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Metabolomic profiling of samples from pediatric patients with asthma unveils deficient nutrients in African Americans

Obesity and vitamin D deficiency are common in asthma cases. Decreased levels of citrate, ketone bodies, and two amino acids histidine (His) and glutamine (Gln), were observed in asthma cases compared to controls. Metabolites for lipid metabolism lost significance after controlling for comorbid obesity.

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Highlights

Asthma is a major health issue in African Americans

Metabolomics represents a powerful approach to understand the metabolism in asthma

We observed decreased citrate, ketone bodies, and amino acids in the plasma

Supplementation of nutrients that are deficient may be beneficial for asthma care

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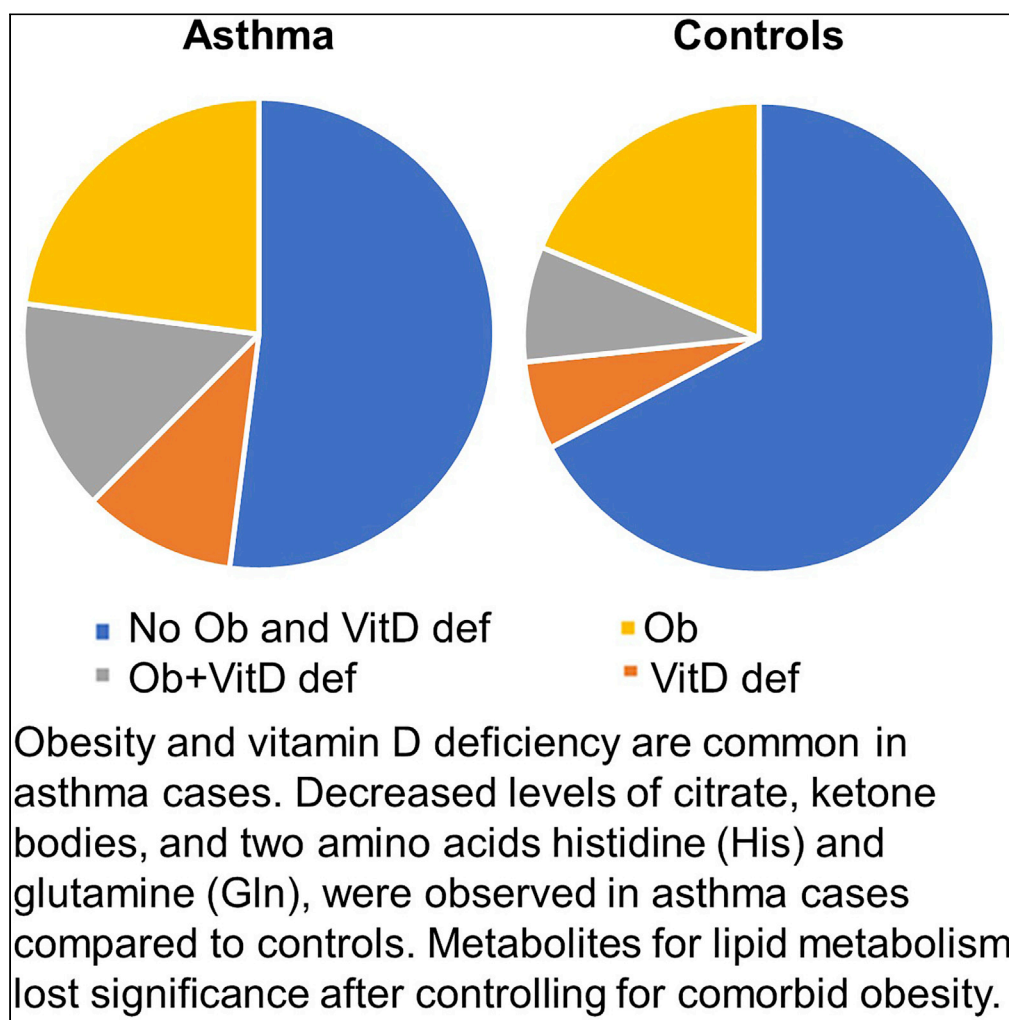# Metabolomic profiling of samples from pediatric patients with asthma unveils deficient nutrients in African Americans

