The systematic use of metabolomic epidemiology, biobanks, and electronic medical records for precision medicine initiatives in asthma: findings suggest new guidelines to optimize treatment

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Analysis

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Abstract

The application of large-scale metabolomic profiling provides new opportunities to realize the potential of omics-based precision medicine with regard to asthma. We leveraged over 14,000 individuals from four distinct epidemiological studies. We identified and independently replicated seventeen steroid metabolites that were significantly reduced in individuals with prevalent asthma. Importantly steroid levels were reduced among all individuals with asthma regardless of medication use; however, the largest reduction was associated with inhaled corticosteroids use (ICS) that was further confirmed in a four-year ICS clinical trial. Cortisol levels extracted from electronic medical records confirmed that cortisol is reduced among asthmatics taking ICS over the entire 24-hour period, compared with all other groups. Clinical-grade adrenal suppression in asthmatics on ICS, resulting from substantial reductions in steroid metabolites, represents a larger public health problem than previously recognized. Regular cortisol testing may identify at-risk individuals, enabling personalized treatment modifications and improving overall patient care.

Introduction

Asthma imparts a tremendous global health and economic burden, affecting over 350 million people worldwide¹⁻⁴, with annual asthma-related costs surpassing \$80 billion/year in the USA. Any improvement in treatment will therefore have significant public health ramifications. While several genetic variants have been determined to influence an individual's asthma liability⁵⁻⁸, asthma also has substantial environmental triggers⁹ and the majority of cases arise from complex interactions between both factors.

Metabolomics, the systematic analysis of small molecules in a biological sample, provides an integrated profile of biological status, reflecting the 'net results' of genetic, transcriptomic, proteomic, and environmental interactions, making it ideally suited to the study of asthma. The clinical potential of asthma metabolomics is well-demonstrated through the improvement in predictive accuracy of asthma phenotypes and the improved understanding of underlying biology it has provided relative to genetics or environmental factors alone⁸. While a number of metabolomic signatures of asthma-relevant phenotypes have been reported to date⁸; there are several drawbacks to these studies that have limited their overall clinical impact, including a lack of independent replication¹⁰⁻¹³ and small sample sizes¹¹. The identification and validation of metabolic signatures for asthma phenotypes using multiple large, well-characterized epidemiological cohorts and clinical trials with comprehensive coverage of the global metabolome is imperative for the translational potential of metabolomics to be fully realized^{11,13-21}.

As a composite measure that captures environmental exposures, the metabolome can inform our understanding of the biological impact that specific medications, such as inhaled corticosteroids (ICS), may have on individuals with asthma. Long-term use of ICS among moderate to severe asthmatics is common. While several studies have examined the biological impact that prolonged ICS use may have, significant limitations in study design have limited their overall utility, resulting in conflicting findings²²⁻

²⁶. Moreover, the current guidelines on ICS use are inconsistent on dosing and effective symptom control²⁷, resulting in confusion.

Metabolomic findings from epidemiological studies may be further enhanced for clinical translation by integrating them with Electronic Medical Records (EMR)²⁸, as many validated and clinically approved 'non-omic' biomarkers and clinical tests have quantifiable relationships with metabolites and metabolomic pathways^{29,30}. In this study, we utilized metabolomics to investigate prevalent asthma and the impact of ICS use, leveraging multiple cohorts in combination with clinical tests available via EMR to explore a metabolomic-driven precision medicine approach for optimized asthma management.

Results

Subjects

We applied an analytic framework leveraging over 14,000 individuals from four epidemiological studies to discover and validate asthma associated metabolites and provide clinical recommendations that optimize ICS treatment recommendations: 1) Discovery cohort: European Prospective Investigation of Cancer (EPIC-Norfolk, n=10,754); 2) Replication cohort: Mass General Brigham Biobank-Asthma (MGBB-Asthma, n=610); 3) ICS randomized clinical trial: Childhood Asthma Management Program (CAMP, n=1,120); and 4) Biobank/EMR-based adrenal suppression cohort: (MGBB-Cortisol, n=2,235). Clinical characteristics of the EPIC-Norfolk, MGBB and CAMP subjects are summarized in **Table 1**. EPIC-Norfolk asthmatics (N=661) and controls (N=10,093) differed significantly by smoking status (P=0.003) and marginally by gender (P=0.08). Asthma cases (N=287) in MGBB-Asthma significantly differed from controls (N=323) by sex (P<0.001), race (P=0.002), ICS intake (P<0.001) and BMI (P<0.001).

