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Metabolomic profiling reveals extensive adrenal suppression due to inhaled corticosteroid therapy in asthma

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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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Code availability

All code for data processing and analyses are available via GitHub at:
https://github.com/CDNMBioinformatics/PartnersBiobank_asthma_metabolomics
https://github.com/CDNMBioinformatics/RPDR
https://github.com/CDNMBioinformatics/RPDR_ACTH
https://github.com/CDNMBioinformatics/PEGASU_Scripts

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Abstract

The application of large-scale metabolomic profiling provides new opportunities for realizing the potential of omics-based precision medicine for asthma. By leveraging data from over 14,000 individuals in four distinct cohorts, this study identifies and independently replicates seventeen steroid metabolites whose levels were significantly reduced in individuals with prevalent asthma. Although steroid levels were reduced among all asthma cases regardless of medication use, the largest reductions were associated with inhaled corticosteroid (ICS) use, as confirmed in a four-year low-dose ICS clinical trial. Effects of ICS use on steroid levels were dose-dependent, however, significant reductions also occurred with low-dose ICS use. Using information from electronic medical records (EMR), we found that cortisol levels were substantially reduced throughout the entire 24-hour daily period in asthma patients treated with ICS compared with those untreated and non-asthma patients. Moreover, asthma patients treated with ICS showed significant increases in fatigue and anemia as compared to those without ICS use. Adrenal suppression in asthma patients treated with ICS may therefore represent a larger public health problem than previously recognized. Regular cortisol monitoring of asthma patients treated with ICS is needed to provide the optimal balance between minimizing adverse effects of adrenal suppression while capitalizing on the established benefits of ICS treatment.

Keywords

asthma; metabolomics; precision medicine; biobanks; inhaled corticosteroids; steroid resistance; adrenal suppression

Asthma imparts a tremendous global health and economic burden, affecting over 350 million people worldwide[1-4], with annual asthma-related costs surpassing $80 billion/year in the USA. Any improvement in biologic understanding or treatment will therefore have significant public health ramifications. While several genetic variants have been determined to influence an individual's asthma liability[5-8], asthma also has substantial environmental triggers[9] 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 alone[8]. While a number of metabolomic signatures of asthma-relevant phenotypes have been reported to date[8], there are several drawbacks of these studies that have limited their overall clinical impact, including a lack of independent replication[10-13] and small sample sizes[11]. 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[11,13-18].

Inhaled corticosteroid (ICS) use has formed the foundation of care for individuals with moderate to severe asthma[19] with tremendous benefit; however, the impact of ICS use on adrenal insufficiency has been a persistent concern[20-24]. While multiple studies have examined the effect of ICS use on adrenal suppression, significant limitations in study design have limited their overall utility[21,25-31], resulting in conflicting findings[32]. To add further complexity, not only are there genetic variants that influence asthma liability[5-8] but also those that influence steroid response[33-38] and susceptibility to adrenal suppression[39]. As a composite measure that captures both the influence of these genetic susceptibilities together with the impact of ICS use, the metabolome is ideally suited to inform our understanding of the biological impact of these ICS medications while simultaneously incorporating individual genetic variation. The effect of ICS use on individuals with asthma is imperative to understand, particularly given the recent changes to the Global Initiative for Asthma (GINA) guidelines[1] that now recommend “as needed” low dose ICS use for the preferred reliever option among adolescents and adults.

Metabolomic findings from epidemiological studies may be further enhanced for clinical translation by integrating them with Electronic Medical Records (EMR)[40], as many established clinical biomarkers and tests are directly related or are metabolites themselves[41,42]. 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

Cohorts studied

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 (Fig. 1): 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) Validation of ICS effect in a randomized clinical trial: Childhood Asthma Management Program (CAMP, n=1,120); and 4) Assessment of ICS effect using clinical cortisol measurements extracted from EMRs: (EMR-Cortisol, n=2,235). Clinical characteristics of the EPIC-Norfolk, MGBB and CAMP subjects are summarized in Table 1. EPIC-Norfolk

asthma cases (N=661) and controls (N=10,093) differed significantly by smoking status (P=0.003) and marginally by sex (P=0.08). Asthma cases (N=287) and controls (N=323) in MGBB-Asthma significantly differed by sex (P<0.001), race (P=0.002), ICS treatment (P<0.001) and body mass index (BMI) (P<0.001). Asthma cases (N=383) and controls (N=1852) in EMR-Cortisol significantly differed by sex ($P=3.9x10^{-14}$), race ($P=1.4x10^{-3}$), ICS treatment ($P<2.2x10^{-16}$) and adrenal insufficiency diagnosis (P=0.03). All CAMP subjects had asthma.

Global metabolomic profiling and replication of findings

Nine-hundred and seventy-three metabolites 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 (Supplementary Table 1). Thirty-four of those were significantly reduced in asthma cases compared with controls (range of ORs=0.65-0.81; range of P-values=$1.4x10^{-27}$–$1.5x10^{-7}$) and were annotated to canonical curated pathways for corticosteroids, pregnenolone, and androgenic steroids (Extended Data Fig. 1A). Importantly, while only 40 of the 973 (4.1%) metabolites on the global profiling platform were annotated to steroid sub-pathways, 34 of these metabolites (85% of the total steroids measured) reached stringent Bonferroni statistical significance. Only one other metabolite, Glycosyl-N-palmitoyl-sphingosine, a sphingolipid, reached statistical significance and was associated with increased levels in asthma cases (OR=1.19, $P=7.6x10^{-6}$) (Supplementary Table 1, Extended Data Fig. 1B).

Twenty-five of the 35 significant metabolites in EPIC were quantified and available for analysis in MGBB-Asthma. Seventeen of the 25 metabolite associations replicated in MGBB-Asthma with a consistent direction of effect at an FDR threshold of 5%; 15 of which also met the more stringent Bonferroni threshold (Table 2, Fig. 2A). All 17 were annotated to major steroid hormone biosynthesis sub-pathways, specifically corticosteroid, pregnenolone, and androgenic steroid pathways with their identity confirmed by Metabolon (Table 2, Extended Data Fig. 2).

Discovery and Replication for Metabolite-Asthma Associations

The 34 corticosteroid, androgenic and pregnenolone steroid metabolites that were measured in MGBB-Asthma, were at lower levels in both asthma cases on and off ICS when compared with controls (Supplementary Table 2, Fig. 2B), with the largest decreases observed in asthma cases on ICS. There was also a nominally significant decrease in 12 out of 34 (35%) of steroids metabolites when comparing asthma cases with ICS treatment to those not treated with ICS (nominal p-value<0.05) (Supplementary Table 2). While progestin steroids also demonstrated these general trends, the reductions were was not as robust (Supplementary Table 2).

