, The loading coefficient plot between 9.000 and 0.500 ppm corresponding to the built OPLS-DA model between asthmatic children and controls. Only metabolites with P -values < 0.05 were labeled. The x-axis indicates the buckets according to the metabolite assignment with variable identity, and the loading score for each variable is labeled on the y-axis $p(ctr)[1]$ according to the centering. In addition, the $abs(p(corr))[1]$ refers to the absolute value of the correlation coefficient between two groups. OPLS-DA, orthogonal partial least squares discriminant analysis; EBC, exhaled breath condensate

metabolisms in the airways of children with asthma. To our knowledge, putative involvement of pyruvate and methane metabolic pathways in the pathophysiology of childhood asthma has not been reported previously.

Our findings add new evidence that EBC metabolomic signature, including lactate, formate, butyric acid, and isobutyrate, has better discriminative property for stable asthma in children than conventional tests such as FeNO or spirometry. Our results are in line with the findings in an Italian case-control study of 17 children with asthma and 11 controls, which showed a success rate of 86% for selected NMR signals in discriminating two groups.¹¹ In a British case-control study of 82 adults with asthma and 35 healthy volunteers, Ibrahim et al reported that a classification model consisting of five NMR spectral regions successfully differentiated asthmatics from health subjects, with AUC of 0.84 and 0.91 in training and test sets, respectively.¹² One main difference between this study and

the above-mentioned studies is the choice of control subjects. The above two studies enrolled healthy subjects as controls, whereas the present study included non-asthmatic children who were either healthy or had other allergic diseases as the control group, resembling the real-world practices because physicians need to differentiate patients with asthma from those without asthma, but possibly with or without other allergic diseases. As such, we think that the ability of EBC metabolomic signature differentiating asthmatic children from non-asthmatic controls including subjects with and without other allergic diseases will be more useful in clinical practice than the one determined only using study sample with asthmatic children and non-allergic healthy controls. Taken together, these findings support that EBC metabolomic signature has a discriminative potential for asthma diagnosis in the real-world clinical settings.

Our data suggest that pyruvate and methane metabolisms may be specifically altered in the airways of asthmatic children, to the

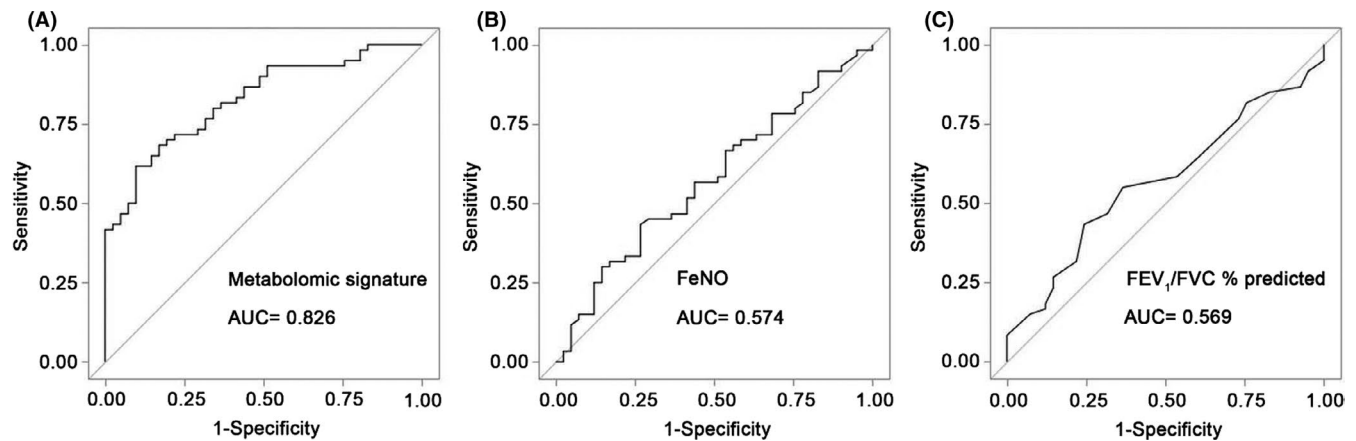


FIGURE 2 Receiver operating characteristic (ROC) curves of (A) EBC metabolomic signature, (B) FeNO, and (C) FEV₁/FVC % predicted for predicting Asian children with stable asthma. ROC, Receiver operating characteristic; EBC, exhaled breath condensate; FeNO, fraction of exhaled nitric oxide; FEV₁/FVC, forced expiratory volume in 1 second/ forced vital capacity