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SUMMARY

Plasma metabolomics represents a potentially powerful approach to understand the biochemical mechanisms of nutrition and metabolism in asthma. This study aims to acquire knowledge on plasma metabolites in asthma, which may provide avenues for nutrition therapy, as well as explanations for the observed effects in existing therapies. This study investigated 249 metabolites from 18 metabolite groups in a large cohort of African American population, including 602 pediatric patients with asthma and 593 controls, using a nuclear magnetic resonance (NMR) metabolomics platform. Decreased levels of citrate, ketone bodies, and two amino acids histidine (His) and glutamine (Gln), were observed in asthma cases compared to controls. Metabolites for lipid metabolism lost significance after controlling for comorbid obesity. For the first time, this study depicts a broad panorama of lipid metabolism and nutrition in asthma. Supplementation or augmentation of nutrients that are deficient may be beneficial for asthma care.

INTRODUCTION

Asthma is a major health issue in African Americans. According to the Centers for Disease Control and Prevention (CDC), African Americans children have a 60% greater prevalence of asthma at 14.3%, and 7.1-fold greater risk of death due to asthma, than non-Hispanic white (Ebell et al., 2019).

With the rapid progress of omics technologies, plasma or serum metabolomics has become an accessible approach to probe the biomechanics of human diseases that may help uncovering noninvasive metabolites, in particular for nutritional/diet interventions (James and Parkinson, 2015). Important insights have been acquired about, for example, systemic inflammation and oxidative stress in asthma using metabolomics (Nambiar et al., 2020).

Diet and nutrition have been suggested as important risk factors for the development and management of obstructive lung diseases such as asthma (Berthon and Wood, 2015). In patients with asthma, diet manipulation is considered to have beneficial effects on systemic inflammation and oxidative stress, and may play protective roles in asthma development and improving asthma symptoms (Alwarith et al., 2020). However, our understanding of changes of plasma metabolites in asthma remains unclear.

The objective of this study is to acquire knowledge on plasma metabolites in asthma, which may provide novel avenues for nutrition therapy, as well as explanations for the observed effects in existing therapies. For this purpose, we performed a metabolomic study in a large cohort of pediatric patients with asthma of African American ancestry (n = 602) in comparison with 593 controls without asthma. This study used a nuclear magnetic resonance (NMR) metabolomic technology with high reproducibility, including 249 selected metabolites related to common human diseases from 18 metabolite groups, with 98 metabolites of lipoprotein subclasses, 70 metabolites of relative lipoprotein lipid concentrations, 18 metabolites of fatty acids, 10 metabolites of disease-related amino acids, as well as metabolites for glycolysis metabolites, ketone bodies, inflammation, etc. A number of nutrients correlated with lower levels of asthma, with beneficial effects being observed for the supplementation of these nutrients by previous studies (Hernández et al., 2019; Poynter et al., 2020; Smith et al., 2013).

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Table 1. Correlation between asthma and metabolite levels[a]

	Controlled For Age & Sex			Controlled For Age, Sex, Obesity and VitD deficiency				
	r	P (2-tailed)	Df	r	P (2-tailed)	df	Group	Subgroup
Citrate	−0.136	2.48E-06	1191	−0.115	6.90E-05	1189	Glycolysis-related metabolites	
Acetoacetate	−0.092	0.002	1190	−0.083	0.004	1188	Ketone bodies	
β-Hydroxybutyrate	−0.087	0.003	1187	−0.082	0.005	1185	Ketone bodies	
Histidine	−0.093	0.001	1190	−0.072	0.013	1188	Amino acids	
Glutamine	−0.086	0.004	1158	−0.067	0.022	1156	Amino acids	
Ratio of saturated fatty acids to total fatty acids (SFA%)	−0.061	0.036	1190	−0.064	0.027	1188	Fatty acids	Fatty acid ratios
Free cholesterol to total lipids ratio in medium VLDL (FC% in mVLDL)	−0.06	0.039	1191	−0.062	0.033	1189	Relative lipoprotein lipid concentrations	Medium VLDL ratios
Acetate	−0.065	0.024	1189	−0.061	0.037	1187	Ketone bodies	

Beta: standardized beta coefficient; t: t-statistic; FC% in mVLDL: Free cholesterol to total lipids ratio in medium VLDL; VLDL: very low density lipoprotein; SFA%: Ratio of saturated fatty acids to total fatty acids.
[a]Point-Biserial partial correlation t-tests were used to measure the relationship between asthma and metabolite levels. r: Point-Biserial correlation coefficient; df: Degrees of Freedom; VitD: Vitamin D; VLDL: very low-density lipoprotein.