Two steroid metabolites, cortisol and cortisone, were measured in CAMP. While there was no significant difference in cortisol or cortisone between the treatment groups at baseline (cortisol: β=0.09; 95% CI=−0.37, 0.55; P=0.70 and cortisone: β=0.19; 95% CI=−0.30, 0.68; P=0.45), at the end of the four year clinical trial, both metabolites were associated with reduced levels in subjects randomized to low dose ICS (Budesonide) intake compared

to those who received nedocromil or placebo (cortisol: β=−0.87; 95% CI=−1.50, −0.24; P=6.8x10^{3} and cortisone: β=−0.60; 95% CI=−1.20, −0.01; P=0.04). These associations remained significant after extensive interrogation of potential confounding by asthma severity (Supplementary Table 3).

In MGBB-Asthma, significant inverse associations were observed between cortisol levels and fluticasone ICS dose measured continuously (β=−0.004; 95% CI=−0.007, −0.001; P=3.0e-03), ordinally (β=−0.29; 95% CI=−0.44, −0.14; P=2.0e-04), and in dose categories. Levels were lower in individuals on “low dose” (44-200 mcg, β=−0.22; 95% CI=−0.43, −0.01; P=0.04) and “moderate to high dose” (>200 mcg, β=−0.64; 95% CI=−0.97, −0.32; P=1.5e-04) ICS relative to those with “no ICS use”. Furthermore, the observed reductions in cortisol and cortisone observed in CAMP subjects were also a result of low-dose ICS.

Quantification of cortisol and assessment of adrenal insufficiency in the EMR

The EMR-Cortisol subjects (N=2,235) are summarized in Table 1. The asthma cases and controls differed by sex (P=3.9x10^{-14}), race (P=1.4x10^{-5}), ICS treatment (P<2.2x10^{-16}) and adrenal insufficiency diagnosis (P=0.03). We determined that asthma cases on ICS treatment had the lowest minimum cortisol levels throughout a 24-hour daily period, compared to asthma cases without ICS treatment (β=−1.74 mcg/dL; 95% CI=−2.90, −0.59; P=3.3x10^{-3}) and controls not treated with ICS (β=−2.86 mcg/dL; 95% CI=−3.96, −1.76; P=3.7x10^{-7}). The largest differences occurred in early morning, the time when individuals are most vulnerable to asthma attacks[43,44] (Fig. 3). There was no difference between asthma cases on ICS and controls on ICS (P=0.11); however, ICS controls may also have other comorbid and confounding diagnoses that make this relationship difficult to assess.

Further within the EMR, there were 755 mild asthma cases with adrenal insufficiency testing, including 200 asthma cases with and 555 asthma cases without ICS use.

Of the four adrenal insufficiency symptoms tested, we observed significant increases among asthma cases with ICS use compared to those without for symptoms of fatigue (OR=2.27; 95% CI=1.61, 3.22; p=3.2x10^{-6}) and anemia (OR=2.28; 95% CI=1.57, 3.35; p=2.0x10^{-5}). No significant differences were observed for weight loss or hyperpigmentation (Supplementary Table 4).

DISCUSSION

In this study, we used four independent cohorts to demonstrate the translational utility of metabolomics for asthma in a precision medicine framework. There were several key findings from this work. First, substantial reductions in seventeen endogenous steroid metabolites were associated with prevalent asthma, including two primary hypothalamic-pituitary-adrenal axis (HPA) steroid hormones that are biomarkers for adrenal suppression[45], DHEA-S and cortisol. Further interrogation demonstrated that this reduction was strongly associated with ICS use among asthma cases. In addition, EMR-extracted measures of cortisol demonstrated a global reduction among asthma cases with ICS use so pronounced that throughout the entire 24-hour period, the average peak cortisol levels among this group did not even attain the average lowest cortisol levels among all other groups. Second,

we observed a dose-effect relationship with steroids and ICS use, and for the first time, we robustly identified a significant association between cortisol and low dose ICS use. Third, we observed that while this reduction in steroids was primarily driven by ICS use, a portion of the observed reduction was independent of ICS use, suggesting that the reduced steroid levels also represent a fundamental characteristic of pathophysiology of asthma as a disease. Finally, for asthma cases, we identified significant associations between adrenal insufficiency symptoms and ICS use.

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 poor study design, with the vast majority being case reports[20,21,25-29,31], having small to modest samples sizes[30], short trial periods[46-48] or a limited range of ICS dose[46,49]. A more comprehensive interrogation is therefore recommended[32]. Studies focused on adrenal insufficiency and ICS use, both within asthma, and more broadly, are often limited in statistical power[30] due to the low prevalence of adrenal suppression diagnoses, but have identified associations with high dose ICS use[23]. Taken together, while acknowledged as a potential harmful side effect, clinical suppression of the HPA-axis from ICS therapy, particularly at low doses has been considered to have minimal long-term systemic ramifications on adrenal function[21,46] and while there is an acknowledged potential effect, it has been largely with regard to high dose ICS therapy[23,47,50,51]. Using multiple well-powered cohorts, designed to address a series of scientific inquiries, this study clarifies existing confusion and expands understanding regarding the effect of ICS therapy for asthma in multiple domains. Most importantly, we demonstrate robust evidence of the broad-based reduction in endogenous steroids and that this reduction is present even at low ICS doses. This study also considered the impact of long-term ICS use including 25 years of EMR data and a four-year clinical trial. While long-term ICS use remains a primary treatment recommendation[52,53], until now, few studies have examined the impact of ICS treatment on steroid levels over this length of time, particularly in the context of both a both a rigorous RCT and with real life EMR data. Finally, this study also considered the impact of ICS on the 24-hour daily diurnal variation of cortisol by extracting cortisol measurements from baseline adrenal insufficiency testing, something that has not yet been described using large scale real-life populations.