Metabolite associations in the EPIC-Norfolk cohort

We identified 973 metabolites that remained for analysis after quality control. In EPIC-Norfolk, 35 (3.6%) were significantly associated with prevalent asthma after multiple testing corrections using Bonferroni threshold (**Table S3**). Thirty-four of these metabolites were significantly reduced in asthmatics compared with controls (range of ORs=0.65-0.81; range of P-values= 1.4×10^{-27} - 1.5×10^{-7}) and were annotated to canonical curated pathways for corticosteroids, pregnenolone, and androgenic steroids (**Figure S1A**). Glycosyl-N-palmitoyl-sphingosine (OR=1.19, P= 7.6×10^{-6}); a sphingolipid had increased levels in asthmatics (**Table S3, Figure S1B**).

Replication in MGBB-Asthma cohort

Seventeen of the 35 significant metabolites were replicated in MGBB-Asthma at an FDR threshold of 5%; 15 of which also met the more stringent Bonferroni threshold (**Table 2**). All 17 metabolites were reduced in asthmatics compared to controls (**Figure 2A**) and were annotated to major steroid hormone biosynthesis sub-pathways, specifically corticosteroid, pregnenolone, and androgenic steroid pathways (**Table 2, Figure S2**).

Metabolite-ICS associations in MGBB-Asthma and CAMP

In MGBB all corticosteroid, androgenic, and pregnenolone steroids were lower in both asthmatics on and off ICS when compared with controls (**Table S4, Figure 2B**). These reductions were larger and more significant among asthmatics on ICS than among asthmatics off ICS; however, steroid reductions were still evident in asthmatics off ICS when compared with controls. While progestin steroids also demonstrated this general trend in all comparisons between asthmatics and controls, the reduction was not as robust (**Table S5-S8**, further details in supplement).

Cortisone and cortisol levels in CAMP collected at the end of the four-year trial also demonstrated reduced concentrations in subjects randomized to ICS as compared to those receiving nedocromil or placebo (cortisol: $b=-0.87$; 95% CI=-1.50, -0.24; $P=6.8 \times 10^{-3}$ and cortisone: $b=-0.60$; 95% CI=-1.20, -0.01; $P=0.04$). Importantly, there was no significant difference at baseline (pre-ICS randomization) between the groups (cortisol: Estimate=0.09; 95% CI=-0.37, 0.55; $P=0.70$ and Cortisone: Estimate=0.19; 95% CI=-0.30, 0.68; $P=0.45$). These results are consistent with systemic suppression of the steroid pathway metabolites in asthmatics with prolonged ICS use.

Clinical Quantification of Cortisol: MGBB-Cortisol

The MGBB-Cortisol subjects ($N=2,235$) are summarized in **Table 1**. The asthmatics and controls differed by sex ($P=3.9 \times 10^{-14}$), race ($P=4.3 \times 10^{-5}$), ICS intake ($P<2.2 \times 10^{-16}$) and adrenal insufficiency diagnosis ($P=0.03$). We determined that asthmatics with ICS intake had the lowest minimum cortisol levels throughout a 24-hour period. Pairwise comparisons between the subgroups demonstrated that asthmatics on ICS had significantly lower minimum cortisol levels compared to asthmatics without ICS intake ($b=-1.74$ mcg/dL; 95% CI=-2.90, -0.59; $P=3.3 \times 10^{-3}$) and controls without ICS intake ($b=-2.86$ mcg/dL; 95% CI=-3.96, -1.76; $P=3.7 \times 10^{-7}$) throughout the 24-hour period with the largest difference occurring in early morning, the time when individuals are most vulnerable to asthma attacks^{31,32} (**Figure 3**). A comparison with controls on ICS was marginally significant ($P=0.055$); however, this group may have other comorbid and confounding diagnoses that make this relationship difficult to assess.

The Research Patient Data Registry (RPDR) identified 16,929 individuals with asthma and 23.4% (3,969) met the criteria for regular ICS use, as defined for MGBB-Asthma. Of the 1,021 (25.7%) asthmatics with ICS use that were tested for adrenal insufficiency, 31.0% ($n=316$) met the clinical diagnostic criteria for adrenal suppression (defined as a peak level <500 nmol/L)³³.

