Brief report

Lipid mediators are detectable in the nasal epithelium and differ by asthma status in female subjects

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Background: Lipid mediators, bioactive products of polyunsaturated fatty acid metabolism, contribute to inflammation initiation and resolution in allergic diseases; however, their presence in lung-related biosamples has not been fully described.

Objective: We aimed to quantify lipid mediators in the nasal airway epithelium and characterize preliminary associations with asthma.

Methods: Using liquid chromatography–mass spectrometry, we conducted a pilot study to quantify 56 lipid mediators from nasal epithelial samples collected from 11 female participants of an outpatient asthma clinic and community controls (aged 30-55 years). We examined the presence of each compound using descriptive statistics to test whether lipid mediators could distinguish subjects with asthma (n = 8) from control subjects (n = 3) using linear regression and partial least squares discriminant analysis.

Results: Fifteen lipid mediators were detectable in all samples, including resolvin (Rv) D5 (RvD5), with the highest median concentrations (in pg/μg protein) of 13-HODE (126.481), 15-HETE (32.869), and 13-OxoODE (13.251). From linear regression adjusted for age, prostaglandin E_2 (PGE2) had a trend ($P < .1$) for higher concentrations in patients with severe asthma compared to controls (mean difference, 0.95; 95% confidence interval, −0.04 to 1.95). Asthma patients had higher scores on principal component 3 compared to controls (mean difference, 2.42; 95% confidence interval, 0.89 to 3.96), which represented lower levels of proresolving 15-HEPE, 19,20-DiHDPA, RvD5, 14-HDHA, 17-HDHA, and 13-HOTrE. Most of these compounds were best at discriminating asthma cases from controls in partial least squares discriminant analysis.

Conclusion: Lipid mediators are detectable in the nasal epithelium, and their levels distinguish asthma cases from controls. (J Allergy Clin Immunol 2022;150:965-71.)

Key words: *Lipids, asthma, nasal airway epithelium, proresolving lipids, oxylipins, resolvins, PUFAs, EPA, DHA, AA*

INTRODUCTION

A growing body of literature supports the role of bioactive lipids in the pathogenesis and severity of asthma, including both pro- and anti-inflammatory products from the metabolism of polyunsaturated fatty acids such as eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and arachidonic acid (AA). AA produces several proinflammatory bioactive lipids, including leukotrienes (LTs) and prostaglandins, that contribute both to the initiation of inflammation and to chronic inflammation in lung diseases, including asthma severity and exacerbation.[1,2] Similarly, isoprostanes, which are products of AA peroxidation, have been implicated in asthma exacerbation,[3] and significant lipid peroxidation occurs in children with severe asthma (SA).[4] An additional consequence of upregulated oxidative stress response involves the generation and liberation of bioactive lipids, including prostaglandins, LTs, and proresolving lipoxins (LX). Conversely, both EPA and DHA can be metabolized to produce proresolving lipids (called specialized proresolving mediators)[5] that promote resolution of inflammation in many tissues[6,7] and may be protective against the pathogenesis of asthma.[8,9] Resolvins, DHA metabolites, are anti-inflammatory, enhance phagocytosis, and are reduced in SA.[10-13]

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Abbreviations used
AA: Arachidonic acid
ALA: α-Linolenic acid
DGLA: Dihomo-γ-linolenic acid
DHA: Docosahexaenoic acid
DPA: Docosapentaenoic acid
EPA: Eicosapentaenoic acid
LA: Linoleic acid
LT: Leukotriene
LX: Lipoxin
MMA: Mild to moderate asthma
PC: Principal component
PGE2: Prostaglandin E_2
RV: Resolvin

While the mechanistic roles and therapeutic potential of bioactive lipids continue to be explored in asthma and allergic disease, a major roadblock remains the detection of bioactive lipids in accessible biospecimens representative of the lung. For example, while some groups have reported the presence of resolvins in plasma or serum, this has been the subject of some controversy.[14] Therefore, we aimed to quantify and characterize lipid mediators in the nasal epithelium, an established proxy of lower airway tissues for other molecular markers, including DNA methylation[15] and gene expression.[16]

In this pilot study, we investigated 56 lipid mediators in nasal epithelial samples collected from 11 female participants of clinical studies from the Program for Control of Asthma in Bahia (ProAR), Salvador, Brazil.[17] We aimed to establish the presence and concentrations of various lipid mediators in the nasal epithelium and to characterize preliminary associations with asthma and asthma severity.

RESULTS AND DISCUSSION

We randomly selected 11 ProAR participants with nasal airway epithelial samples collected as part of the Consortium on Asthma Among African-Ancestry Populations in the Americas, including 3 nonatopic, nonasthma control subjects, 4 atopic cases of mild to moderate asthma (MMA), and 4 atopic cases of SA (see the Methods section in this article's Online Repository at www.jacionline.org). Asthma was defined using the 2006 criteria of the Global Initiative Against Asthma. Atopy was determined using ImmunoCAP technology and was defined as having a Phadiatop level of ≥0.35 kU/L (Thermo Fisher Scientific, Waltham, Mass). All participants were female, with an average age of 40 years at sample collection (range, 30-55 years; see Table E1 in the Online Repository). A panel of 56 bioactive lipid mediators was quantified (in pg/μg protein) from nasal epithelial cells using well-established liquid chromatography–mass spectrometry methods at the Mass Spectrometry Core Facility in the University of Colorado Skaggs School of Pharmacy and Pharmaceutical Sciences[18-20] (see the Methods section in the Online Repository).

Fifteen of 56 lipid mediators were detectable at or above the limit of quantification in all 11 samples (Fig 1), as follows: 15-HETE, 5,15-DiHETE, 12-HHTrE, PGE2, $PGF_2\alpha$ isomers, and 8-HETE derived from AA; 13-HOTrE derived from α-linolenic acid (ALA); 15-HETrE derived from dihomo-γ-linolenic acid (DGLA); 14-HDHA, 17-HDHA, 11-HDoHE, and resolvin (Rv) D5 derived from DHA; and 13-HODE, 13-OxoODE, and 9,10-DiHOME derived from linoleic acid (LA) (for compound annotations, see Table E2 in the Online Repository at www.jacionline.org). Among these, 13-HODE, 15-HETE, and 13-OxoODE were present in the highest concentrations in the nasal epithelium, with median concentrations of 126.481, 32.869, and 13.251 pg/μg protein, respectively. An additional 8 compounds were detectable at or above the limit of quantification in at least 8 samples and were therefore included in analyses.