To date, large-scale precision medicine initiatives have primarily focused on other omic data types, particularly genetics. This study clearly demonstrates the potential that metabolomics offers in the realm of precision medicine initiatives. Starting with global metabolomic profiling in large scale and well-powered epidemiological cohorts, we identified and validated a robust metabolic signature with endogenous steroid metabolites. While the following steps often necessitate additional laboratory work, in several cases, as is demonstrated here with cortisol, a clinically relevant biomarker may be immediately obvious, expediting the steps toward clinical translation. As the extent of clinically relevant metabolites, including both clinical biomarkers and drugs, identified via global metabolomic profiling continues to increase, the clinical potential of metabolomics should not go underappreciated. When considering precision medicine approaches, additional incorporation of other relevant omic findings, such as underlying genetic predisposition, should also be considered. Existing evidence suggests that the impact of ICS use may

be even more extreme for individuals with genetic susceptibility to corticosteroid-induced adrenal suppression[39]. Identifying such at-risk individuals and considering specific treatment course modifications that are most efficacious to this group may build on the current findings to further define personalized approaches for asthma treatment specific to ICS use and may motivate future scientific research in this area. More in-depth research is suggested[54] and merited and in future studies. As the heterogeneity of asthma as a disease is well established, both from clinical classifications and underlying genetic susceptibility[55], analogous precision medicines recommendations may continue to optimize treatment approaches for specific groups. A central point relevant to precision medicine that may be gleaned from these findings is that metabolomics has tremendous potential to be utilized as an initial step towards precision medicine that may be further informed by other omic data types.

Treatment with ICS has formed the foundation of care for individuals with moderate to severe asthma since they were introduced into the standard management of asthma over 30 years ago[19]. They have been instrumental in reducing asthma exacerbations and improving overall quality of life[56]. Yet, while the effectiveness of ICS as a treatment for asthma should not go underappreciated, the risks of ICS use that have been further clarified and substantiated through this study, should also be considered. These findings are of particular interest given recent changes to the GINA guidelines[1]. In 2021, GINA released updated treatment recommendations for asthma in adolescents and adults that recommended two alternate tracks for treatment. Track 1 recommends “as needed” low dose ICS as the preferred reliever approach while Track 2 recommends short acting beta agonist (SABA) as the alternate reliever approach. The updated recommendations make a notable shift toward increased ICS use and away from SABA[1], which has been the primary reliever recommendation for the last 50 years. Our findings not only found a dose effect relationship with ICS use and cortisol, but also demonstrated a significant decrease in cortisol at low ICS doses. This highlights the urgent need to quantify the impact of low dose ICS use on adrenal suppression more carefully. Such information may have a substantial impact on these updated treatment recommendations, and an overall shift in mindset towards an increased awareness of these adverse side effects and more stringent indications needed to prescribe ICS, particularly when considering higher ICS dosages and frequencies.

It is important, however, to note that simple measures can be implemented to mitigate the systemic side effects of ICS use. Clinical recommendations for regular monitoring of adrenal hormones, particularly morning cortisol measurements[51,57], among individuals with ICS use is one clear and inexpensive clinical recommendation that may identify at-risk individuals and enable treatment modifications prior to significant and potentially permanent long-term complications. Critical evaluation of the prescribed ICS dose is also imperative, with a focus on identifying the minimal dose for overall symptom control. This is even more relevant as prior ICS dose research reported that in cases where maximal efficacy was achieved at a dose of 500 mcg/day, 90% of the overall benefit was achieved at doses of 100 to 250 mcg/day[58,59], suggesting that for most individuals, substantial reductions in ICS dose may have minimal clinical impact while simultaneously substantially reducing the overall extent of adrenal suppression. As such, when considering the clinical implications of these findings, for asthma cases that require ICS use, regular cortisol monitoring while simultaneously

utilizing the minimal ICS dose for overall symptom control, will likely provide the optimal balance between minimizing adverse adrenal suppression and capitalizing on the established benefits of ICS treatment.

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. We therefore created a robust binary variable for ICS use in MGBB-Asthma using information on prescription counts. While we did not have complete information on the specific details of ICS prescription lengths that likely leads to some misclassification; this misclassification would result in a bias towards the null hypothesis and the effects we identified would remain highly significant. We also recognize that ICS use in inherently confounded with asthma severity. We have addressed this by adjusting for measures of asthma severity in some analyses and by restricting our analysis to mild asthma cases in others. While the approaches implemented can account for part of this confounding, ultimately the use of ICS RCTs or rigorous pharmacoepidemiologic studies are required to disentangle the effects of ICS use and asthma severity more clearly. Second, we validated our findings in using the CAMP RCT, at which time the participant were adolescents, which represents a younger age range than the other cohorts in this study. 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. Despite this, our findings were consistent across these age ranges, which may point more to the overall generalizability of these findings. Third, metabolomic profiling was performed in a different laboratory for CAMP and did not have the broader range of steroid metabolites. We recognize that the variation in the breadth of metabolomic measurements varies by laboratory. Despite these differences, we were able to refine and further validate the robustness of our findings over the four populations we utilized in this study. 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.

This study utilized four independent cohorts with over 14,000 individuals to demonstrate the translational utility of metabolomics for asthma in a precision medicine framework. With a sequential set of scientific inquiries, we utilized global metabolomics to identify substantial reductions in endogenous steroid metabolites associated with prevalent asthma cases compared with controls. With additional clinical data extracted from EMR, as well as extracted cortisol measures, we further demonstrated that this reduction was strongly associated with ICS use among asthma cases and more substantially than previously recognized, with significant effects even at low ICS doses. With these findings, a clinical framework of recommendations specific to asthma cases with ICS use may begin. The utilization of metabolomic data in this study, demonstrates the central role it may play in establishing an initial framework for precision medicine that may be further informed by other omic data types, in addition to enhancing biologic understanding both on the side of clinicians and researchers.

METHODS

Overview of cohorts and study populations

Discovery Study Population: EPIC-Norfolk—EPIC-Norfolk[60] is a subset of the EPIC[60] multi-center longitudinal cohort study. It includes 25,000 men and women of predominantly European descent. At baseline (between 1993 and 1998), participants provided blood samples and completed a health and lifestyle questionnaire. Plasma samples were stored in the gas phase of liquid nitrogen at −175 degrees Celsius for long-term storage. Asthma status was ascertained based on a combination of self-report of previous diagnosis ("Has the doctor ever told you that you have any of the following: Asthma"?), and any additional cases ascertained at a baseline health check performed by a registered nurse.

Plasma metabolomic data generated on a subset of 10,754 participants were used as the discovery population in this analysis (Table 1). Of these, 661 (6%) individuals had asthma (as previously defined), while the remainder were considered non-asthmatic controls. Individuals with a self-reported previous doctor's diagnosis of bronchitis, and/or emphysema at baseline were excluded. Participants were largely unfasted and provided consent to participate in the study.