Discussion

In this study, we used four independent cohorts to demonstrate the translational utility of metabolomics. We identified significant and robust reductions in steroid metabolites among asthma cases using ICS that place them at serious risk for adrenal suppression with as many as thirty percent reaching clinically diagnosable levels. There were three key findings. First, seventeen steroid metabolites had substantially

reduced levels in prevalent asthma cases compared with controls, including marked reductions in the two primary hypothalamic-pituitary-adrenal axis (HPA) steroid hormones that are biomarkers for adrenal suppression³⁴, DHEA-S and cortisol. Second, we observed that this reduction in steroids was primarily, but not exclusively driven by ICS use in asthmatics, suggesting that the reduced steroid levels represent both a fundamental characteristic of the asthma phenotype and a result of ICS use. Third, not only were cortisol levels consistently reduced among asthmatics on ICS compared with all other groups throughout an entire 24-hour diurnal period, but this group had the largest variation in levels throughout the day, with a steep reduction in cortisol levels during the early morning hours, a peak time for asthma exacerbations to occur^{31,32}. Prior studies have correlated low cortisol with decreased asthma control³⁵, suggesting that this reduction may pose a further threat during this vulnerable time^{31,32}. The global reduction in cortisol levels was so pronounced that on average, peak cortisol levels among asthmatics on ICS during an entire 24-hour period, were lower than the average minimum cortisol levels among any of the other groups at any time.

To date, multiple studies have investigated the potential adverse side effects of ICS use for asthma; however, the overall utility of these studies to assess adrenal suppression has been hampered by small or modest samples sizes, short trial periods^{22,25}, and a limited range of ICS dose^{22,36}, with few resounding conclusions; a more comprehensive interrogation is therefore recommended²⁷. While acknowledged as a potential harmful side effect, clinical suppression of the HPA-axis from ICS therapy alone has been considered unusual with minimal long-term systemic ramifications on adrenal function^{22,23} that may only be apparent at high doses^{25,26}. In contrast to the existing scientific literature, in our population there was a consistent, pronounced, and clinically relevant reduction (as defined by a pronounced increase in adrenal suppression diagnoses) in adrenal function over a range of ICS use and dose. The utilization of 25 years of EMRs from individuals diagnosed with asthma and ICS use, cortisol measurements from a 4-year ICS trial, and extensive clinical-grade cortisol testing from over 2,000 individuals enables a robust evaluation of the long-term impact of ICS use on adrenal function and suggests increased rates of adrenal and sub-adrenal suppression among asthmatics as a result of ICS use.

The majority of moderate to severe asthmatics use ICS as the first line of treatment to improve control of persistent asthma. It remains an integral part of their long-term treatment protocol^{37,38} and one of the most effective and efficacious treatments to date. In the short term, these benefits likely outweigh the long-term side effects. However, patient treatment is optimized with proactive monitoring of circulating steroid levels to prevent permanent adrenal suppression. We observed that 31 percent of asthma cases with ICS use tested met the clinical criteria for an adrenal suppression diagnosis. While this is likely an overestimate due to potential selection bias in ordering tests for adrenal suppression when clinically suspected, if these were the only diagnosable cases out of all the entire RPDR, including the other 74.3 percent of asthmatics using ICS who were not tested, this would still suggest that eight percent of all asthmatics using ICS have cortisol levels low enough to classify as adrenal insufficiency diagnosis.

The degree of pronounced steroid suppression upon extended ICS use among asthma cases suggests that proactive monitoring of cortisol levels has the potential to identify and prevent adrenal suppression through altered treatment approaches. This becomes even more imperative, as cases of adrenal suppression are often problematic to diagnose, symptoms can be missed due to the range of presentation of the disease, and the broad range of negative side effects can even result in life-threatening complications that are critical to avoid^{27,38–42}. The institution of regular cortisol testing enables the identification of marked decreases in steroid levels prior to significant and potentially permanent long-term complications from clinical-grade adrenal suppression. Moreover, yearly monitoring is inexpensive (adrenocorticotrophic hormone test (ACTH) simulation test)⁴³ and manageable in most primary care settings and may decrease the overall public health burden of this ICS adverse effect. Individual optimization of treatment protocols does not necessarily imply omitting ICS use in all cases, but potentially changing the dosage, the frequency, or supplementation with additional medications may result in more efficacious outcomes in some individuals, while preventing adverse adrenal suppression ramifications in others.