In descriptive analysis, 3 compounds showed a trend for a linear relationship between concentration and asthma severity group, including 13-HODE, PGE2, and RvD5 (Fig 2). Only 9,10-DiHOME levels were significantly different across the 3 groups ($P < .05$, Kruskal-Wallis test), with the lowest levels among patients with SA. While many of these lipid mediators have been previously detected and quantified in urine,[21] plasma,[20] and tissues more proximal to the lung,[22] to our knowledge, the detection of DHA-derived metabolite RvD5 has only been detected in cerebral spinal and synovial fluids.[23] In addition, reported measurements of RvD1 in serum and plasma may be due to differences in technical methods, including the use of internal standards and stringency in applying qualifying ions.[14,18,23] RvD5 acts by the activation of DRV1, the D-series resolvin receptor 1 expressed on human neutrophils, lymphocytes, and monocytes and macrophages, as well as in vascular tissues (vascular smooth muscle cells and endothelial cells).[24] RvD5 has been reported to play roles in infection and the immune system; notably, it inhibits inflammatory pain in male, but not female, mice.[23]

We tested for differences between asthma groups using multivariable linear models adjusted for age. A separate regression was performed for each lipid mediator quantified in at least 8 samples ($m = 23$) and the top 3 principal components (PCs) of those 23 compounds. Despite our small sample size, we identified PGE2 with a trend ($P < .1$) for higher concentrations in SA cases compared to controls (mean difference, 0.95; 95% confidence interval, −0.0 to 1.95). A similar trend has been previously described in sputum, where the higher PGE2 levels found in subjects with SA and subjects with moderate asthma compared to controls were thought to reflect ongoing anti-inflammatory response.[22] In addition to its classical proinflammatory effects, the AA metabolite PGE2 also has been shown to prevent allergen-induced airway bronchoconstriction, hyperresponsiveness, and inflammation.[25]

We also identified significant differences between groups ($P < .05$) for scores on PC3 (Fig 3). Asthma cases had higher scores on PC3 compared to controls (mean difference, 2.42; 95% confidence interval, 0.89 to 3.96). Results were similar comparing the SA (mean difference, 2.25; 95% confidence interval, 0.42 to 4.07) or MMA (mean difference, 2.63; 95% confidence interval, 0.75 to 4.51) to controls. Higher scores on PC3 reflected higher concentrations of 13-OxoODE, RvD1, LXA4 isomers, TXB2, and LXB4, and lower concentrations of 15-HEPE, 19,20-DiHDPA, 13-HOTrE, RvD5, 14-HDHA, 17-HDHA, 11-HDoHE, and 8-HETE, and as determined by PC loadings more extreme than ±0.2 (see Fig E2 in the Online Repository at www.jacionline.org). Many of the compounds displaying strong negative correlations with PC3 scores (ie, those with loadings less than −0.2) have proresolving activity and are derived from

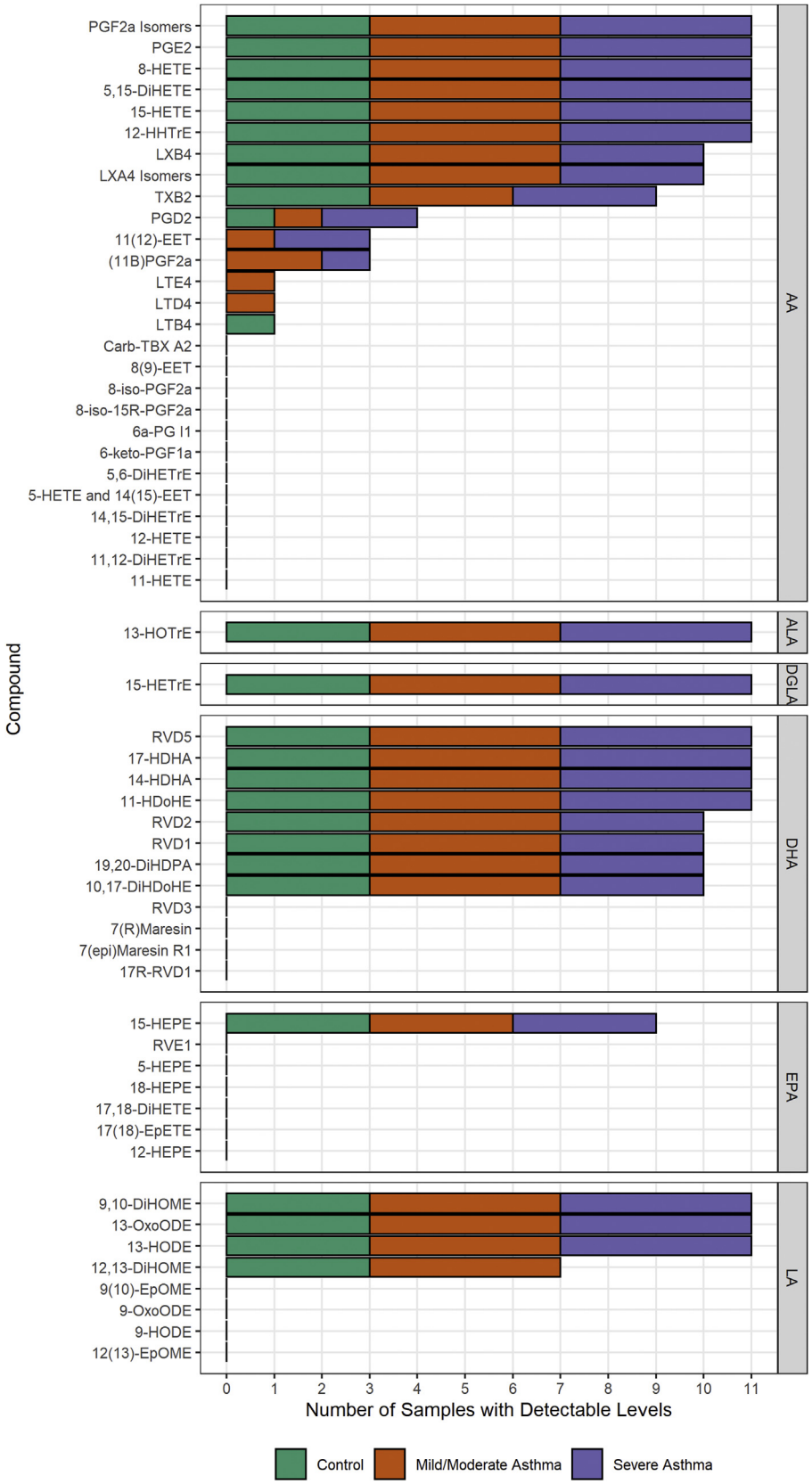

FIG 1. Lipid mediator array content and detection performance in nasal epithelium by parent fatty acid precursor and asthma severity group. Detectable levels defined as sample analyte levels at or above the limit of quantification (LOQ).