Replication Study Population: MGBB-Asthma—The MGBB (https://biobank.partners.org) is a collection of DNA, serum, and plasma samples from 81,502 fully consented subjects linked to the Research Patient Data Registry (RPDR), a data warehouse that gathers data from multiple electronic medical record (EMR) systems and stores it in a SQL Server database. Researchers may query the RPDR using an online query tool; RPDR currently contains data on 4.6M patients, with 227M encounters, and approximately 900M distinct, coded clinical facts stored in the database dating back to 1986 including demographic data, diagnoses (e.g., ICD-9/ICD-10 codes), procedures (e.g., Current Procedural Terminology (CPT) codes), pharmacy data (*e.g.* RxNorm), inpatient and outpatient encounter information, provider information, laboratory data, imaging, and pathology data. We applied a validated phenotyping algorithm[61] for asthma diagnosis and identified 287 individuals with asthma (positive predictive value > 85%) and 323 controls (negative predictive value > 99%) to generate the MGBB-Asthma population (total N = 610 subjects, Table 1). Completed questionnaires including the demographic details on the biospecimen collection are collected from the individuals who enroll in the Biobank. Non-fasting plasma samples for the MGBB-Asthma cohort were collected between October 2010 and March 2017 and were stored immediately (within 4 hours) in an −80 degrees freezer. Controls were randomly selected from the pool of individuals without asthma with available plasma samples.

Metabolomic Profiling for EPIC-Norfolk and MGBB-Asthma—In both studies, metabolomic profiling was conducted by Metabolon Inc. (Durham, NC, USA). The profiling methods have been described in detail[62]. In short, four non-targeted Liquid Chromatography Couple Mass Spectroscopy (LCMS) platforms were performed enabling the broadest coverage of the metabolome: 1) Amines and polar metabolites that ionize in the positive ion mode; 2) Central metabolites and polar metabolites that ionize in the negative ion

mode; 3) Polar and non-polar lipids; 4) Free fatty acids, bile acids, and metabolites of intermediate polarity. All reagents and columns for this project were purchased in bulk from a single lot and all instruments were calibrated for mass resolution and mass accuracy daily. Coefficients of variation were measured in blinded QC samples randomly distributed among study samples, and batch variation is controlled for. Metabolites were identified by their mass-to-charge ratio (m/z), retention time (rt), and through a comparison to a library of purified known standards. Peaks were quantified using the area-under-the-(ROC) curve. Metabolite measures were median normalized across run days (with medians set to 1).

In EPIC-Norfolk, measurements were made in citrate plasma samples taken at baseline, for two sets of samples, each consisting of approximately 6,000 quasi-randomly selected individuals. Individuals with high levels of metabolite missingness were excluded. Metabolites present in at least 30 asthma cases in both measurement sets were included in the analyses. Metabolite measures were natural log transformed, windsorized at 5 SD and standardized ($\mu = 0$, SD = 1). Analyses were performed within each of the two metabolite measurements sets individually, and results were meta-analyzed to pool the associations from the two measurement sets, using a fixed effects inverse variance weighted meta-analysis. In the MGBB-Asthma, missing metabolite values were imputed by replacement with half the minimum value for each metabolite in all samples. Metabolites that are of unknown identity (or with format X-nnnnn) can be quantified and tracked, and therefore Metabolon is confident that they represent biologically relevant molecules and not analytical artefacts. Metabolites with an interquartile range of zero were excluded from further analysis (n=129) with 904 metabolites remaining for the analysis (Supplementary Table 5).

Randomized controlled trial (RCT) of ICS: CAMP—CAMP is a double blind RCT that randomized 1,041 children with mild-to-moderate asthma aged 5 to 12 years to low dose inhaled steroid budesonide (200 mcg), nedocromil (8 mg) or matching placebos, treatment twice daily during 4.3 years of the trial[63-65]. Samples for the CAMP clinical trial were obtained from the clinical sites at the randomization visit between December 1993 and September 1995 and again at the end of the clinical trial between December 1997 and December 1999 and were stored in an −80-degree freezer. Cortisone and cortisol were both among the metabolites measured via global untargeted metabolomic profiling at the Broad Institute using 560 serum samples (Table 1) collected from CAMP individuals at baseline and four years later at the end of the trial. The quality control information is detailed elsewhere[66]. The RCT design of CAMP was used to further validate the cause-effect relationship between long-term ICS use and changes in cortisone and cortisol levels.

ICS effect and clinical cortisol measurements: EMR-Cortisol—We queried the RPDR in MGBB to identify individuals who obtained clinical cortisol measurements from cortisol testing (most often as a part of adrenal insufficiency testing, specifically the adrenocorticotropic hormone (ACTH) stimulation test). Given the diurnal variation in cortisol levels, we obtained and extracted detailed information on specifications of the cortisol measurements, including the time and date of the blood draw. For individuals with multiple cortisol measurements on different dates, we recorded the minimum, maximum, mean, and median cortisol values, only considering the initial measurement from any single

visit. To avoid confounding by other medications and to avoid misclassification due to controls that may have taken ICS medications for reasons other than asthma, we used a stringent measure of ICS treatment: subjects having a count of greater than or equal to at least 10 prescriptions of their most common ICS medication were categorized as “ICS use”, while subjects with less than 10 prescription counts of their most common ICS medication were categorized as “no ICS use”. We then stratified these groups based on asthma affection status and ICS treatment, resulting in the following four categories: 1) no asthma/no ICS use; 2) no asthma/ICS use; 3) asthma/no ICS use; 4) asthma/ICS use. Subjects with COPD were excluded from this analysis. In total, we identified 2,235 individuals that were included in the EMR-Cortisol cohort (Table 1). Twenty-three of these subjects overlapped with MGBB-asthma.

Statistical Analysis

Overview of Analytic Approach—An illustration of our analytic strategy is presented in Fig. 1. Briefly, we utilized a discovery and replication approach to identify metabolites (predictor variable) associated with prevalent asthma (outcome variable), using the EPIC-Norfolk (discovery) and biobank-derived Mass General Brigham Biobank-Asthma (MGBB-Asthma) (replication) cohorts. As these findings implicated 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 using cortisol and cortisone measures from the four-year ICS RCT, CAMP[63,65]. Using cortisol as a biomarker for adrenal suppression from ICS use, we queried the MGBB EMR to identify individuals with cortisol measures to create the EMR-Cortisol cohort and assessed the impact of ICS use on these cortisol measures. Analyses in the EPIC-Norfolk cohort were performed in Stata 14.2. All other analyses were performed in R version 4.0.3[67]. The p-values presented are two-sided and have been adjusted for multiple comparisons. The EPIC-Norfolk study was approved by the Norwich Local Ethics Committee (REC Ref. 7898CN01). 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 (Protocol#: 1999-P-001549/29, the Partners Human Research Committee (PHRC), # 2002P000331, ClinicalTrials.gov Identifier: NCT00000575). All study participants provided written consent at enrollment. Consent from the guardian and assent was obtained from all children participating in the CAMP trial.