While other omic data types are touted for their potential use in precision medicine, this study demonstrates the expediency to clinical translation that metabolomic profiling offers. Because a large number of clinical tests currently in use are measured metabolites, there are often cases where the metabolites of interest from untargeted analyses are already measured in clinical practice. This was the case in the present study, in which the observed reduction of multiple steroid metabolites led to the exploration of the use of cortisol testing to assess adrenal suppression in asthmatics on ICS. This approach enables investigators to more efficiently translate metabolomic findings into clinical practice than for other omic data types that would require substantial follow-up time and resources.

Despite the strengths of the reported findings, several limitations should be noted. First, our discovery cohort (EPIC-Norfolk) did not have information on ICS use. In MGBB, we created a robust algorithm to identify ICS use. We acknowledge that there is likely misclassification; however, this misclassification would result in a bias towards the null hypothesis and the effects we identified would remain highly significant. Therefore, we do not believe that this impacted the overall conclusions of the study. Moreover, we verified our findings in an RCT, which represents a more robust study design. Second, the CAMP RCT utilized children and while at the end of the trial, they were mostly adolescents; this differs from the other cohorts that utilized primarily adults. It is important to note that there may be important differences between ICS response in adults and adolescents that should be studied in more detail. Third, metabolomic profiling was performed in a different laboratory for CAMP and did not have the broader range of steroid metabolites. Despite these differences, we were able to validate and further refine our findings over the four populations we utilized. Finally, while it is important to realize the potential of large EMR databases, it is equally important to recognize that this information is derived from an overrepresentation of individuals with illness and may bias the data or result in confounding by indication. Acknowledging this limitation, we excluded individuals with common relevant comorbid conditions, such as COPD.

In conclusion, our results suggest regular monitoring of steroid levels among asthma cases with long-term ICS use, which is not currently commonplace, is merited to identify the optimal clinical regimen for individuals with asthma at risk of serious adrenal suppression and determine the clinical impact of low cortisol levels in this population. This could potentially improve overall health and reduce health care spending. Integrating metabolomics data from epidemiological studies with existing clinical biomarkers obtained via EMRs may enhance the interpretation of metabolomic data as it relates to health and current medical practices, in addition to enhancing comprehension both on the side of researchers and of clinicians.

Methods

Statistical Analysis

Overview of Analytic Approach

A detailed summary of the four cohorts utilized in this study, including cohort generation, details on asthma diagnosis and ICS use, metabolomic profiling and quality control procedures is provided in the supplement (**Methods & Table S1-S2**). An illustration of our analytic strategy is presented in **Figure 1**. Briefly, we utilized a discovery and replication approach to identify metabolites associated with prevalent asthma, using the EPIC-Norfolk (discovery) and biobank-derived Mass General Brigham Biobank-Asthma (MGBB-Asthma) (replication) cohorts. As these findings implicated potential involvement of steroid-associated metabolites, we then assessed the impact of ICS use on the replicated asthma-associated metabolites within MGBB-Asthma cohort and further evaluated the impact of ICS use on steroid metabolites using cortisol and cortisone measures from the four-year double-blind longitudinal ICS randomized controlled trial (RCT), Childhood Asthma Management Program (CAMP)^{44,45}. Using cortisol as a biomarker for subclinical steroid suppression from ICS use, we queried the MGBB EMR to identify individuals with cortisol measures to create the MGBB-Cortisol cohort and assessed the impact of ICS use on these cortisol measures. Some analyses in the EPIC-Norfolk cohort were done in Stata. All other analyses were performed in R version 3.6.3⁴⁶. The EPIC-Norfolk study was approved by the Norwich Local Ethics Committee (REC Ref. 78 98CN01). The research work for Biobank cohorts was approved by the IRB of Mass General Brigham (# 2015P000983, #2014P001109). For CAMP data, all study procedures were approved by the Institutional Review Board of the Brigham and Women's Hospital (the Partners Human Research Committee (PHRC), # 2002P000331). All study participants provided written consent at enrollment.