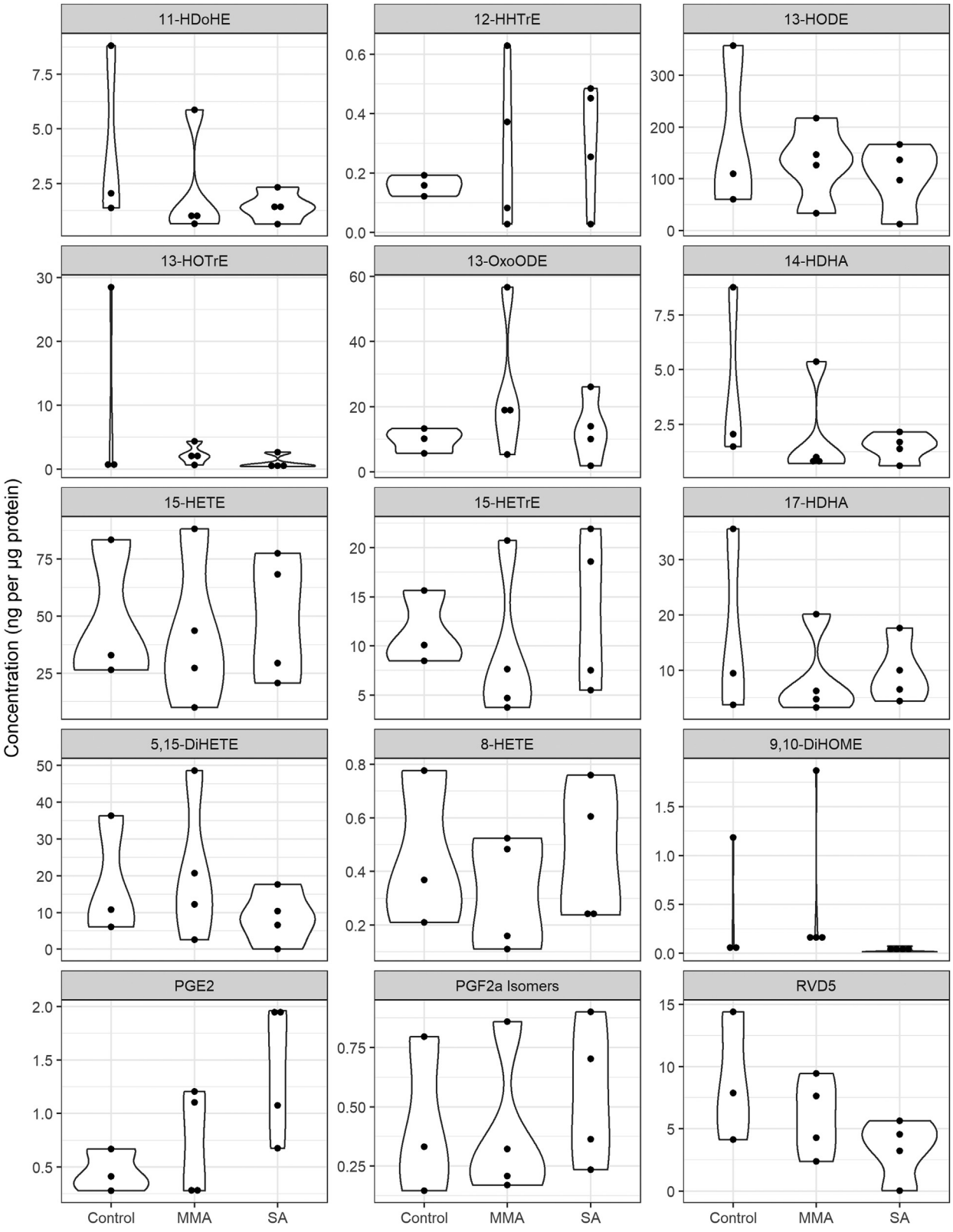

FIG 2. Distribution by asthma status of the 15 lipid mediators with complete quantification in the nasal epithelium.