Discovery and Replication for Metabolite-Asthma Associations—Multivariable logistic regression models were employed in EPIC-Norfolk to assess the association between log-transformed metabolite levels with asthma affection status where the number of participants varied for each metabolite. Models were adjusted for age, sex, 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)[68] is <5%.

Steroid Metabolites by ICS treatment in MGBB-Asthma—Using the MGBB, we obtained information on asthma medication use (Supplementary Table 6. We created a binary measure of inhaled corticosteroid (ICS) use as the outcome using information on the total number of ICS prescriptions. To identify the optimal binary threshold, we considered several cutoffs for ICS prescription count: >0, ≥4, ≥6 and ≥10. Importantly, our findings were robust to all four prescription cutoff selections. A count of four prescriptions was selected as the threshold to define a binary cut-off for ICS. Specifically, subjects with at least four ICS prescriptions were categorized as using ICS while subjects with less than four ICS prescription counts were categorized as not treated with ICS. The following medications were utilized to create the prescription count: Beclomethasone, Dipropionate, Budesonide, Ciclesonide, Inhaled dexamethasone, Flunisolide, Fluticasone, Fluticasone/Salmeterol, Mometasone and Triamcinolone. An ordinal measure of asthma severity was created using EMR indications of mild intermittent, mild persistent, moderate persistent, and severe persistent asthma. A binary indicator of OCS use was defined by at least one prescription of the following oral medications in the past year: Dexamethasone, Methylprednisolone, Prednisolone, Prednisone. Any healthy controls with intake of ICS and individuals with COPD were excluded from the analysis.

To quantify the relative reduction in steroid metabolite levels based on ICS use, MGBB-Asthma subjects were stratified by their asthma affection status. Asthma cases were further stratified by ICS treatment. This resulted in four sub-group comparisons: 1) asthma cases versus controls; 2) asthma cases with ICS treatment versus controls; 3) asthma cases not treated with ICS versus controls; 4) asthma cases with ICS treatment versus asthma cases not treated with ICS. Multivariable logistic regression models using pairwise comparisons adjusted for age, gender, race, BMI and smoking status were utilized to compare metabolite levels between groups. Analyses comparing asthma cases with ICS and without ICS treatment were additionally adjusted for asthma severity and oral corticosteroid use in the past year.

Evaluation of the ICS dose on cortisol in MGBB-Asthma—We extracted the identified individuals from MGBB-Asthma prescribed Fluticasone propionate, over the 5 years prior to plasma collection and extracted the median fluticasone dosage level. Dose was considered both as a continuous measure and in groups defined using the GINA 2021[1] guidelines of "no ICS use", "low ICS dose" (44-200 mcg) and "moderate to high ICS dose" (>200 mcg). This resulted in 82 individuals that were categorized in the "low dose" group (44-200 mcg), and 20 individuals were categorized in the "moderate to high dose" group (>200 mcg). There were 81 individuals that were categorized in the "No ICS" group.

Linear models were utilized with dose as the predictor variable, and adjusted for age, sex, race, smoking status, BMI and the frequency of the medication use while cortisol was the response variable. Dosage was measured using the actual dosage values, and as both an ordinal and categorical variable using the GINA 2021 classifications described above. A trend test was used to evaluate the significance of the ordinal dose variable on cortisol while

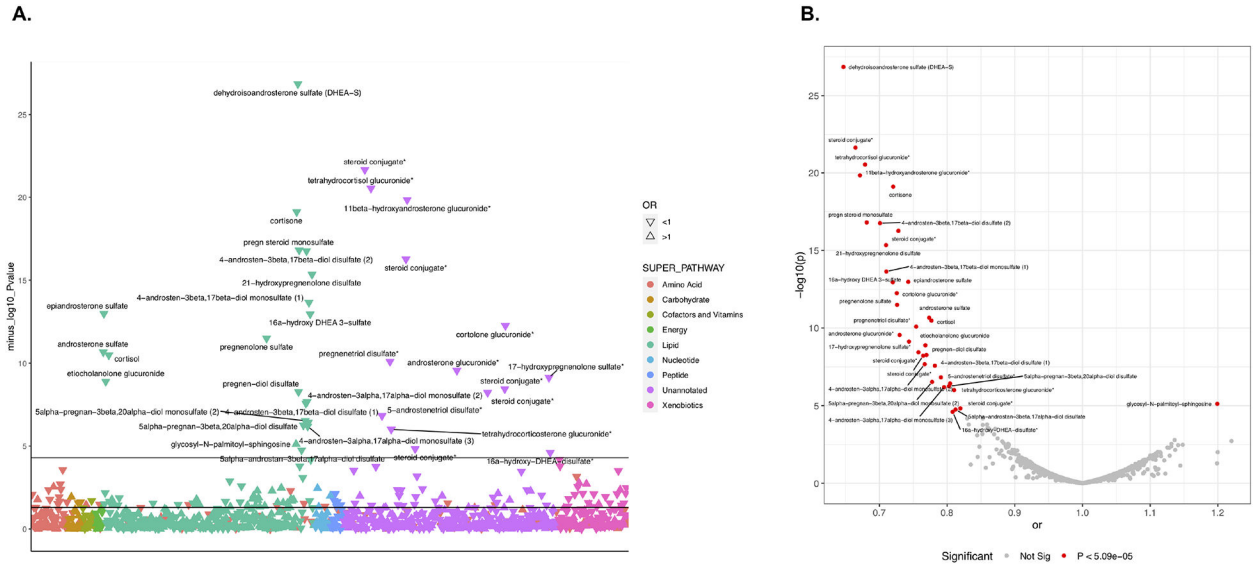
individual estimates were generated when comparing low ICS dose use and moderate to high ICS dose to the reference group with no ICS use.

ICS effect on cortisone and cortisol 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 low dose ICS (budesonide) verses 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 puberty directly influences steroid levels. We further performed a rigorous assessment of the impact of potential confounders on the ICS and steroid metabolite associations observed in CAMP, including measures of FEV_1, cumulative hospitalizations, emergency room visits, total eosinophils, and total Immunoglobulin E (IgE). Because CAMP is an RCT that specifically randomized individuals to low-dose ICS budesonide (200mcg), the findings provide a direct estimate of low dose ICS use on cortisone and cortisol.

Quantification of Cortisol and adrenal insufficiency within the EMR—We investigated minimum cortisol levels (mcg/dL) recorded in EMR-Cortisol subjects throughout a 24-hour period, stratified as above 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 HSD[69] 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.