Discovery and Replication Analyses for Metabolite-Asthma Associations

Multivariable logistic regression models were employed in EPIC-Norfolk to assess the association between log-transformed plasma concentrations of each metabolite with asthma affection status. Models were adjusted for age, sex, body mass index (BMI) and smoking status (current, former and never). We did not adjust for ethnicity, as the EPIC-Norfolk population is mostly White (99.7%). To correct

for multiple testing, we applied a stringent Bonferroni significance threshold (0.05/number of statistical tests in EPIC-Norfolk). In MGBB-Asthma, multivariable logistic regression models adjusted for age, sex, race, BMI and smoking status were used to replicate the associations between the Bonferroni significant metabolites and asthma case status. An association was considered “replicated” if: 1) The effect estimate (Odds Ratio) is in the same direction as the initial association finding and 2) The False Discovery Rate (FDR)⁴⁷ is <5%. To quantify the relative reduction in steroid metabolite levels based on ICS use, asthmatics were stratified into four sub-groups: 1) no asthma/no ICS use; 2) no asthma/ICS use; 3) asthma/no ICS use; 4) asthma/ICS use. Multivariable logistic regression models using pairwise comparisons adjusted for age, gender, race, and BMI were utilized to compare metabolite levels between groups. All models were adjusted for age, gender, race, and BMI.

Establishment of a temporal relationship between cortisone, cortisol, ICS using the CAMP RCT

We utilized multivariable linear regression models and individuals from CAMP to further assess the relationship between cortisol and cortisone levels in children randomized to ICS (budesonide) versus those randomized to nedocromil or placebo. Considering both baseline and the end of the four-year clinical trial, the models were adjusted for age, gender, race, BMI, and an interaction variable between age and randomized ICS-use for the end of the trial model, as the children were in various stages of puberty and puberty directly influences steroid levels.

Clinical Quantification of Cortisol: MGBB-Cortisol

We investigated minimum cortisol levels (mcg/dL) recorded in MGBB-Cortisol subjects throughout a 24-hour period stratifying on asthma status and ICS use. To account for differences in sample availability across the 24-hour period, subjects were binned into three time categories based on sample collection time: 4:00am-12:00pm, 12:00pm-6:00pm and 6:00pm-4:00am. The mean cortisol levels were subjected to smoothing interpolation using loess curve regression fitting. Tukey’s HSD⁴⁸ test was used to identify significant differences between the asthma/ICS subgroups. Pairwise comparisons between the subgroups were also performed using generalized linear models, adjusted for collection time, age, gender and race.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Requests for raw data, analyzed data and materials will be reviewed by the cohort and contact PIs for the studies to determine if the request is subject to intellectual property or confidentiality obligations. Data and materials that can be shared will be released using a Material Transfer Agreement. Appropriate IRB approvals may be required to access de-identified data in particular data from electronic medical health records. The EPIC-Norfolk data can be requested by bona fide researchers for specified scientific purposes via the study website (<https://www.mrc-epid.cam.ac.uk/research/studies/epic-norfolk/>). Data

will either be shared through an institutional data sharing agreement or arrangements will be made for analyses to be conducted remotely without the need for data transfer.

Code availability

The R Code for data processing and analyses can be made available for research purposes upon request from the corresponding author.

Declarations

Author Contributions: PK and IDS had full access to the data and take responsibility for the data integrity and accuracy of the analysis. JALS and CL contributed to conceptualization of the study; PK performed the quality control and statistical downstream data analyses for Mass General Brigham Biobank (MGBB) cohorts and comparison to the EPIC-Norfolk datasets. PK also performed the replication in the Childhood Asthma Management Program (CAMP). IDS performed the quality control and regression analysis for the EPIC-Norfolk cohort. MS contributed to the data pulls and acquisition from MGBB. AW contributed to ascertainment of the inhaled medications in MGBB. KM contributed to the downstream analyses. DIS performed the cost-effectiveness analysis. PK and JALS prepared the original draft of the manuscript; PK, JALS, RSK, IDS, CL, KM, AD, SHC, MH, MM, MC, HMK, KLS, AW, ACW, YV, and CEW contributed to the statistical interpretation and critical revision of the manuscript; PZ, CEW, and CC contributed to the metabolomic data generation. JALS, STW, CL, NJW, EWK contributed to funding acquisition; all authors reviewed the final manuscript.