Variable	Cutoff value	Sensitivity (%)	Specificity (%)	Positive predictive value(%)	Negative predictive value (%)
EBC metabolomic signature	1.01	61.7%	90.2	90.2	61.7
FeNO, ppb	15.49	43.3	73.2	70.3	46.9
FEV ₁ /FVC % predicted, %	98.50	43.3	75.6	72.2	47.7

TABLE 2 Cutoff, sensitivity, specificity, and positive and negative predictive values for EBC metabolomic signature, FeNO, and FEV₁/FVC % predicted in discriminating asthma

Abbreviations: EBC, exhaled breath condensate; FeNO, fraction of exhaled nitric oxide; FEV₁/FVC, forced expiratory volume in 1 second/ forced vital capacity.

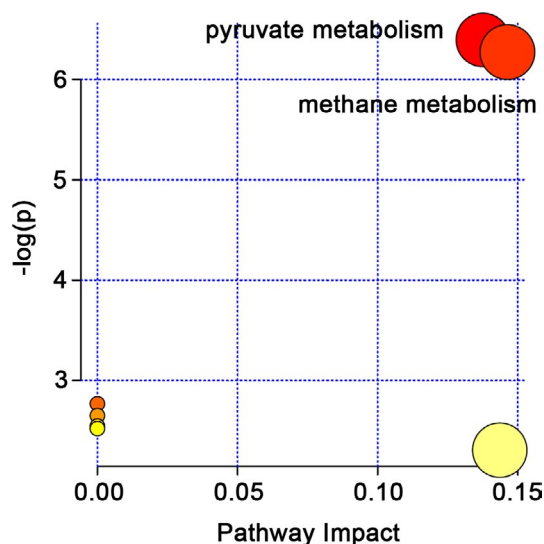
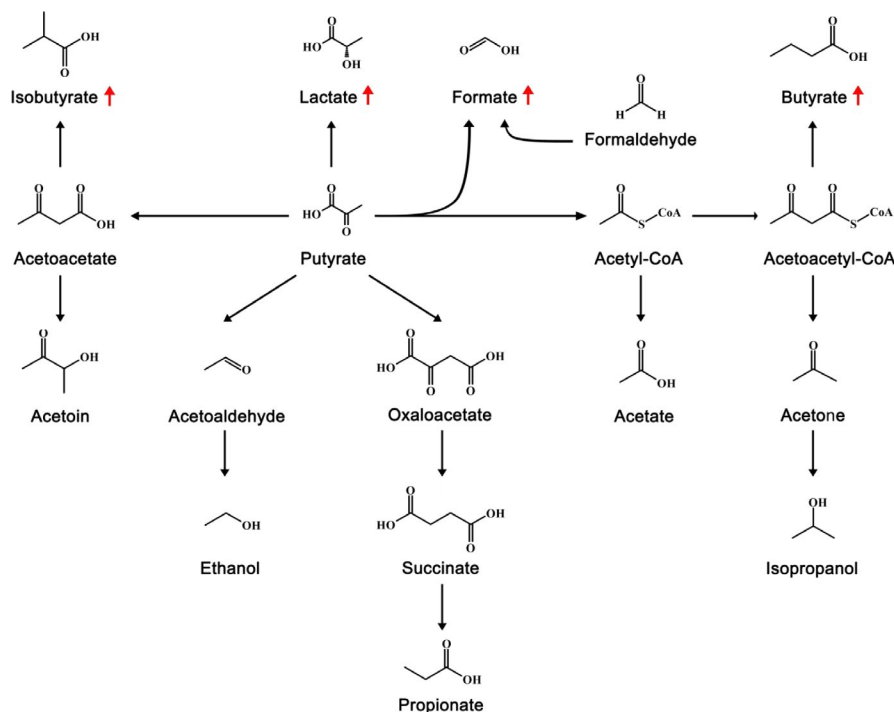


FIGURE 3 The pathway impact analysis of EBC samples based on selected metabolites responsible for the class discrimination. Altered metabolic pathways between asthmatic children and non-asthmatic controls were identified. Both pyruvate and methane metabolic pathways are potentially involved in class separation with statistically significant *P*-values

best of our knowledge, which have not been reported. In line with our findings, Maniscalco et al reported the involvement of pyruvate, methane, and glyoxylate and dicarboxylate metabolic pathways in EBC samples of adults with obesity-related asthma phenotype.¹³ Pyruvate and methane metabolisms are important pathways involved in the requirement of cellular energy for converting glucose into carbohydrates, fatty acid and energy through the Krebs cycle in mammals. Pyruvate, the byproduct of glucose, has been reported to be a potent endogenous antioxidant and free radical scavenger, showing potent anti-inflammation activity.^{13,23} Ostroukhova et al reported that pyruvate dehydrogenase kinase is upregulated in activated T cells to switch glucose metabolism from oxidative phosphorylation to aerobic glycolysis, resulting in increased lactate production and promoting T-cell activation and airway inflammation.²⁴ Ostroukhova et al also recognized that serum lactate level is significantly increased in clinically stable asthmatic adults.²⁴ Meert et al²⁵ found that hyperlactatemia and lactic acidosis were common in children with acute severe asthma, supporting the role of increased lactate production in the pathophysiology of childhood asthma.