We further queried the EMR for asthma cases that were tested for adrenal insufficiency within the last five years. Information was extracted on the presence or absence of four primary adrenal insufficiency symptoms[70,71] including fatigue, weight loss, hyperpigmentation and anemia using ICD10 codes. Presence of ICS use/treatment was defined as four or more ICS prescriptions and absence of ICS was defined as no ICS prescriptions. To account for potential confounding by asthma severity, we restricted this analysis to "mild" asthma cases (n=755), as identified by a physician's report via the EMR. Logistic regression models were applied to this set of mild asthma cases, with ICS use as the primary exposure of interest and each adrenal insufficiency symptom as the outcome, while adjusting for age, sex, race, BMI, and smoking (former and current).

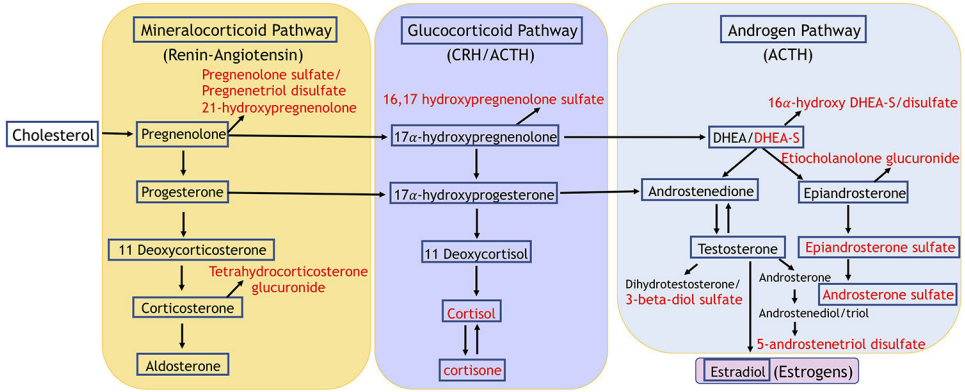
Extended Data



Extended Data Fig. 1. Plasma metabolites in EPIC-Norfolk cohort.

A. Manhattan plot of metabolites sorted by their pathways on x-axis and negative log10 of P-value on the y-axis. The cut off horizontal lines on the y-axis highlight the metabolites significantly associated with asthma outcome at a P-value <0.05 and at the Bonferroni threshold (n=35 metabolites, P-value<$5.14x10^{-5}$). The legend key shape and color show the direction of effect for the metabolites and the main pathway they belong to, respectively.

B. Volcano Plot showing the effect size of the metabolites with OR on the x-axis and negative log10 of P-value on the y-axis. The metabolites colored in red are significant at a Bonferroni threshold of P-value<$5.14x10^{-5}$. Multivariable logistic regression models were used to obtain odds ratio and p-values comparing asthma cases with controls (A, B).



Extended Data Fig. 2. Principal steroid hormone biosynthesis pathways with mineralocorticoid, glucocorticoid and androgen metabolites highlighting the replicated metabolites between EPIC-Norfolk cohort and Mass General Brigham Biobank.

Our annotated metabolites colored in red have been mapped to these pathways with their precursors or intermediates.

Abbreviations: CRH: Corticotropin releasing hormone; ACTH: Adrenocorticotropic hormone

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Role of the Funder/Sponsor:

The external funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Data availability

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/). Requests for the other datasets can be made by researchers via a data use agreement for specific scientific inquiries. Datasets for the other cohorts is subject to controlled access. These restrictions apply, given the sensitive nature of patient data and the possibility of identifying individuals via the use of electronic medical records in conjunction with omic data. Please contact the corresponding author to create a data use agreement. The corresponding author will respond within 10 days to your request.

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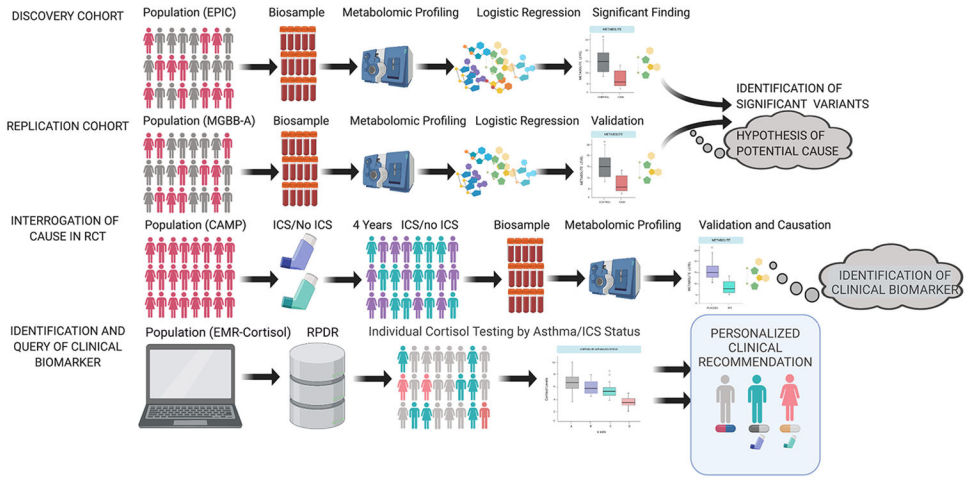

Fig. 1. Overall study design.

The study incorporates four independent cohorts: the EPIC Norfolk cohort, the Mass General Brigham Biobank (MGBB)-Asthma cohort, the CAMP randomized controlled trial (RCT) and the EMR-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.

Abbreviations: MGBB-A, Mass General Brigham Biobank (MGBB)-Asthma; RCT, Randomized Controlled Trial; CAMP, Childhood Asthma Management Program; ICS, inhaled corticosteroids; EMR, Electronic Medical health Record; RPDR, Research Patient Data Registry

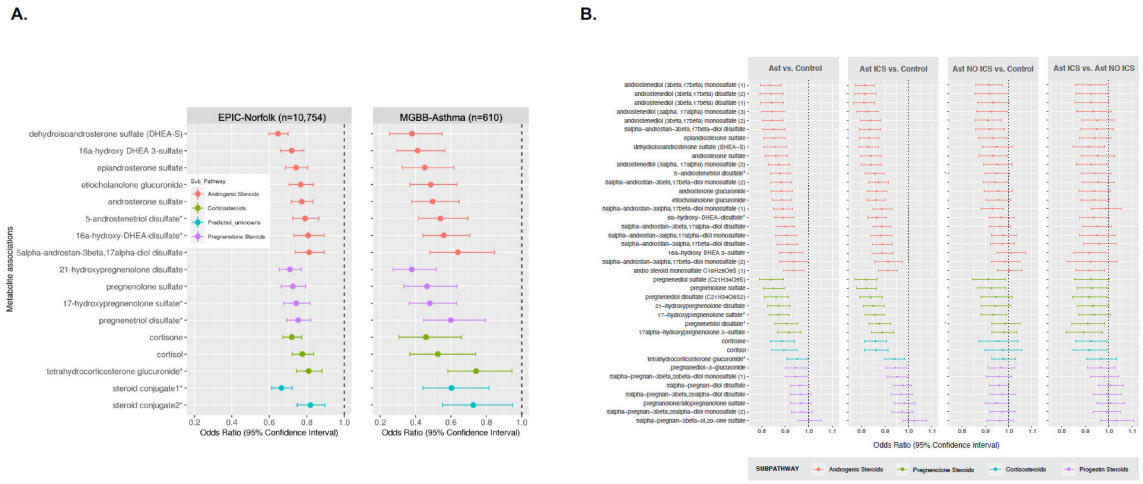

Fig. 2. Plasma steroid metabolite associations in EPIC-Norfolk cohort and Mass General Brigham Biobank (MGBB)-Asthma cohort.