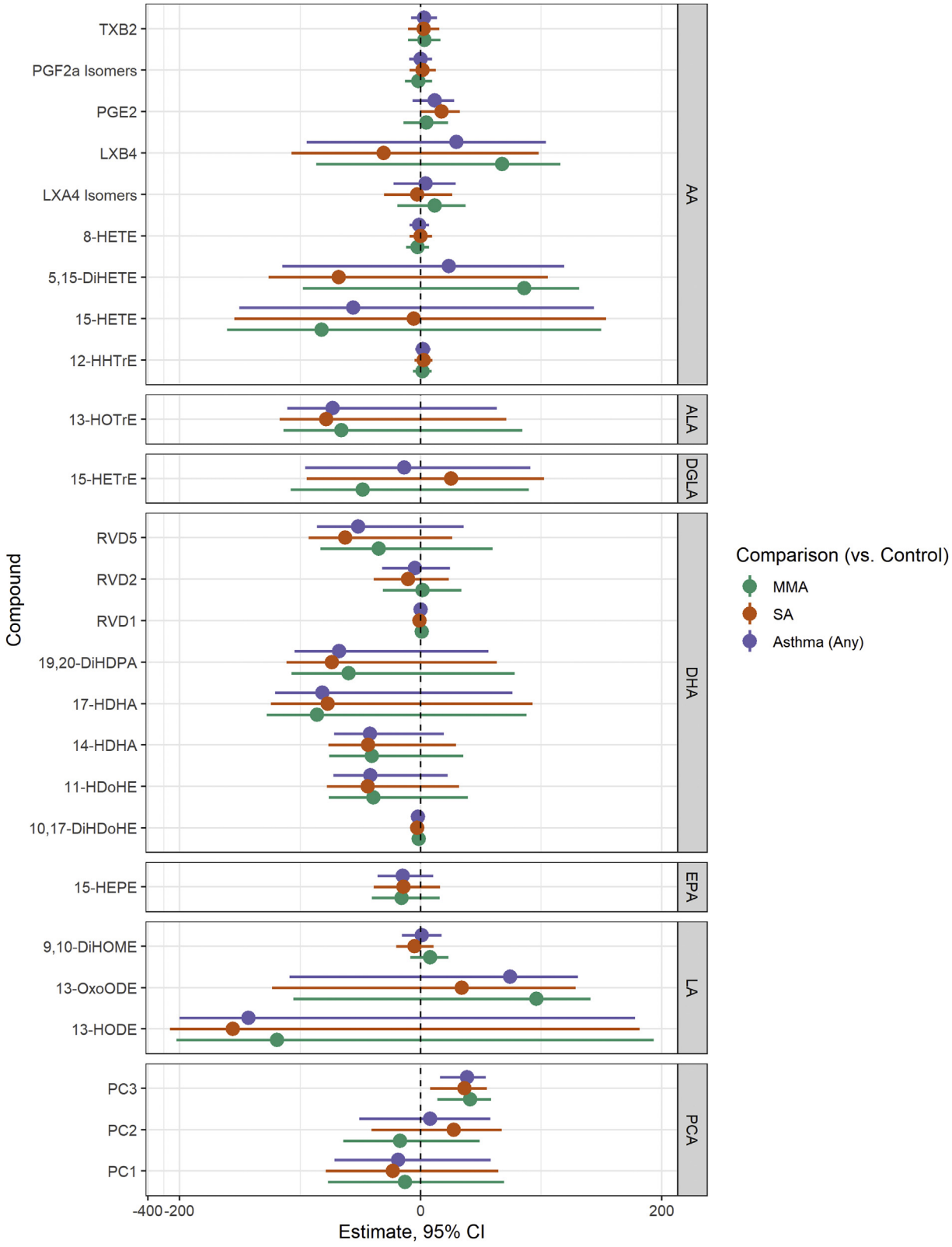

FIG 3. Mean lipid mediator differences between asthma cases compared to controls, and asthma severity group compared to controls. Results from 2 multivariable linear regression models adjusted for age, grouped by precursor/parent fatty acid.

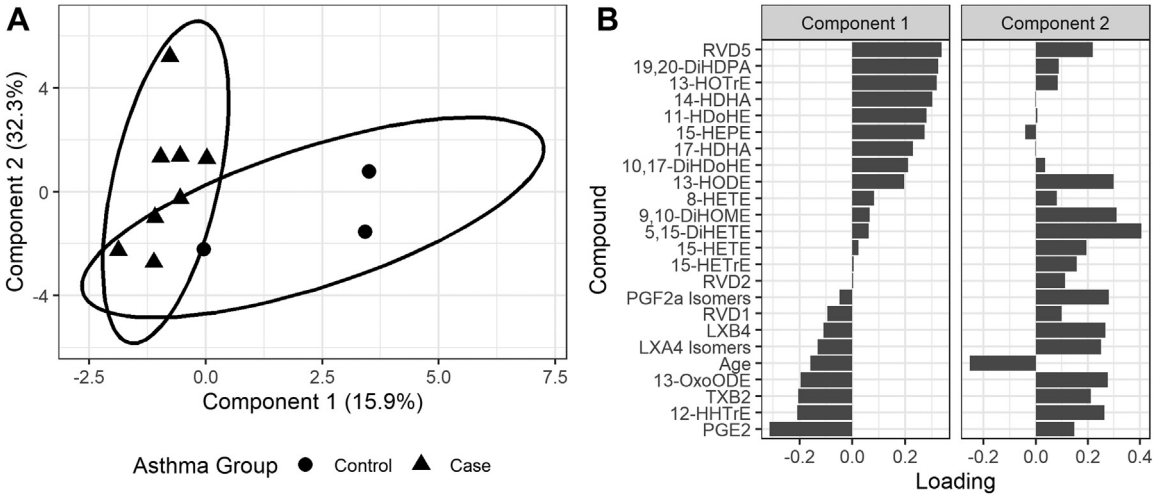

FIG 4. Multivariate PLS-DA results for discrimination of asthma cases and controls by 23 quantitative lipids and age. **A**, Discrimination of 3 controls and 8 asthma cases by PC1 and PC2. **B**, PLS-DA loadings. *PLS-DA,* Partial least squares discriminant analysis.

the anti-inflammatory precursor fatty acids EPA (15-HEPE), DHA (19,20-DiHDPA, RvD5, 14-HDHA, 17-HDHA, 11-HDoHE), and ALA (13-HOTrE). The mean difference of these lipids was similar whether comparing SA/MMA or asthma (any) to controls (Fig 3), suggesting the results were not driven by exposure to inhaled corticosteroids, which was definitional to the SA group. While the PC loadings for compounds such as 13-OxoODE were consistent with bivariate and linear regression results, the relationship of this compound with asthma status seems more important when considered in context of other metabolites (in multivariate PC analysis) than independently. Multivariate analyses such as these that consider multiple analytes simultaneously may better reflect biological systems and could provide novel insights into underlying processes.

We also tested whether multivariate profiles of lipid mediators could discriminate between asthma cases and controls using partial least squares discriminant analysis. Asthma controls and cases were modestly separated by PC1 (Fig 4, *A*). The loadings plot (Fig 4, *B*) shows the compounds with the highest contributions to separation between case and control groups in PC1, which include RvD5, 19,20-DiHDPA, 13-HOTrE, 14-HDHA, 11-HDoHE, 15-HEPE, 17-HDHA, PGE2, 12-HHTrE, and TXB2—many of these the same compounds that contributed to PC3.

In this pilot study, we achieved detection and quantification of lipid mediators derived from the precursor fatty acids AA, ALA, DGLA, EPA, docosapentaenoic acid (DPA), DHA, and LA in nasal epithelium. Despite a small sample size, we found preliminary evidence that patients with asthma had higher levels of PGE2 and lower levels of a combination of certain proresolving compounds (15-HEPE, 19,20-DiHDPA, 13-HOTrE, RvD5, 14-HDHA, 17-HDHA, 11-HDoHE, and 8-HETE) compared to controls. Given the growing evidence that bioactive lipids contribute to the pathogenesis and severity of asthma, combined with the inability to detect some of these compounds in plasma and serum, these novel data establish a baseline and rationale for their measurement in this accessible tissue for larger asthma and allergy studies.

Many thanks to the ProAR participants for their contributions to the study.

Key messages

- **Fifteen lipid mediators were present at detectable levels in the nasal epithelium of all subjects, including RvD5, a compound that has been inconsistently detected in other tissues.**
- **Patients with asthma had higher levels of PGE2, an AA metabolite with both proinflammatory and proresolving effects, and lower levels of EPA-, DHA-, and ALA-derived proresolving lipid mediators compared to controls.**

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METHODS

Study population and case definitions

The Program for Control of Asthma in Bahia (ProAR) is a clinical study of asthma in Salvador, Brazil.[E1] The ProAR study population comprises 3 groups: (1) SA requiring a combination of long-acting beta-agonists and regular inhaled corticosteroids (according to the standard definition at the time; the clinic was initiated in 2003); (2) milder forms of asthma receiving no regular treatment and only as-needed bronchodilators; and (3) a control group with no asthma. In 2013, a 2-fold case–control study was selected from the ProAR cohort, in which 473 SA subjects, 452 subjects with milder forms of asthma, and 454 controls without asthma were evaluated (all unrelated), to investigate risk factors, endophenotypes, and biomarkers of SA.[E2] All patients with asthma had previously undergone an evaluation by a specialist to confirm the diagnosis using the criteria of the Global Initiative against Asthma[E3] and to exclude conditions that could interfere in the evaluation of asthma control, followed by a thorough evaluation (2013-19).