RESULTS

As shown by the Point-Biserial partial correlation between asthma and metabolite levels, and controlling for age and sex, 37 metabolites from 10 groups showed p < 0.05. Notably, a number of metabolites for lipid metabolisms are significant, e.g., 13 metabolites for relative lipoprotein lipid concentrations, 11 metabolites for lipoprotein subclasses, and several metabolites for cholesterol and fatty acids. In addition, many of these metabolites are for high-density lipoprotein (HDL) particles, and have lower levels in asthma cases.

Considering the clinical association of asthma and obesity, as well as the common comorbidities of obesity and VitD deficiency in asthma cases, we performed further partial correlation analysis between asthma and the metabolite levels, controlled for age, sex, obesity status, and VitD deficiency. As expected, significantly fewer (or only 8) metabolites with p < 0.05 in the initial test retained p < 0.05 after additionally controlled for obesity and VitD deficiency (Table 1).

Decreased citrate levels in asthma

Lower citrate is correlated with asthma with a $p = 6.90 \times 10^{-5}$, and remains statistically significant after Bonferroni correction for multiple testing (i.e., $\alpha = 0.05/249 =$ significance threshold of 2.01×10^{-4}). In addition, lower citrate is correlated with increased age, asthma, obesity, and VitD deficiency (Table 2).

Decreased levels of ketone bodies in asthma

The two main ketone bodies—acetoacetate and β-Hydroxybutyrate (βOHB)—as well as acetate, have lower levels in asthma cases with p < 0.05. Both age and asthma status are correlated with levels of the two main ketone bodies—i.e., lower levels in older individuals and asthma cases. In contrast, lower levels of acetate were seen in younger individuals and asthma cases (Table 2).

Decreased levels of two amino acids in asthma

Lower plasma levels of two amino acids histidine (His) and glutamine (Gln) were seen in asthma cases with p < 0.05. Both asthma and obesity are correlated with lower levels of His and Gln. In addition, older age is correlated with higher levels of His (Table 2).

Metabolites for lipid metabolism correlated with asthma

A diagnosis of asthma correlated with lower levels of FC% in mVLDL [Free cholesterol to total lipids ratio in medium very low-density lipoprotein (VLDL)], and lower ratio of saturated fatty acids (SFA) to total fatty

Table 2. Linear regression between asthma and metabolite levels[a]

Glycolysis related metabolites	Citrate	Standardized Coefficients		
	Linear regression	Beta	t	P
	age	−0.171	−5.938	3.79E-09
	sex	−0.036	−1.255	0.210
	asthma	−0.115	−3.994	6.90E-05
	Obesity	−0.083	−2.818	0.005
	Vitamin D deficiency	−0.059	−2.012	0.044
Ketone bodies	Acetate			
	age	0.105	3.555	3.94E-04
	sex	−0.039	−1.325	0.185
	asthma	−0.062	−2.090	0.037
	Obesity	0.003	0.096	0.923
	Vitamin D deficiency	−0.028	−0.925	0.355
	Acetoacetate			
	age	−0.128	−4.353	1.46E-05
	sex	−0.020	−0.705	0.481
	asthma	−0.084	−2.858	0.004
	Obesity	−0.013	−0.444	0.657
	Vitamin D deficiency	−0.041	−1.379	0.168
	β-Hydroxybutyrate			
	age	−0.090	−3.061	0.002
	sex	−0.027	−0.938	0.348
	asthma	−0.083	−2.816	0.005
	Obesity	0.017	0.569	0.570
	Vitamin D deficiency	−0.044	−1.456	0.146
Amino acids	Histidine			
	age	0.172	5.962	3.28E-09
	sex	−0.029	−1.002	0.317
	asthma	−0.072	−2.494	0.013
	Obesity	−0.115	−3.883	1.09E-04
	Vitamin D deficiency	−0.033	−1.110	0.267
	Glutamine			
	age	0.029	0.966	0.334
	sex	−0.016	−0.553	0.581
	asthma	−0.068	−2.283	0.023
	Obesity	−0.099	−3.252	1.18E-03
	Vitamin D deficiency	−0.034	−1.104	0.270
Relative lipoprotein lipid concentrations	FC% in mVLDL			
	age	0.129	4.410	1.13E-05
	sex	0.039	1.356	0.175
	asthma	−0.063	−2.137	0.033
	Obesity	0.045	1.505	0.133
	Vitamin D deficiency	−0.020	−0.672	0.502
Fatty acids	SFA%			
	age	−0.132	−4.513	7.02E-06
	sex	−0.061	−2.105	0.035