A. Plasma metabolites significantly associated with asthma in the EPIC-Norfolk cohort and replicated in the MGBB-Asthma cohort (n=17 metabolites). The dotted line indicates the cut off for odds ratio (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. **B.** Steroid metabolite associations between asthma cases and controls; asthma cases with ICS treatment and controls; asthma cases not treated with ICS and controls; and asthma cases with ICS treatment and not treated with ICS in the MGBB-Asthma cohort. The dotted line indicates the cut off for OR< or >1 for direction of effect. Individual values shown for each metabolite as odds ratios along with their lower and upper 95% confidence intervals in A and B. The legend key color shows the sub-pathway they belong to (A, B). Generalized linear model was used to compare groups (A, B); Statistical significance was determined using a bonferroni threshold and false discovery rate of 0.05 (A, B).

Abbreviations: Ast, asthma cases; ICS, inhaled corticosteroids

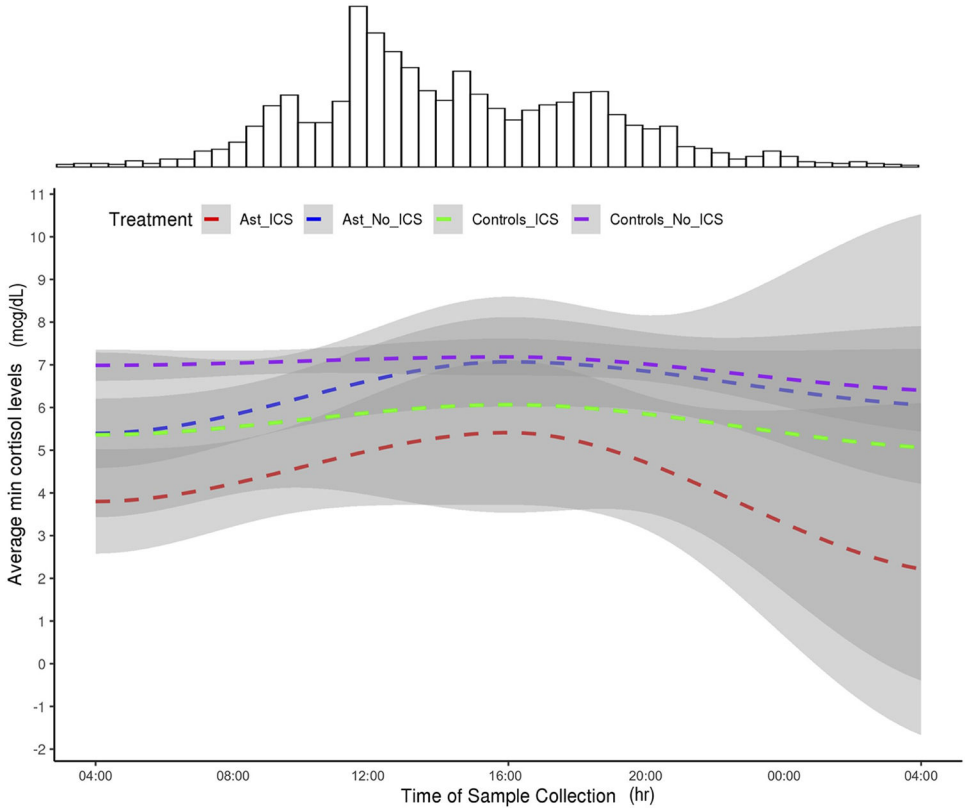

Fig. 3. Quantification of Cortisol in the EMR-Cortisol cohort.

Top, histogram showing the frequency of subjects during the indicated times of sample collection (24-hour daily period) on the x-axis of the bottom graph. Bottom, graph showing the aggregated minimum cortisol levels for four groups of subjects during the indicated times of sample collection on the x-axis. Subjects were binned into three time categories (04:00-12:00, 12:00-18:00, 18:00-04:00) based on the availability of subjects and smoothed using loess curve fitting. Legend label is colored based on four categories: Ast_ICS: asthma cases with ICS treatment (Average=4.2 mcg/dL; SD=5.2); Ast_No_ICS: Asthma cases without ICS treatment (Average=6.03 mcg/dL; SD=5.1); Controls_ICS: Controls with ICS treatment (Average=5.58 mcg/dL; SD=4.6); Controls_No_ICS: Controls without ICS treatment (Average=7.02 mcg/dL; SD=5.8). P-value for asthma cases with ICS treatment compared to controls without ICS treatment (Mean difference=−2.80 mcg/dL; 95% CI=−1.4, −4.2; $P=1.9x10^{-6}$). Tukey's HSD 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.

Abbreviations: Ast, Asthma cases; ICS, inhaled corticosteroids

Table 1.