Formate is involved in one carbon metabolism and can be endogenously produced from serine, glycine, methionine, choline, methanol, and formaldehyde in mammals. Beside, formate can also be formed as a side product of fermentation and methane

FIGURE 4 Proposed scheme illustrating the metabolic pathways of EBC metabolites discriminating children with stable asthma. EBC, exhaled breath condensate



metabolism of anaerobic bacteria in the gut and then enters the circulation.²⁶ Greenwald et al identified significantly higher formate concentrations in EBC samples from children with asthma than from healthy controls, supporting our findings in this study.²⁷ The EBC formate may represent a marker of depleted airway S-nitrosothiols,²⁷ a class of endogenous airway smooth muscle relaxants that are depleted in asthmatic patients.²⁸ Formate is also a downgrading product of formaldehyde, which has been reported to exacerbate inflammatory state of A549 alveolar and Beas-2B bronchial cell lines,²⁹ as well as in male Wistar rats.³⁰ Ron-Harel et al reported that ablation of *SHMT2* gene encoding the enzyme involved in mitochondrial catabolism of serine to formate leads to impairment of T-cell proliferation and survival,³¹ suggesting the involvement of formate in T-cell regulation. Taken together, elevated formate concentrations in EBC samples of asthmatic children in the current study might indicate the presence of airway smooth muscle contraction and airway inflammation.

Butyrate and its structural isomer isobutyrate are short-chain fatty acids (SCFAs) produced by gut bacterial fermentation of dietary fibers. There is mounting evidence that butyrate possess anti-inflammatory properties.³² Recently, butyrate has been shown to attenuate airway inflammation in ovalbumin-sensitized mice.³³ In the Protection against Allergy-Study in Rural Environments (PASTURE) birth cohort study, children with the highest levels of SCFAs, especially butyrate and propionate, in feces at the age of 1 year had a reduced risk of subsequent asthma between 3 and 6 years.³⁴ Chiu et al found a significantly reduced fecal butyrate levels in children with asthma,³⁵ supporting the role of butyrate in modulating immunological responses in asthma. Further studies are needed to elucidate the roles of butyrate and isobutyrate in the pathophysiology of childhood asthma.

This study has some limitations. First, the study subjects only included Asian children in Taiwan, which might limit the generalization of these findings to other Asian countries and ethnicities. Second, the number of subjects in this study is relatively small, but the findings were consistent across two independent study samples. Third, the enrolled asthmatic children had stable asthma and did not have acute exacerbation of asthma in the preceding 4 weeks, which may obscure the differences in metabolomic profiles between children with and without asthma. Fourth, the use of concomitant medications in a few study subjects might be a concern. Concomitant medications might or might not affect the metabolomic profiles observed in this study. The results should be interpreted with caution.

In summary, our findings in this study add evidence supporting the role of EBC signature to discriminate stable asthma in Asian children in the community, with a discriminative property outperforming conventional clinical tests such as FeNO or spirometry.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Ju Chang-Chien: Data curation (lead); Formal analysis (lead); Investigation (equal); Methodology (equal); Project administration (supporting); Validation (lead); Writing-original draft (lead); Writing-review & editing (equal). **Hsin-Yi Huang:** Data curation (supporting);

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

Supporting Information

Metabolomic Differences of Exhaled Breath Condensate Among Children With and Without Asthma

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Table S1. Demographic and clinical characteristics of study children in the training and validation sets.