For determination of atopy, blood samples were sent to the Dermatology, Allergy, and Clinical Immunology Laboratory at the Johns Hopkins Asthma and Allergy Center. Using the UniCap 250 system (Pharmacia & Upjohn, Peapack, NJ), the laboratory performed Phadia ImmunoCAP blood tests to detect allergen-specific IgE against several aeroallergens, including food (FP5E), mite–roach (HX2), animal dander (EX2), weed (WX1), grass (GX2), tree (TX3 and RTX10), and mold (MX2). Cases with Phadiatop level of ≥0.35 kU/L were considered atopic.

For this pilot study, we selected 11 female participants comprising 4 subjects with atopic SA, 4 subjects with atopic MMA, and 3 nonasthmatic, nonatopic control subjects.

Lung function

Spirometry was performed with a Koko spirometer (Ferraris Medical, Louisville, Colo) for measurement of FEV_1, FVC, and FEV_1/FVC ratio before and 15 minutes after inhaling 400 μg of bronchodilator (salbutamol), as recommended by the American Thoracic Society/European Respiratory Society.[E4,E5] Airway obstruction was assessed as FEV_1/FVC ratio lower than the lower limit of normal, and FEV_1 bronchodilator responsiveness was determined by the increase in FEV_1 postbronchodilator of <12% and 200 mL using spirometric reference equations developed for Brazilian adults.[E4,E6]

Nasal airway epithelium sample collection

Lipids were measured from the nasal epithelial cells collected as previously described by our group.[E7] Briefly, after inspecting the patient's nostrils with an otoscope, a brush was inserted through the nose and rolled for ~4 seconds. The brush was removed and placed into a tube with 400 μL of phosphate-buffered saline. The tube was closed and agitated for a few seconds. Then it was centrifuged for 3 minutes at 800 × *g*. The supernatant was removed and the pellet resuspended with 100 μL of methanol 70%. It was stored in a −80°C freezer until shipment to the University of Colorado, Denver, for sample processing.

Oxylipin sample preparation

All standards and internal standards used for liquid chromatography–tandem mass spectrometry analysis of AA-, DHA-, and LA-derived lipid mediators were purchased from Cayman Chemicals (Ann Arbor, Mich). All high-performance liquid chromatography (HPLC) solvents and extraction solvents were HPLC grade or better.

Nasal brush cell suspension samples were pretreated for solid phase extraction (SPE). Briefly, cells were lysed by diluting samples to a 70% methanol and 1% ethanol solution by adding 700 μL methanol, 10 μL of the internal standard solution (10 pg/μL each of 5(S)-HETE-d8, 8-iso-$PGF_2\alpha$-d4, 9(S)-HODE-d4, LTB4-d4, LTD4-d5, LTE4-d5, PGE2-d4, $PGF_2\alpha$-d9, and RvD2-d5 in ethanol), and sufficient water to create 1 mL of solution. The samples were then centrifuged for 10 minutes at 4°C at 14,000 rpm. The sample supernatant was then dried in a vacuum centrifuge at 55°C until dry, then immediately reconstituted in 1.0 mL of 90/10 water/methanol before purification by SPE. Sample cell pellets were tested for protein content with the Pierce bicinchoninic acid assay (Pierce, Rockford, Ill) and analyzed using a SpectraMax 190 (Molecular Devices, San Jose, Calif).

Specialized proresolving mediators from cells were isolated and purified using SPE as follows. The reconstituted extracts were loaded on a Strata-X 33 μm, 30 mg/1 mL SPE column (Phenomenex, Torrance, Calif) preconditioned with 2 volumes of 1.0 mL methanol followed by 2 volumes of 1.0 mL. The SPE column was then washed with 10% methanol and eluted directly into a reduced surface activity/maximum recovery glass autosampler vial with 1.0 mL of methyl formate. The methyl formate was evaporated completely from the vial with a stream of nitrogen, and the SPE cartridge was then eluted with 1.0 mL of methanol directly into the same autosampler vial. The methanol was evaporated to dryness with a stream of nitrogen and the sample reconstituted with 20 μL of ethanol. The samples were analyzed immediately or frozen at −70°C until analysis.

Liquid chromatography–mass spectrometry

Quantitation of lipid mediators was performed using 2-dimensional reverse-phase HPLC tandem mass spectrometry following previously described protocols that optimize accuracy and precision.[E8] The HPLC system consisted of an Agilent 1290 autosampler (Agilent Technologies, Santa Clara, Calif), an Agilent 1200 binary SL loading pump (pump 1), an Agilent 1290 binary analytical pump (pump 2), and a 6-port switching valve. Pump 1 buffers consisted of 0.1% formic acid in water (solvent A) and 9:1 vol:vol acetonitrile:water with 0.1% formic acid (solvent B). Pump 2 buffers consisted of 0.01% formic acid in water (solvent C) and 1:1 vol:vol acetonitrile:isopropanol (solvent D).

Extracted sample (5 μL) was injected onto an Agilent SB-C18 2.1 × 5 mm, 1.8 μm trapping column using pump 1 at 2 mL/min for 0.5 minutes with a solvent composition of 97% solvent A:3% solvent B. At 0.51 minutes, the switching valve changed the flow to the trapping column from pump 1 to pump 2. The flow was reversed, and the trapped lipid mediators were eluted onto an Agilent Eclipse Plus C-18 2.1 × 150 mm, 1.8 μm analytical column using the following gradient at a flow rate of 0.3 mL/min: hold at 75% solvent A:25% solvent D from 0 to 0.5 minutes, then a linear gradient from 25% to 75% D over 20 minutes, followed by an increase from 75% to 100% D from 20 to 21 minutes, then holding at 100% D for 2 minutes. During the analytical gradient, pump 1 washed the injection loop with 100% B for 22.5 minutes at 0.2 mL/min. Both the trapping column and the analytical column were re-equilibrated at starting conditions for 5 minutes before the next injection.