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

Discovery cohort: EPIC-Norfolk				
Number of subjects	**All subjects (n = 10,754)**	**Asthma (n = 661)**	**No asthma (n = 10,093)**	***P*-value***
Age, mean (SD)	59.73 (9.0)	59.24 (9.1)	59.76 (8.9)	0.14
BMI kg/m^2, mean (SD)	26.19 (3.7)	26.10 (3.9)	26.20 (3.7)	0.53
Sex, n (%)				0.08
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 (%)				0.76
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)	
Smoking, n (%)				
Never	5,012 (46.6)	308 (46.6)	4,704 (46.6)	0.003
Former	4,573 (42.5)	306 (46.3)	4,267 (42.3)	
Current	1,169(10.9)	47(7.1)	1,122(11.1)	
Replication cohort: MGBB-Asthma				
Number of subjects	**All subjects (n = 610)**	**Asthma (n = 287)**	**No asthma (n = 323)**	***P*-value***
Age, mean (SD)	32.7 (5.3)	33.1 (6.6)	32.4 (3.7)	0.11
BMI kg/m^2, mean (SD)	25.6 (6.5)	28.3 (8.0)	23.2 (3.1)	$<2.2x10^{-16}$ $2x10^{-16}$
Sex, n (%)				
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)	
Oral corticosteroid intake, n (%)				
No	265 (43.4)	265 (92.3)	NA	NA
Yes	17 (2.8)	17 (5.9)	NA	NA
Asthma severity scale				
No mild, moderate or severe asthma	72 (11.8)	72 (25.1)	NA	NA
Mild intermittent or persistent	146 (23.9)	146 (50.9)	NA	NA

Discovery cohort: EPIC-Norfolk				
Number of subjects	**All subjects (n = 10,754)**	**Asthma (n = 661)**	**No asthma (n = 10,093)**	***P*-value***
Moderate persistent	50 (8.2)	50 (17.4)	NA	NA
Severe persistent	19 (3.1)	19 (6.6)	NA	NA
Smoking, n (%)				$1.2x10^{-3}$
No	482 (79)	210 (73.2)	272 (84.2)	
Yes	128 (21)	77 (26.8)	51 (15.8)	

Validation cohort: Randomized clinical trial of ICS use - CAMP				
Number of subjects	**All subjects (n = 1041)**	**Baseline**	**End of trial**	***P*-value***
		(n = 560 at both time points)		
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)	
Nedocromil + Placebo	730 (70.1)	409 (73)	409 (73)	

Validation cohort: Assessment of ICS effect in a clinical cohort EMR-Cortisol				
Number of subjects	**All subjects (n = 2,235)**	**Asthma (n = 383)**	**No asthma (n = 1,852)**	***P*-value***
Age, mean (SD)	56.1 (16.2)	55.2 (16)	56.3 (16.3)	0.20
Sex, n (%)				$3.9x10^{-14}$
Female	1377 (61.6)	302 (78.9)	1075 (58.0)	
Male	858 (38.4)	81 (21.1)	777 (42.0)	
Race, n (%)				$1.4x10^{-3}$
African American	122 (5.5)	34 (8.9)	88 (4.8)	
White	1959 (87.7)	317 (82.8)	1642 (88.7)	
Asian	52 (2.3)	7 (1.8)	45 (2.4)	
Other	102 (4.6)	25 (6.5)	77 (4.2	
Inhaled steroid intake, n (%)				$<2.2x10^{-16}$
No	2079 (93.0)	268 (70)	1811 (97.8)	
Yes	156 (7.0)	115 (30)	41 (2.2)	
Adrenal Insufficiency diagnosis, n (%)				0.03
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; oral corticosteroid intake (OCS) and asthma severity were only extracted for asthma cases (therefore corresponding P-values cannot be determined) and OCS was not available for five subjects. Data on inhaled and oral medications were not available in EPIC-Norfolk cohort.

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, therefore NA is indicated for P-values.

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	$1.4x10^{-27}$	0.38	0.25, 0.55	$1.1x10^{-6}$
Steroid conjugate *	Corticosteroid	0.68	0.61, 0.72	$2.9x10^{-21}$	0.60	0.44, 0.81	$1.1x10^{-3}$
Cortisone	Corticosteroid	0.72	0.67, 0.77	$7.8x10^{-20}$	0.46	0.31, 0.66	$6.2x10^{-5}$
21-hydroxypregnenolone disulfate	Pregnenolone Steroid	0.71	0.65, 0.77	$4.5x10^{-16}$	0.47	0.33, 0.63	$2.9x10^{-6}$
Epiandrosterone sulfate	Androgenic Steroid	0.74	0.69, 0.80	$1.1x10^{-13}$	0.41	0.29, 0.57	$1.0x10^{-7}$
16a-hydroxy DHEA 3-sulfate	Androgenic Steroid	0.81	0.66, 0.79	$2.5x10^{-5}$	0.64	0.48, 0.85	$1.9x10^{-3}$
Pregnenolone sulfate	Pregnenolone Steroid	0.73	0.66, 0.79	$3.2x10^{-12}$	0.38	0.27, 0.52	$3.4x10^{-9}$
Androsterone sulfate	Androstane Steroid	0.77	0.72, 0.83	$2.1x10^{-11}$	0.45	0.33, 0.62	$1.1x10^{-6}$
Cortisol	Corticosteroid	0.78	0.72, 0.84	$3.3x10^{-11}$	0.53	0.37, 0.74	$2.9x10^{-4}$
pregnenetriol disulfate *	Pregnenolone Steroid	0.73	0.69, 0.82	$3.2x10^{-12}$	0.60	0.45, 0.79	$4.6x10^{-4}$
17-hydroxypregnenolone sulfate *	Pregnenolone Steroid	0.74	0.68, 0.82	$7.4x10^{-10}$	0.48	0.36, 0.63	$1.9x10^{-7}$
etiocholanolone glucuronide	Androstane Steroid	0.79	0.71, 0.84	$1.5x10^{-7}$	0.49	0.37, 0.63	$1.7x10^{-7}$
5-androstenetriol disulfate *	Androstane Steroid	0.79	0.72, 0.86	$1.5x10^{-7}$	0.50	0.38, 0.65	$3.1x10^{-7}$
Tetrahydrocorticosterone glucuronide‡‡	Corticosteroid	0.81	0.74, 0.88	$9.9x10^{-7}$	0.74	0.58, 0.94	0.015
Steroid conjugate *‡‡	Corticosteroid	0.68	0.75, 0.90	$2.9x10^{-21}$	0.73	0.55, 0.95	0.020
5alpha-androstan-3beta, 17alpha-diol disulfate	Androgenic Steroid	0.81	0.74, 0.89	$1.8x10^{-5}$	0.56	0.44, 0.71	$1.1x10^{-6}$
16a-hydroxy-DHEA-disulfate *	Androgenic Steroid	0.81	0.73, 0.89	$2.5x10^{-5}$	0.54	0.42, 0.70	$2.6x10^{-6}$

Generalized linear model was used to compare asthma cases with controls; Statistical significance was determined using a bonferroni threshold and false discovery rate of 0.05.

‡The *P*-value thresholds for declaring significance was Bonferroni in both EPIC-Norfolk ($P<5.14x10^{-5}$) and MGBB-Asthma ($P<2x10^{-3}$)

‡‡Replicated metabolites in MGBB-Asthma at FDR threshold only

Abbreviations: MGBB: Mass General Brigham Biobank

Metabolite names with an asterisk * indicate that the identity of the metabolite has not been confirmed based on an analytical standard