(Continued on next page)

Table 2. *Continued*

Glycolysis related metabolites	Citrate	Standardized Coefficients		
	asthma	−0.065	−2.206	0.028
	Obesity	0.055	1.850	0.065
	Vitamin D deficiency	−0.022	−0.720	0.472

[a]Linear regression t-tests were used to estimate p values (two-tailed).

acids (SFA%). In addition, older age is correlated with both higher levels of FC% in mVLDL and lower levels of SFA% (Table 2).

DISCUSSION

Lower citrate in asthma

Levels of metabolites in the citrate cycle are altered in asthma related to hypoxia as reviewed by Kelly et al.(Kelly et al., 2017) Increased workload of inspiratory muscles and hypoxia, in addition to high turnover rate of the citrate cycle in asthma (Papamichael et al., 2021), may deplete energy stores and lower citrate level. We observed lower citrate levels in individuals with asthma with statistical significance after correction for multiple testing (Bonferroni corrected p = 0.017). No blood sample in this study was treated with citrate anticoagulant. Bone is the major source of citrate in plasma, which releases citrate into plasma during osteoclast bone resorption. Plasma citrate levels are mainly regulated by parathyroid hormone (PTH, upregulation of plasma citrate level) and calcitonin (CT, downregulation of plasma citrate level) (Costello and Franklin, 2016). The age effect observed in this study (i.e. higher citrate in younger age) may be explained by higher osteoclast activity and bone turnover in children than young adults (Jürimäe, 2010). The link between obesity and bone metabolism is complex (Cao, 2011; Hou et al., 2020). In children and young adults, obesity is associated with suppressed bone turnover (Fintini et al., 2020; Viljakainen et al., 2014), which may explain the lower level of citrate in obesity. The correlation between VitD deficiency and lower citrate level observed in this study may be due to decreased activity of osteoclast in VitD deficiency (Bikle, 2012).

The roles of citric acid cycle metabolites in the hyperresponsiveness and airway remodeling in asthma are related to the function of ten-eleven translocation (TET) enzymes in airway smooth muscle (SM) cells, as highlighted by a recent study (Huang, 2020; Yeung et al., 2020). TET1 has been demonstrated to prevent house dust mite (HDM)-induced allergic airway inflammation in a mouse model (Burleson et al., 2019). Hypocitricemia may lead to decreased TET activity, and thus contribute to the development of asthma. The finding of our study may suggest citrate supplementation as a potential new therapy for patients with asthma of African American ancestry.

Lower ketone bodies in asthma

Increased energy demands and hypoxia in asthma (Kelly et al., 2017) may increase the consumption of ketone bodies. The two main ketone bodies as a pair of redox couples, acetoacetate and βOHB, have lower levels in asthma cases as shown in this study. In addition to providing energy, signaling roles of ketone bodies in health and disease have been suggested by previous studies as reviewed by Puchalska and Crawford (Puchalska and Crawford, 2021). βOHB is a stress response molecule and plays key roles in maintaining redox homeostasis in response to environmental and metabolic challenges (Rojas-Morales et al., 2020). In particular, immunomodulatory and anti-inflammatory effects of βOHB by suppressing activation of the NLRP3 inflammasome have been demonstrated (Youm et al., 2015). NLRP3 (NOD-, LRR-, and pyrin domain-containing protein 3) is an intracellular sensor that detects variety of pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs), and results in the formation and activation of the NLRP3 inflammasome (Swanson et al., 2019; Zhao and Zhao, 2020). Ketone body augmentation has been suggested to have therapeutic effects in HDM-induced asthma (Poynter et al., 2020).