Mass spectrometric analysis was performed on an Agilent 6490 triple-quadrupole mass spectrometer in negative ionization mode. The drying gas was 250°C at a flow rate of 15 mL/min. The sheath gas was 350°C at 12 mL/min. The nebulizer pressure was 35 psi. The capillary voltage was 3500 V. Data for lipid mediators were acquired in "dynamic MRM" mode using experimentally optimized collision energies obtained by flow injection analysis of authentic standards. Calibration standards for each lipid mediator were analyzed over a range of concentrations from 0.25 to 250 pg on the column. Calibration curves for each lipid mediator were constructed by Agilent Masshunter Quantitative Analysis software. Samples were quantitated using the calibration curves to obtain the on-column concentration, followed by multiplication of the results by the appropriate dilution factor derived from the initial sample volume and the protein concentration data gathered from the bicinchoninic acid assay to obtain the concentration (in pg/μg protein).

For statistical analysis, measurements at the limit of detection (LOD) were calculated as the concentration for each analyte at which the lowest calibration standard injected repeatedly (n = 5) produced a response signal-to-noise ratio of ≥5. Measurements at limit of quantification (LOQ) were calculated with an analysis of the same repeated reference standard injections where the average accuracy of the most dilute from protein concentration data to obtain the concentration (in pg/mL).[E8] LOD, LOQ, and range of detectable levels for each compound are provided in Table E2.

Statistical analysis

We evaluated patterns of missingness for the 11 nasal epithelium samples across the 56 lipid mediator analytes. An analyte quantified at or above the LOQ in at least 8 samples was treated as a continuous/quantitative variable in statistical analyses (number of analytes, $m = 23$; 15 of these were quantified at or above the LOQ in all 11 samples). For the 1 to 3 samples with low analyte abundance for each of the compounds analyzed quantitatively, we substituted the corresponding LOD or LOQ value for analysis. Analytes quantified in 4 to 7 samples were dichotomized into present/absent ($m = 2$). Analytes quantified in 3 or fewer samples were excluded from statistical analysis ($m = 31$). Comparisons between groups were conducted in 2 ways: by comparing asthma cases (SA and MMA) to controls, and by comparing SA cases to MMA cases to controls as a 3-level categorical variable.

We evaluated the pairwise relationships between all analytes and phenotypic participant characteristics by using the Spearman correlation coefficient (Fig E1). After examining analyte distributions using histograms and violin plots by group, we used linear regression to test for differences between groups for each quantitative analyte, adjusting for age at nasal epithelium sample collection. Because of the established biological connections and high correlation between analytes, we also conducted a PC analysis of all quantitative analytes ($m = 23$, Fig E2) and examined whether scores on the first 3 PCs (which explained >80% of the variance) differed between groups using the same linear regression framework. Logistic regression models adjusted for age were used to test for differences between groups for analytes treated as present/absent.

Partial least squares discriminant analysis (PLS-DA)[E9] in the mixOmics R package[E10] was used to identify lipid mediators that contributed the most to discrimination between the asthma cases and controls. The $m = 23$ quantitative compounds and age were included in the PLS-DA model.

Ethics

Nasal epithelial samples were collected in accordance with approved protocols from the Colorado multiple institutional review board (approval COMIRB 17-1807) and the national ethics committee of Brazil (approval CAAE 94096318.4.0000.5577).

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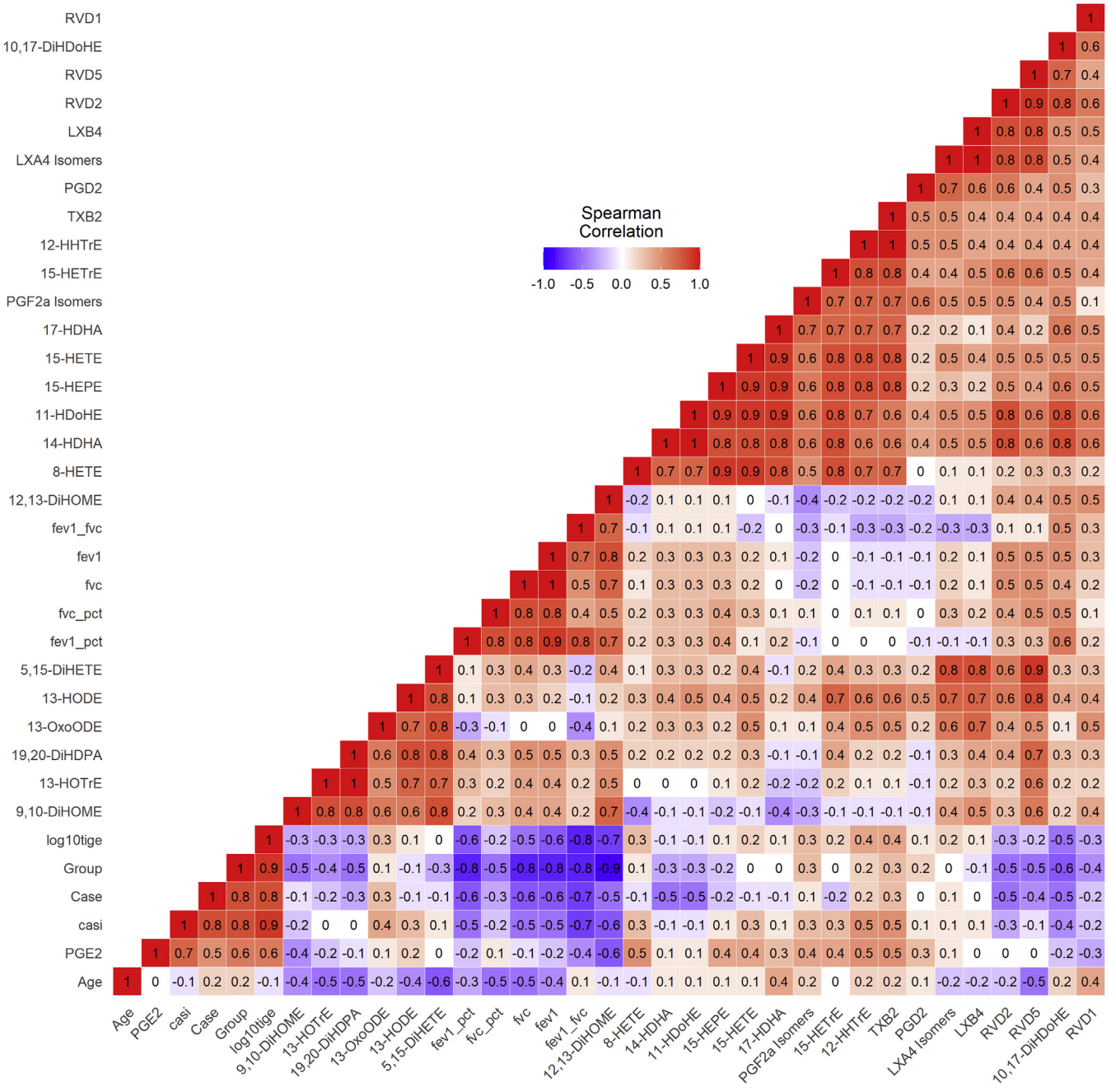

FIG E1. Spearman rank correlations between lipid mediators in the nasal epithelium (quantitative or present/absent, $m = 25$) and participant characteristics in ProAR (n = 11). Case indicates asthma vs control; group, SA vs MMA vs control.