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Tables

Table 1. Clinical characteristics of subjects in the large discovery epidemiologic cohort (EPIC-Norfolk) and replication in two biobank/electronic medical record (EMR)-based cohorts (MGBB-Asthma, MGBB-Cortisol) and an inhaled corticosteroid (ICS)-randomized clinical trial (CAMP (Childhood Asthma Management Program))

<i>Discovery cohort: EPIC-Norfolk</i>				
Number of subjects	All subjects (n = 10,754)	Asthmatics (n = 661)	Non asthmatics (n = 10,093)	P-value*
Age, mean (SD)	59.73 (8.96)	59.24 (9.05)	59.76 (8.95)	0.142
BMI kg/m ² , mean (SD)	26.19 (3.71)	26.10 (3.86)	26.20 (3.70)	0.529
Sex, n (%)				0.076
Female	5,759 (53.6)	376 (56.9)	5,383 (53.3)	
Male	4,995 (46.4)	285 (43.1)	4,710 (46.7)	
Race, n (%)				0.181
African American	9 (0.1)	1 (0.2)	8 (0.1)	
White	10,684 (99.7)	655 (99.5)	10,029 (99.8)	
Asian	9 (0.1)	1 (0.2)	8 (0.1)	
Other	9 (0.1)	1 (0.2)	8 (0.1)	
Inhaled steroid intake, n (%)	NA	NA	NA	NA
No				
Yes				
Smoking, n (%)				0.003
No	5,012 (46.6)	308 (46.6)	4,704 (46.6)	
Yes	5,742 (53.4)	353 (53.4)	5,389 (53.4)	
<i>Replication cohort: MGBB-Asthma</i>				
Number of subjects	All subjects (n = 610)	Asthmatics (n = 287)	Non asthmatics (n = 323)	P-value*
Age, mean (SD)	32.7 (5.3)	33.1 (6.6)	32.4 (3.7)	0.110
BMI kg/m ² , mean (SD)	25.6 (6.5)	28.3 (8.0)	23.2 (3.1)	<2.2x10 ⁻¹⁶
Sex, n (%)				2x10 ⁻¹⁰
Female	359 (58.9)	208 (72.5)	151 (46.7)	
Male	251 (41.1)	79 (27.5)	172 (53.3)	
Race, n (%)				9.8x10 ⁻³
African American	51 (8.4)	34 (11.8)	17 (5.3)	
White	475 (77.9)	222 (77.4)	253 (78.3)	
Asian	28 (4.6)	10 (3.5)	18 (5.6)	
Other	56 (9.2)	21 (7.3)	35 (10.8)	
Inhaled corticosteroid intake, n (%)				<2.2x10 ⁻¹⁶
No	413 (67.7)	90 (31.4)	323 (100)	
Yes	172 (28.2)	172 (59.9)	0 (0)	
Smoking, n (%)				1.2x10 ⁻³
No	482 (79)	210 (73.2)	272 (84.2)	
Yes	128 (21)	77 (26.8)	51 (15.8)	
<i>Validation cohort: Randomized controlled trial of ICS use - CAMP</i>				
Number of subjects	All subjects (n = 1041)	Baseline (n = 560 at both time points)	End of trial	P-value*
Age, mean (SD)	8.8 (2.1)	8.8 (2.1)	12.8 (2.1)	<2.2x10 ⁻¹⁶
BMI kg/m ² , mean (SD)	18.1 (3.5)	18.0 (3.3)	21.2 (4.5)	<2.2x10 ⁻¹⁶
Sex, n (%)				NA
Female	420 (40.3)	201 (35.9)	201 (35.9)	
Male	621 (59.7)	359 (64.1)	359 (64.1)	
Race, n (%)				NA
African American	138 (13.3)	82 (14.6)	82 (14.6)	
White	711 (68.3)	395 (70.5)	395 (70.5)	
Hispanic	98 (9.4)	56 (10)	56 (10)	
Other	94 (9.0)	27 (4.8)	27 (4.8)	
Treatment, Steroid use (%)				NA
Budesonide	311 (29.9)	151 (27)	151 (27)	
Nedocramil + Placebo	730 (70.1)	409 (73)	409 (73)	
<i>Validation cohort: Clinical cohort of MGBB-Cortisol</i>				
Number of subjects	All subjects (n = 2,235)	Asthmatics (n = 383)	Non asthmatics (n = 1,852)	P-value*
Age, mean (SD)	56.1 (16.2)	55.2 (16)	56.3 (16.3)	0.200
Sex, n (%)				3.9x10 ⁻¹⁴