Variable	Asthmatics	Non-asthmatics	P-value ^a
Training set			
Number	60	41	
Age, years, mean $\pm$ SD	6.3 $\pm$ 0.3	6.4 $\pm$ 0.3	.122
Gender, male, <i>n</i> (%)	33 (55.0)	22 (53.7)	.894
BMI, kg/m ² , mean $\pm$ SD	15.9 $\pm$ 2.5	15.9 $\pm$ 2.3	.991
Rhinitis, <i>n</i> (%)	43 (71.7)	15 (36.6)	<.001
Eczema, <i>n</i> (%)	27 (45.0)	6 (14.6)	.001
FeNO, ppb, mean $\pm$ SD	16.8 $\pm$ 14.9	13.3 $\pm$ 13.5	.228
FEV ₁ /FVC % predicted, %, mean $\pm$ SD	99.3 $\pm$ 6.3	100.8 $\pm$ 4.7	.210
Concomitant medication, <i>n</i> (%)			
Leukotriene receptor antagonist	9 (15.0)	0 (0.0)	.009
ICS	5 (8.3)	0 (0.0)	.058
ICS-LABA	2 (3.3)	0 (0.0)	0.238
Intranasal corticosteroids	1 (1.7)	0 (0.0)	.406
Antihistamines	5 (8.3)	1 (2.4)	.218
Validation set			
Number	32	32	
Age, years, mean $\pm$ SD	6.4 $\pm$ 0.4	6.4 $\pm$ 0.4	.822
Gender, male, <i>n</i> (%)	16 (50.0)	15 (46.9)	.802
BMI, kg/m ² , mean $\pm$ SD	15.6 $\pm$ 1.7	15.6 $\pm$ 1.8	.999
Rhinitis, <i>n</i> (%)	22 (68.8)	9 (28.1)	.001
Eczema, <i>n</i> (%)	14 (43.8)	3 (9.4)	.002
FeNO, ppb, mean $\pm$ SD	24.9 $\pm$ 20.2	12.3 $\pm$ 12.4	.004
FEV ₁ /FVC % predicted, %, mean $\pm$ SD	99.9 $\pm$ 6.9	102.3 $\pm$ 4.4	.101
Concomitant medication, <i>n</i> (%)			
Leukotriene receptor antagonist	5 (15.6)	0 (0.0)	.020
ICS	1 (3.1)	0 (0.0)	.313
ICS-LABA	1 (3.1)	0 (0.0)	.313
Intranasal corticosteroids	2 (6.3)	0 (0.0)	.151
Antihistamines	3 (9.4)	1 (3.1)	.302

Note:

Values shown are mean $\pm$ standard deviation or number (%) of subjects where appropriate. Values in bold are statistically significant at $P < .05$.

^a Chi-squared tests were used for categorical variables, and Student's *t* tests for continuous variables.

Abbreviations: SD, standard deviation; BMI, body mass index; FEV₁/FVC, forced expiratory volume in 1 second/ forced vital capacity; ICS, inhaled corticosteroids; LABA, long acting β_2 sympathomimetic agonists.

Table S2. Relative abundance and fold change of EBC metabolites between asthmatic children and non-asthmatic controls.

Metabolite	Mean $\pm$ SD		Fold change	P-value ^a
	Asthmatics (n=60)	Non-asthmatics (n=41)		
Formate, a.u. ^b	0.0015 $\pm$ 0.0012	0.0012 $\pm$ 0.0007	1.25	.042
Methanol, a.u.	0.0585 $\pm$ 0.0365	0.0671 $\pm$ 0.0424	0.87	.281
Acetone, a.u.	0.0236 $\pm$ 0.0302	0.0225 $\pm$ 0.0218	1.05	.832
Butyric acid, a.u.	0.0022 $\pm$ 0.0010	0.0018 $\pm$ 0.0009	1.22	.034
Acetate, a.u.	0.0198 $\pm$ 0.0193	0.0174 $\pm$ 0.0139	1.14	.476
Alanine, a.u.	0.0054 $\pm$ 0.0028	0.0050 $\pm$ 0.0028	1.08	.482
Acetoin, a.u.	0.0017 $\pm$ 0.0011	0.0018 $\pm$ 0.0017	0.94	.826
Lactate, a.u.	0.0155 $\pm$ 0.0126	0.0102 $\pm$ 0.0065	1.52	.006
Ethanol, a.u.	0.3754 $\pm$ 0.0880	0.3832 $\pm$ 0.0895	0.98	.663
Isopropanol, a.u.	0.1561 $\pm$ 0.0376	0.1599 $\pm$ 0.0385	0.98	.618
Isobutyrate, a.u.	0.0277 $\pm$ 0.0051	0.0258 $\pm$ 0.0039	1.07	.049
Propionate, a.u.	0.0106 $\pm$ 0.0092	0.0087 $\pm$ 0.0039	1.22	.158

Note:

^a Values in bold are statistically significant at $P < .05$.

^b The relative integral areas under the various peaks are calculated from nuclear magnetic resonance spectra and plotted as a.u.

Abbreviations: EBC, exhaled breath condensate; SD, standard deviation.