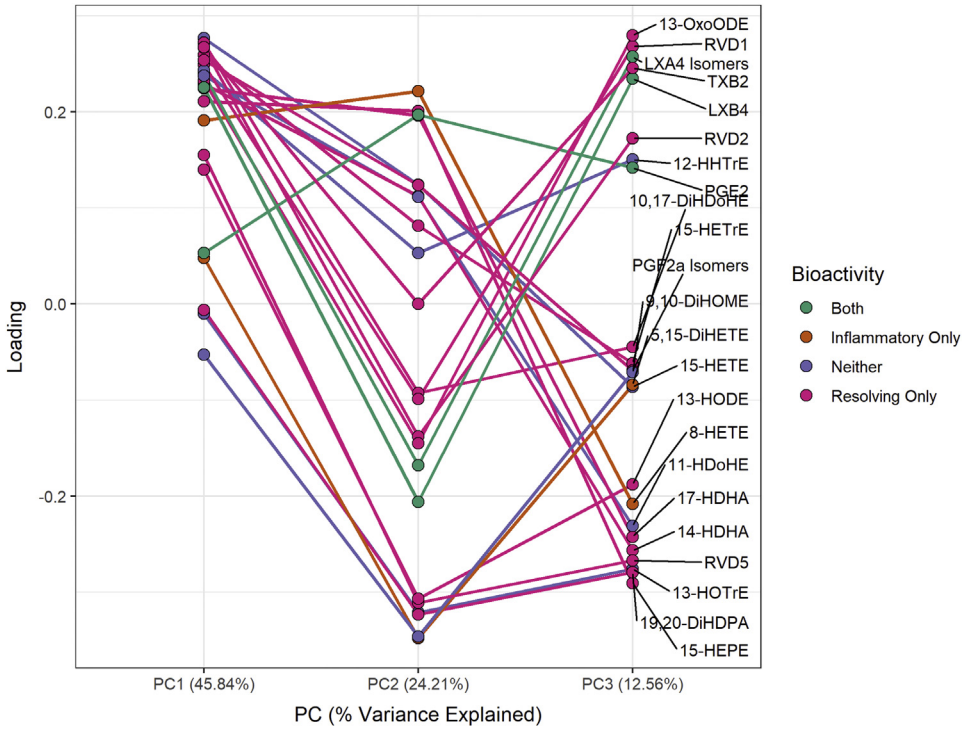

FIG E2. PC analysis loadings plot for lipid mediators ($m = 23$).

TABLE E1. Pilot study subject characteristics

Characteristic	Control (n = 3)	MMA (n = 4)	SA (n = 4)
Female sex, no. (%)	3 (100%)	4 (100%)	4 (100%)
Age (years), median (min, max)	37.0 (30.0, 45.0)	43.0 (30.0, 55.0)	40.5 (38.0, 41.0)
Phadiatop level, median (min, max)	0.100 (0.100, 0.100)	5.12 (2.13, 8.67)	4.50 (0.590, 8.55)
Total IgE (log_{10}), median (min, max)	0.820 (0.643, 1.03)	2.06 (1.88, 2.23)	2.47 (2.01, 3.00)
FEV_1, median (min, max)	2.91 (2.65, 3.28)	2.07 (1.93, 2.92)	1.41 (1.06, 1.97)
FEV_1%, median (min, max)	92.0 (90.0, 101)	83.5 (71.0, 95.0)	48.5 (35.0, 85.0)
FVC, median (min, max)	3.36 (2.89, 4.00)	3.01 (2.27, 3.62)	2.25 (2.01, 2.70)
FVC%, median (min, max)	90.0 (79.0, 101)	90.5 (83.0, 98.0)	63.5 (54.0, 96.0)
FEV_1/FVC, median (min, max)	0.870 (0.820, 0.920)	0.770 (0.650, 0.850)	0.630 (0.510, 0.730)

TABLE E2. Lipid mediator concentration ranges (in pg/μg protein) in nasal epithelium

