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RESEARCH LETTER

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Metabolomic profile in preschool children with transient wheezing and early onset asthma

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Abstract
Introduction: Early diagnosis of childhood asthma is a challenge; so we questioned whether metabolomic analysis could differentiate persistent recurrent wheezing from transient wheezing in preschoolers. *Methods*: Case-control study with individuals aged 4–6 years and 11 months with three or more episodes of wheezing due to bronchospasm was carried out in an allergy outpatient clinic and metabolomics laboratory from July 2021 to February 2023. Two groups were formed: persistent wheezers with multiple trigger attacks after the fourth year of life; and transient wheezers without wheezing for at least 12 months after the third year of life. Those with other wheezing disorders were excluded.
Results: This study was carried out on 29 children with a mean age of 4.9 (±0.6) years—19 (65%) persistent wheezers and 10 (35%) transient wheezers. Sensitization to aeroallergens and the positive asthma predictive index were significantly higher among persistent wheezers. From the plasma hydrogen-1 NMR (^{1}H NMR) spectra, five best subsets were selected to discriminate between the two groups with an accuracy rate of 93.1%. Among the metabolites, valine and citrate showed higher signals and lipids and lipoproteins were higher in transient wheezers.

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Introduction

Asthma is the most common chronic disease of childhood that results in high morbidity and mortality as well as high healthcare costs.[1] The heterogeneity of the clinical aspects of asthma and the presence of different wheezing phenotypes in childhood, such as transient wheezing, can make adequate diagnosis and control of the disease difficult. Thus, the analysis of biomarkers of the inflammatory process of the airways represents an interesting tool for the early diagnosis and monitoring of asthma.[2] Metabolomics has been used for the early and differential diagnosis of different diseases[3,4] and can help identify pathways associated with a complex disease such as asthma, facilitating its diagnosis and allowing early treatment.

The aim of this study was to evaluate whether Nuclear Magnetic Resonance (NMR)-based metabolomics using the plasma of preschool children with recurrent wheezing makes it possible to differentiate those who present transient wheezing from those who persist with wheezing due to multiple triggers, considered to be asthmatics of early onset.

Methods

This case-control study was carried out in the allergy outpatient clinic and metabolomics laboratory of the Federal University of Pernambuco, Recife, Brazil, from July 2021 to February 2023, with individuals aged 4–6 years old and with three or more previous episodes of wheezing. After the caregivers signed the free and informed consent form, two groups were formed: persistent wheezing—children who continued to have attacks caused by multiple triggers after the fourth year of life—and transient wheezing—children without wheezing for at least 12 months after the third year of life. Those with wheezing from other conditions were excluded. The patients' epidemiological data, number of serum eosinophils, total immunoglobulin E (IgE), and prick test for respiratory allergens were recorded. Both groups underwent blood collection—5 mL in a dry tube, which was centrifuged (Fanem model 206 BL centrifuge). The resulting serum was stored in Eppendorf-type tubes, frozen at a temperature of −15°C, with subsequent analysis of the plasma metabolomics using NMR spectrometer.

Statistical analysis

For frequency analysis between groups, Fisher's test was used. Comparison between means of normal variables was performed using the student's t-test. Metabolomic models were built using the MetaboAnalyst 5.0 online platform. The matrix data were preprocessed using sum normalization (SNV) on the samples (rows) and autoscaling on the variables (columns). Subsequently, they were subjected to exploratory Principal Component Analysis (PCA) to investigate whether there was a natural tendency to cluster in the classes of interest as well as to identify anomalous samples. Next, the supervised partial least squares discriminant analysis (PLS-DA) and orthogonal partial least squares discriminant analysis (OPLS-DA) formalisms were used to construct classification models, seeking to differentiate the groups in each study. All models were validated by leave-one-out cross-validation (LOOCV) and tested with 2000 permutations. The Statistica 12.0 program was also used to apply the Linear Discriminant Analysis (LDA) formalism. The models were built using five variables that were selected using Wilks' lambda. All LDA models were validated by LOOCV, and accuracy, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated.

This study was evaluated and approved by the research ethics committee of Hospital da Clínicas at the Federal University of Pernambuco (number 4,762,232).

Results

With a mean age of 4.9 years, 29 children were evaluated with 19 (65%) in the persistent wheezing group and 10 (35%) in the transient wheezing group. Sensitization to aeroallergens and positive asthma predictive index were significantly higher among persistent wheezing patients (Table 1).

From the plasma ^{1}H NMR spectra, five best subsets were selected to discriminate the two groups, with an accuracy rate of 93.1%. Table 2 presents the contingency matrix of

Table 1 Clinical and demographic data of preschool children with persistent and transient wheezing (N = 29).

Clinical data	Persistent wheezing (N = 19)	Transient wheezing (N = 10)	p*
Total	19 (65%)	10 (35%)	
Age (years)#	4.9 (± 0.7)	4.8 (± 0.5)	0.62
First episode of wheezing (months) #	9.8 (± 7.8)	16.7 (± 12)	0.25
Male	63%	80%	0.43
Rhinitis	84%	70%	0.39
Atopic dermatitis	11%	0	0.53
Dust mite sensitization	77%	30%	**0.02**
APIm**	84%	40%	**0.03**
Eosinophilia	812 (± 735)	488 (± 159)	-
Total IgE	1617 (± 2578)	108 (± 83.6)	-

*p-values (Fisher) **Modified Asthma Predictive Index; #Mean ± standard deviation; Student's t-test.

Table 2 Classification matrix of the metabonomic model I (LDA, five variables, 29 samples).