In contrast to acetoacetate and βOHB being generated in liver, plasma acetate is from microbial-derived production in the colon, diet (e.g., vinegar), and endogenous acetogenesis from acetyl-CoA hydrolysis in cytoplasm (Moffett et al., 2020). Citrate, with lower levels among individuals with asthma as observed, is the
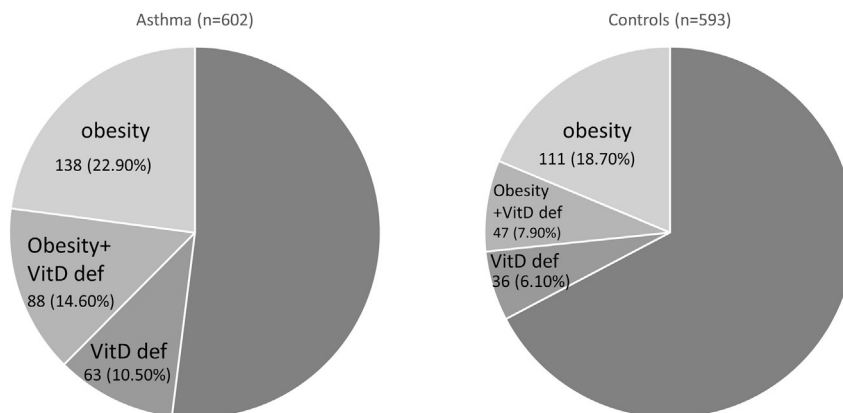
Figure 1. Obesity and VitD deficiency in asthma cases and controls

Obesity and VitD deficiency are common in both cases and controls. For the cases, 22.9% have obesity, 10.5% have VitD deficiency, and 14.6% have both obesity and VitD deficiency. For the controls, 18.7% have obesity, 6.1% have VitD deficiency, and 7.9% have both obesity and VitD deficiency.

source of acetyl-CoA to generate acetate in cytoplasm in various tissues including liver, intestine, and brain (Hernández et al., 2019; Moffett et al., 2020). Acetate supplementation has been shown to increase the number and function of anti-inflammatory Treg cells and to decrease systemic pro-inflammatory cytokine levels (Hernández et al., 2019; Smith et al., 2013).

In this study, both ketone bodies are inversely correlated with age. An inverse relationship between age and blood ketone bodies in fasting status has been previously observed in children (Saudubray et al., 1981). Higher level of acetate is, however, correlated with increasing age as shown in these pediatric subjects. On the other hand, acetate levels were found to decline slightly in older adults (Skutches et al., 1979). These age effects highlight the importance of controlling for age in ketone body augmentation and acetate supplementation.

Lower His and Gln in asthma

Lower levels of plasma His and Gln were correlated with asthma. His is an essential amino acid (EAA). Lower His level in autoinflammatory states (e.g., rheumatoid arthritis) have been observed for a long time (Gerber et al., 1976). His supplementation for its anti-inflammatory and antioxidant effects have been investigated in a wide range of conditions with potential benefits, including autoinflammatory diseases like rheumatoid arthritis, inflammatory bowel disease, and atopic dermatitis (Holeček, 2020). The increased His level by age, as observed here, is supported by a previous study (Lepage et al., 1997). We observed a lower His level in obesity, while significantly lower serum His levels have been previously found in obese women (Niu et al., 2012). His supplementation reduces high-fat diet-induced body weight increase and ameliorates inflammation and oxidative stress of adipose tissue in female obese rat models (Sun et al., 2014).

It is important to emphasize that His is the precursor of histamine. Histamine plays a central role in allergic reactions mediated by mast cells (Amin, 2012). In asthma, histamine contributes to airway obstruction by inducing the bronchoconstriction of smooth muscle, secretion of mucous glycoprotein, and airway submucosal edema (Yamauchi and Ogasawara, 2019). His decarboxylase (HDC) catalyzes the generation of histamine from His, and the expression of HDC is elevated in asthma (Yamauchi, 1996). The potential effects of His supplementation on histamine reaction in asthma may warrant further investigation.