Female	1377 (61.6)	302 (78.9)	1075 (58)	4.3x10 ⁻⁵
Male	858 (38.4)	81 (21.1)	777 (42.2)	
Race, n (%)				
African American	122 (5.5)	34 (8.9)	88 (4.8)	
White	1914 (85.6)	302 (78.9)	1612 (87)	
Hispanic	49 (2.2)	16 (4.2)	33 (1.8)	
Asian	48 (2.1)	6 (1.6)	42 (2.3)	
Other	102 (4.6)	25 (6.5)	77 (4.2)	<2.2x10 ⁻¹⁶
Inhaled steroid intake, n (%)				
No	2079 (93)	268 (70)	1811 (97.8)	
Yes	156 (7)	115 (30)	41 (2.2)	0.030
Adrenal Insufficiency diagnosis, n (%)				
No	1640 (73.4)	263 (68.7)	1377 (74.4)	
Yes	595 (26.6)	120 (31.3)	475 (25.6)	

Missing data: In MGBB-Asthma, BMI was missing for seven subjects; Inhaled steroid intake was missing for 25 subjects

* Significance of difference was evaluated using chi-squared test for categorical variables and two-sample t-test for continuous variables. In CAMP, the categorical variables sex, race and treatment did not change with time point.

Abbreviations: BMI, body mass index; SD, standard deviation; CAMP, Childhood Asthma Management Program

Table 2. Plasma metabolites significantly associated with asthma in EPIC-Norfolk with replication in MGBB-Asthma.

Metabolite	Metabolite Subclass	EPIC-Norfolk (N=10,754*)			MGBB-Asthma (N=610)		
		OR	95% CI	P-value*	OR	95% CI	P-value [‡]
Dehydroisoandrosterone sulfate (DHEA-S)	Androgenic Steroid	0.65	0.60, 0.70	1.4×10^{-27}	0.38	0.25, 0.55	1.1×10^{-6}
Steroid conjugate*	Corticosteroid	0.68	0.61, 0.72	2.9×10^{-21}	0.60	0.44, 0.81	1.1×10^{-3}
Cortisone	Corticosteroid	0.72	0.67, 0.77	7.8×10^{-20}	0.46	0.31, 0.66	6.2×10^{-5}
21-hydroxypregnenolone disulfate	Pregnenolone Steroid	0.71	0.65, 0.77	4.5×10^{-16}	0.47	0.33, 0.63	2.9×10^{-6}
Epiandrosterone sulfate	Androgenic Steroid	0.74	0.69, 0.80	1.1×10^{-13}	0.41	0.29, 0.57	1.0×10^{-7}
16a-hydroxy DHEA 3-sulfate	Androgenic Steroid	0.81	0.66, 0.79	2.5×10^{-5}	0.64	0.48, 0.85	1.9×10^{-3}
Pregnenolone sulfate	Pregnenolone Steroid	0.73	0.66, 0.79	3.2×10^{-12}	0.38	0.27, 0.52	3.4×10^{-9}
Androsterone sulfate	Androstane Steroid	0.77	0.72, 0.83	2.1×10^{-11}	0.45	0.33, 0.62	1.1×10^{-6}
Cortisol	Corticosteroid	0.78	0.72, 0.84	3.3×10^{-11}	0.53	0.37, 0.74	2.9×10^{-4}
pregnenetriol disulfate*	Pregnenolone Steroid	0.73	0.69, 0.82	3.2×10^{-12}	0.60	0.45, 0.79	4.6×10^{-4}
17-hydroxypregnenolone sulfate*	Pregnenolone Steroid	0.74	0.68, 0.82	7.4×10^{-10}	0.48	0.36, 0.63	1.9×10^{-7}
etiocholanolone glucuronide	Androstane Steroid	0.79	0.71, 0.84	1.5×10^{-7}	0.49	0.37, 0.63	1.7×10^{-7}
5-androstenetriol disulfate*	Androstane Steroid	0.79	0.72, 0.86	1.5×10^{-7}	0.50	0.38, 0.65	3.1×10^{-7}
Tetrahydrocorticosterone glucuronide ^{‡‡}	Corticosteroid	0.81	0.74, 0.88	9.9×10^{-7}	0.74	0.58, 0.94	0.015
Steroid conjugate ^{‡‡}	Corticosteroid	0.68	0.75, 0.90	2.9×10^{-21}	0.73	0.55, 0.95	0.020
5alpha-androstan-3beta,17alpha-diol disulfate	Androgenic Steroid	0.81	0.74, 0.89	1.8×10^{-5}	0.56	0.44, 0.71	1.1×10^{-6}
16a-hydroxy-DHEA-disulfate*	Androgenic Steroid	0.81	0.73, 0.89	2.5×10^{-5}	0.54	0.42, 0.70	2.6×10^{-6}