Metabolomic Model	Clinical Diagnosis	
	Persistent wheezing	Transient wheezing
Persistent wheezing	17	2
Transient wheezing	0	10

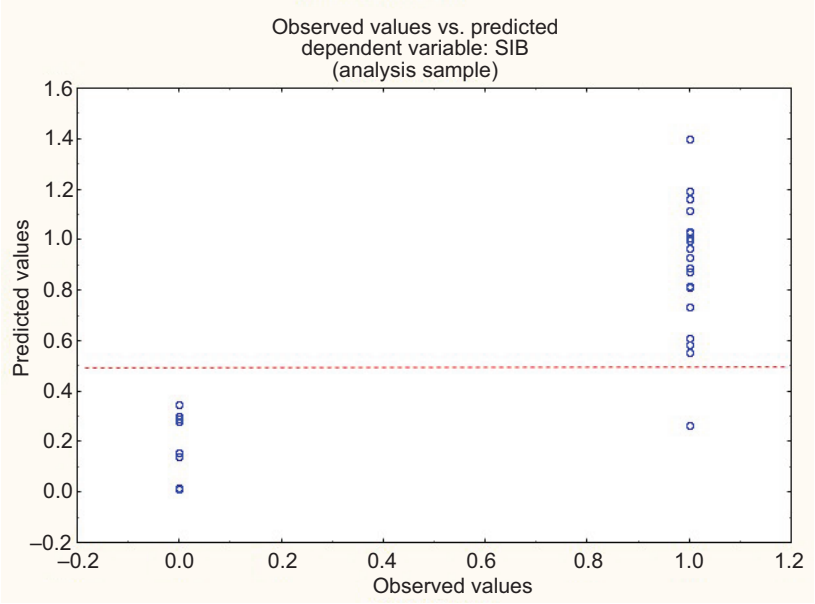

Figure 1 LDA model—plot of predicted values versus observed values. Values in 1 are for persistent wheezers and values in 0 are for transient wheezers.

the LDA model after cross-validation to differentiate the two groups.

Figure 1 presents the graph of values predicted by the LDA metabonomic model versus the values observed for each sample (1 = persistent wheezing; 0 = transient wheezing).

Metabolite identification

The important variables for discrimination in this model were: 0.91 ppm, 1.11 ppm, 1.23 ppm, 2.70 ppm, and 2.78 ppm. According to the discriminant functions, the most intense chemical shifts—δ 1.11 ppm and 2.70 ppm—lead to the persistent sibilant classification and the chemical shifts—δ 0.91 ppm, 1.23 ppm, and 2.78 ppm—are more intense in transient sibilants. The relationship between these variables, the specific phenotypes, and the possible metabolites responsible for discrimination are described in Table 3.

Table 3 Discriminating metabolites, corresponding bins, and relative levels in each wheezing phenotype.

Metabolites	δ (ppm)	Group
VLDL/LDL	0.91	Transient ↑
Valine	1.11	Persistent ↑
VLDL/LDL	1.23	Transient ↑
Citrat	2.70	Persistent ↑
Lipids and lipoproteins	2.78	Transient ↑

LDL, low-density lipoprotein; VLDL, very low-density lipoprotein.

Discussion

Most asthma metabonomic studies are aimed at adults or children over 7 years old due to the ability to confirm the diagnosis with spirometry.[5]

In this study, 29 children were matched by age—19 persistent wheezers and 10 transient wheezers. The persistent group started wheezing earlier, had a higher percentage of sensitization to aeroallergens, and higher mean values of peripheral eosinophils and total IgE. We found that children under 7 years of age who maintained wheezing caused by multiple triggers had a plasma metabolome with a different pattern than children who had been asymptomatic for at least one year. We also identified some potential biomarkers characteristic of each of the two conditions.

Using the selection of the five best subsets to discriminate the two groups by LDA, we obtained a 93.1% accuracy rate after LOOCV validation. Among persistent wheezers, higher levels of the metabolites valine and citrate were identified in serum. Changes in metabolites such as citrate suggest a disturbance related to the arachidonic acid cycle. This, in turn, is a precursor of inflammatory mediators such as leukotrienes, prostaglandins, and sphingomyelins involved in airway inflammation such as rhinitis and asthma.[6]

In a study using plasma and urine samples, the authors found a significantly higher level of histidine in plasma in parallel with lower levels of 1-methylnicotinamide and trimethylamine N-oxide (TMAO) in the urine of children with asthma compared to healthy controls. Significant correlations were observed between plasma 3-hydroxybutyric acid and the excreted volumes of hydroxy acids, which were strongly correlated with plasma levels of leucine and valine.[7] The degradation metabolism of plasma pyruvate and urinary valine, leucine, and isoleucine were significantly

associated with allergic sensitization to childhood asthma, showing that the blood and urine metabolome reflect different metabolic pathways in allergic reactions. Another recent study used the analysis of exhaled breath condensate (EBC) samples from children aged 5–7 years via ^{1}H NMR and OPLS-DA formalism and identified four EBC metabolites—lactate, formate, butyric acid, and isobutyrate—as potential biomarkers of asthma in young children. Analysis of the metabolic pathway revealed alterations in the metabolism of pyruvate and methane in the airways of children with asthma, suggesting that these pathways may be specifically altered in asthmatic children.[8]

Conclusions

In our sample, we found that despite the small number of participants, the metabolomic analyses allowed a good discrimination between children with transient wheezing and persistent wheezing who can be clinically identified as early onset asthmatics. We also identified some putative biomarkers characteristic of each of the two conditions that may have a role as early biomarkers of childhood asthma. This study should be considered preliminary, and further studies are needed to confirm our discrimination models in larger and independent groups of children and to validate the metabolites identified.

Author Contributions

All authors contributed equally to this article.

Conflicts of Interest

We declare that there were no conflicts of interest for this study.

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