Gln is not an EAA, but rather n conditionally essential amino acid, showing deficiency in stress conditions (Morris et al., 2017). A trend of lower Gln level in asthma has been observed previously, albeit not meeting statistical significance (p = 0.07) (Fogarty et al., 2004). As reviewed by Oliveira et al., several studies have suggested that exogenous Gln administration might be beneficial in respiratory diseases, e.g., acute respiratory distress syndrome (ARDS) and asthma (Oliveira et al., 2016). We observed a lower Gln level in obesity. A study by Petrus et al. showed that reduced Gln levels are a metabolic signature of obese white adipose tissue in human (Petrus et al., 2020). Gln administration may attenuate adipose tissue inflammation in obese mouse model and human fat cell models (Petrus et al., 2020).

Lipid levels in asthma

Increased energy demands and hypoxia in asthma (Kelly et al., 2017) may increase lipid mobilization as an alternative energy source. Insulin resistance commonly seen in patients with asthma, especially in those with obesity (Arshi et al., 2010) is closely related to the pathogenesis of dyslipidemia. Dyslipidemia is a key driver of airway inflammation in asthma (Wood et al., 2003). Previous studies have shown that blood lipid levels are associated with childhood asthma, and higher levels of LDL cholesterol and triglyceride have been associated with concurrent asthma, airway obstruction, and aeroallergen sensitization, while higher levels of HDL cholesterol have been associated with improved airway resistance and decreased bronchial responsiveness and aeroallergen sensitization (Vinding et al., 2016). In this study, a number of metabolites for lipid metabolisms showed $p < 0.05$, including metabolites for relative lipoprotein lipid concentrations, metabolites for lipoprotein subclasses, and metabolites for cholesterol and fatty acids. However, obesity is a common comorbidity in asthma, and is the most common cause of lipid disorders. Here, we tested the correlation of metabolomic metabolites and asthma controlled for obesity. The associations of most of metabolites for lipoprotein subclasses were not significant after controlling for obesity, except two metabolites, free cholesterol to total lipids ratio in medium VLDL (FC% in mVLDL) and SFA%.

Levels of FC% in mVLDL were lower in asthma cases compared to controls. However, no significant change was observed for the level of free cholesterol in medium VLDL (M_VLDL_FC), nor was any change observed for total cholesterol (i.e., the sum of free cholesterol and cholesteryl esters) to total lipid ratios in medium VLDL (M_VLDL_C_pct), or cholesteryl esters to total lipid ratios in medium VLDL (M_VLDL_CE_pct). Therefore, the change of FC% in mVLDL in asthma needs to be replicated in independent samples before we venture any biomedical explanation.

Increased SFA have been reported in patients with mild, moderate, and severe asthma by a previous study (Reinke et al., 2017). Instead, lower SFA% were observed in asthma cases in this study. In this regard, unsaturated fatty acids have been suggested to have health benefits, including anti-inflammatory effects (Delmastro-Greenwood et al., 2014). However, different unsaturated fatty acids have been shown to have different inflammatory properties. For example, the two essential nutrients of unsaturated fatty acids, the pro-inflammatory omega-6 (n-6), and the anti-inflammatory omega-3 (n-3) have opposite inflammatory properties in asthma (Wendell et al., 2014). The $p = 0.027$ of SFA% observed in this study, therefore, needs replication in an independent sample. If validated, further investigation would be warranted for the change of the components of unsaturated fatty acids and their pathogenic implications in asthma.

In conclusion, we observed decrease in citrate, ketone bodies, and the two amino acids, His and Gln, in the plasma of asthma cases undergoing metabolomic measurements. This suggests that augmentation or supplementation of these nutrients may be beneficial as asthma treatment. The change of citrate level is statistically significant after correction for multiple testing. Importantly, both the changes of the ketone bodies and the two amino acids lack statistical significance following correction for multiple testing. Thus, validation of these changes in independent samples is required. We do note, however, that a number of previous studies support these observations, while ketone body augmentation, or supplementation of acetate or administration of the two amino acids has been suggested to be potentially beneficial in treating inflammatory diseases such as asthma. Asthma is a complex disease, involving a combination of numerous genetic, environmental, and lifestyle factors (Arruda et al., 2005). Despite the small r values, the correlations identified in this study highlighted the decreased metabolites as risk factors and potential therapeutic targets of asthma, in addition to mechanistic knowledge gained on asthma. Finally, this study urges caution in ascribing changes in lipid metabolism in relation to asthma, as obesity is a common comorbidity, and the most common cause of lipid disorders. Nonetheless, obesity in asthma should not necessarily be regarded as a simple confounder. Obesity, the most common type of metabolic health problems in children and adults, has been suggested as both a major risk factor and a disease modifier for asthma in children and adults (Peters et al., 2018; Tesse et al., 2011).