max N = 10,754^{*} for EPIC-Norfolk, as N varies for each metabolite in this cohort

[‡] The P-value thresholds for declaring significance was Bonferroni in both EPIC-Norfolk ($P < 5.14 \times 10^{-5}$) and MGBB-Asthma ($P < 2 \times 10^{-3}$)

^{‡‡} Replicated metabolites in MGBB-Asthma at FDR threshold only

Abbreviations: MGBB: Mass General Brigham Biobank

Metabolite names with an asterisk * indicate that the metabolite has not been confirmed based on an analytical standard, but we are confident in its identity as confirmed by Metabolon

Figures

Figure 1

Overall study design that incorporates four independent cohorts (1) EPIC-Norfolk cohort; (2) Mass General Brigham Biobank (MGBB)- Asthma/MGBB-A cohort; (3) CAMP randomized controlled trial (RCT); and (4) MGBB-Cortisol cohort. The cohorts were utilized sequentially to identify prevalent asthma metabolites, validate significant metabolites, assess the effect of inhaled corticosteroids (ICS) on the steroid metabolites, and evaluate the utility of cortisol as a biomarker of adrenal suppression among asthmatics on ICS. The details of our approach for each cohort are illustrated below.

A.

B.

Figure 2

Plasma metabolites significantly associated with asthma in EPIC-Norfolk cohort and replicated in Mass General Brigham Biobank (MGBB)-Asthma cohort (n=17 metabolites). Steroid pathway metabolites in MGBB-Asthma cohort were further assessed with subjects stratified by asthma status and ICS use. These analyses are based on logistic regression models (adjusted for confounders). A. The dotted line indicates the cut off for OR< or >1 for direction of effect. Metabolite names with an asterisk * indicate that the

metabolite has not been officially confirmed based on a standard, but we are confident in its identity as confirmed/predicted by Metabolon. B. Steroid metabolite associations between asthma cases and controls; asthma cases with ICS intake and controls; asthma cases without ICS and controls; and asthma cases with ICS intake and without ICS. The dotted line indicates the cut off for $OR < 1$ or $OR > 1$ for direction of effect. max N = 10,754 for EPIC-Norfolk, as N varies for each metabolite in this cohort Abbreviations: Ast, asthmatics; ICS, inhaled corticosteroids

Figure 3

Clinical Quantification of Cortisol: Mass General Brigham Biobank-Cortisol (MGBBCortisol). The top histogram panel shows the frequency of subjects during the 24-hour period. The lower panel shows the aggregated minimum cortisol levels for subjects on the Y-axis associated with the time of their sample collection on the X-axis. Subjects were binned into three time categories based on the availability of

subjects and smoothed using loess curve fitting. Legend label is colored based on four categories: Ast_ICS: asthmatics with ICS intake (Average=4.2 mcg/dL; SD=5.2); Ast_No_ICS: Asthmatics without ICS intake (Average=6.03 mcg/dL; SD=5.1); Controls_ICS: Controls with ICS intake (Average=5.58 mcg/dL; SD=4.6); Controls_No_ICS: Controls without ICS intake (Average=7.02 mcg/dL; SD=5.8). P-value for asthmatics on ICS compared with controls without ICS intake (Mean difference=-2.80 mcg/dL; 95% CI=-1.4, -4.2; P=1.9x10⁻⁶)

Supplementary Files

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