Limitations of the study

Limited by the sample size of the current study, the reported metabolites warrant for replication in independent samples, including different populations other than African American. As a project of the International Hundred Thousand Plus Cohort Consortium, this study aims to assess the research application in asthma, as well as the clinical potential, of a cost-effective NMR metabolomic platform with high reproducibility. In contrast to an untargeted metabolomic platform, the Nightingale NMR platform used in this study

includes 249 selected metabolites. In this case, further study with an untargeted metabolomic technology or a targeted metabolomic platform with defined metabolite markers, e.g., assessing comprehensively the citrate cycle, will be helpful to validate and further clarify the change of related metabolic pathways.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2022.104650.

AUTHOR CONTRIBUTIONS

Conceptualization, H.H. and H.Q.; literature search, H.Q.; Figures and Tables, H.Q.; data analysis, H.Q., J.G., J.Q., and F.M.; data interpretation, H.Q., J.G., J.Q., S.G., I.C., P.S., J.J.C., and H.H.; original draft writing, H.Q. and H.H.; review and revision, H.Q., J.J.C., and H.H.; supervision, H.H.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Plasma	The Committees for the Protection of Human Subjects (IRB), The Children's Hospital of Philadelphia (CHOP)	IRB 16-013278; IRB 10-007590
Deposited data		
Metabolomics data	MetaboLights (Metabolomics experiments and derived information, https://www.ebi.ac.uk/metabolights/)	MTBLS5024

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Dr. Hakon Hakonarson (hakonarson@chop.edu).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- The metabolomic data presented in this study is available through Metabolights (MTBLS5024, www.ebi.ac.uk/metabolights/MTBLS5024).

- There is no original code associated with this work.

- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Patient recruitment and sample collection

This study included 602 pediatric cases (with the upper age limit as 21 years (Hardin et al., 2017)) with asthma (299 males and 303 females, age range 6–21 years old, and 593 controls without asthma (265 males and 328 females, age range 6–21 years old. All cases were recruited at the Center for Applied Genomics (CAG) at the Children's Hospital of Philadelphia (CHOP), with the ancestry of African American confirmed by the genome-wide single nucleotide polymorphism (SNP) genotyping and principal component analysis of population structure. No individuals had a history of sickle cell disease, type 1 diabetes, 22q11.2 deletion syndrome, or human immunodeficiency virus (HIV) infection. Obesity and vitamin D (VitD) deficiency were common in both cases and controls (Figure 1, Table S1). As expected, both obesity and VitD deficiency were more common in asthma cases as both are related to the risk of asthma (Bener et al., 2012; Beuther et al., 2006). This study was approved by the Institutional Review Board (IRB) of the Children's Hospital of Philadelphia. Personal information is all encrypted to ensure no protected health information (PHI) is included and study participants are de-identified. All human subjects or their proxies provided written informed consent.

METHOD DETAILS

Plasma samples were collected and kept according to standard procedures for lipid testing. In a sample of 300 μL, metabolites are quantified in absolute concentrations (e.g. mmol/l) and percentages. 249 metabolites of 18 groups were measured using a NMR metabolomics platform (Table S2, Nightingale Health Ltd;

biomarker quantification version 2020). Absolute concentrations of the metabolites was based on the Bayesian modeling that converted the spectral information by integrated quality control in the NMR spectrometer (Würtz et al., 2017).

QUANTIFICATION AND STATISTICAL ANALYSIS

Metabolite levels were normalized with means and standard deviation (STD), i.e. normalized value = $(X - \overline{X})/s$, where: x = metabolite level, $\overline{X}$ = mean, s = STD. Point-Biserial partial correlations between asthma and metabolite levels were controlled for age, sex, obesity, and VitD deficiency, as covariates (Table S3, Related to Table 1. Correlation between asthma and metabolite levels). Statistical analysis was done by the IBM SPSS Statistics Version 23 software.