Compound	Name	Precursor fatty acid	Pathway	LOD		LOQ		No. samples >LOQ	Median*	Min*	Max*	Analysis type†
				pg/μg protein	No. samples	pg/μg protein	No. samples		pg/μg protein			
(11B)$PGF_{2\alpha}$	11β-prostaglandin F2α	AA	COX	0.014	8	0.028	1	2	0.138	0.035	0.241	None
10,17-DiHDoHE	10(S),17(S)-dihydroxy-4Z,7Z,11E,13Z,15E,19Z-docosahexaenoic acid	DHA	15-LOX	0.003	1	0.028	4	6	0.146	0.042	0.411	Quantitative
11(12)-EET	(±)11,(12)-epoxy-5Z,8Z,14Z-eicosatrienoic acid	AA	CYP	0.003	8	0.028	3	0	—	—	—	None
11,12-DiHETrE	(±)11,12-dihydroxy-5Z,8Z,14Z-eicosatrienoic acid	AA	EH	0.003	11	0.014	0	0	—	—	—	None
11-HDoHE	(±)11-hydroxy-4Z,7Z,9E,13Z,16Z,19Z-docosahexaenoic acid	DHA	ROS	0.014	0	0.028	0	11	1.367	0.633	8.821	Complete
11-HETE	(±)11-hydroxyeicosatetraenoic acid	AA	ROS	0.001	11	0.071	0	0	—	—	—	None
12(13)-EpOME	(±)12(13)epoxy-9Z-octadecenoic acid	LA	CYP	0.014	11	0.028	0	0	—	—	—	None
12,13-DiHOME	12,13-dihydroxy-9Z-octadecenoic acid	LA	EH	0.007	4	0.028	5	2	0.232	0.033	0.431	Present/absent
12-HEPE	(±)-12-hydroxy-5Z,8Z,10E,14Z,17Z-eicosapentaenoic acid	EPA	12-LOX	0.014	11	0.028	0	0	—	—	—	None
12-HETE	(±)12-hydroxy-5Z,8Z,10E,14Z-eicosatetraenoic acid	AA	12-LOX	0.001	11	0.001	0	0	—	—	—	None
12-HHTrE	12S-hydroxy-5Z,8E,10E-heptadecatrienoic acid	AA	COS	0.014	0	0.028	2	9	0.255	0.083	0.630	Complete
13-HODE	(±)-13-hydroxy-9Z,11E-octadecadienoic acid	LA	15-LOX	0.014	0	0.284	0	11	126.481	12.362	357.988	Complete
13-HOTrE	13S-hydroxy-9Z,11E,15Z-octadecatrienoic acid	ALA	15-LOX	0.014	0	0.028	0	11	0.771	0.414	28.515	Complete
13-OxoODE	13-oxo-9Z,11E-octadecadienoic acid	LA	15-LOX	0.014	0	0.028	0	11	13.251	1.824	56.705	Complete
14,15-DiHETrE	(±)14,15-dihydroxy-5Z,8Z,11Z-eicosatrienoic acid	AA	EH	0.003	11	0.028	0	0	—	—	—	None
14-HDHA	(±)14-hydroxy-4Z,7Z,10Z,12E,16Z,19Z-docosahexaenoic acid	DHA	12-LOX	0.001	0	0.001	0	11	1.490	0.626	8.765	Complete
15-HEPE	(±)-15-hydroxy-5Z,8Z,11Z,13E,17Z-eicosapentaenoic acid	EPA	15-LOX	0.071	2	0.071	0	9	1.045	0.247	2.983	Quantitative
15-HETE	(±)15-hydroxy-5Z,8Z,11Z,13E-eicosatetraenoic acid	AA	15-LOX	0.001	0	0.001	0	11	32.869	9.966	88.215	Complete
15-HETrE	(±)14,15-dihydroxy-5Z,8Z,11Z-eicosatrienoic acid	DGLA	15-LOX	0.007	0	0.007	0	11	8.486	3.729	21.936	Complete
17(18)-EpETE	(±)17,18-epoxy-5Z,8Z,11Z,14Z-eicosatetraenoic acid	EPA	CYP	0.028	11	0.071	0	0	—	—	—	None
17,18-DiHETE	(±)17,18-dihydroxy-5Z,8Z,11Z,14Z-eicosatetraenoic acid	EPA	EH	0.014	11	0.014	0	0	—	—	—	None
17-HDHA	(±)17-hydroxy-4Z,7Z,10Z,13Z,15E,19Z-docosahexaenoic acid	DHA	15-LOX	0.001	0	0.001	0	11	6.522	3.254	35.598	Complete
17R-RvD1	7S,8R,17R-trihydroxy-4Z,9E,11E,13Z,15E19Z-docosahexaenoic acid	DHA	15-LOX	0.001	11	0.028	0	0	—	—	—	None

(Continued)

TABLE E2. *(Continued)*

Compound	Name	Precursor fatty acid	Pathway	LOD		LOQ		No. samples >LOQ	Median*	Min*	Max*	Analysis type†
				pg/μg protein	No. samples	pg/μg protein	No. samples		pg/μg protein			
18-HEPE	(±)-18-hydroxy-5Z,8Z,11Z,14Z,16E-eicosapentaenoic acid	EPA	15-LOX	0.014	11	0.028	0	0	—	—	—	None
19,20-DiHDPA	(±)19,20-dihydroxy-4Z,7Z,10Z,13Z,16Z-docosapentaenoic acid	DHA	EH	0.014	1	0.071	1	9	1.810	0.432	24.141	Quantitative
5,15-DiHETE	5S,15S-dihydroxy-6E,8Z,10Z,13E-eicosatetraenoic acid	AA	5-LOX	0.007	0	0.071	1	10	11.484	2.634	48.589	Complete
5,6-DiHETrE	(±)5,6-dihydroxy-8Z,11Z,14Z-eicosatrienoic acid	AA	EH	0.014	11	0.028	0	0	—	—	—	None
5-HEPE	(±)-5-hydroxy-6E,8Z,11Z,14Z,17Z-eicosapentaenoic acid	EPA	5-LOX	0.014	11	0.028	0	0	—	—	—	None
5-HETE and 14(15)-EET	(±)5-hydroxy-6E,8Z,11Z,14Z-eicosatetraenoic acid and (±)14(15)-epoxy-5Z,8Z,11Z-eicosatrienoic acid	AA	5-LOX; CYP	0.001	11	0.001	0	0	—	—	—	None
6a-PG I1	6R,9α-epoxy-11α,15S-dihydroxy-prost-13E-en-1-oic acid	AA	COX	0.003	11	0.028	0	0	—	—	—	None
6-keto-$PGF_1\alpha$	6-oxo-9α,11α,15S-trihydroxy-prost-13E-en-1-oic acid	AA	COX	0.007	11	0.028	0	0	—	—	—	None
7(epi)Maresin R1	7S,14S-dihydroxy-4Z,8E,10E,12Z,16Z,19Z-docosahexaenoic acid	DHA	12-LOX	0.014	11	0.028	0	0	—	—	—	None
7(R)Maresin	7R,14S-dihydroxy-4Z,8E,10E,12Z,16Z,19Z-docosahexaenoic acid	DHA	12-LOX	0.014	11	0.028	0	0	—	—	—	None
8(9)-EET	(±)8,9-epoxy-5Z,11Z,14Z-eicosatrienoic acid	AA	CYP	0.071	11	0.071	0	0	—	—	—	None
8-HETE	(±)8-hydroxy-5Z,9E,11Z,14Z-eicosatetraenoic acid	AA	ROS	0.001	0	0.001	0	11	0.368	0.110	0.777	Complete
8-iso-15R-$PGF_2\alpha$	9α,11α,15R-trihydroxy-(8β)-prosta-5Z,13E-dien-1-oic acid	AA	ROS	0.071	11	0.071	0	0	—	—	—	None
8-iso-$PGF_2\alpha$	9α,11α,15S-trihydroxy-(8β)-prosta-5Z,13E-dien-1-oic acid	AA	ROS	0.028	11	0.028	0	0	—	—	—	None
9(10)-EpOME	(±)9,10-epoxy-12Z-octadecenoic acid	LA	CYP	0.014	11	0.028	0	0	—	—	—	None
9,10-DiHOME	(±)9,10-dihydroxy-12Z-octadecenoic acid	LA	EH	0.003	0	0.014	3	8	0.153	0.050	1.871	Complete
9-HODE	(±)-9-hydroxy-10E,12Z-octadecadienoic acid	LA	NA	0.007	11	0.007	0	0	—	—	—	None
9-OxoODE	9-oxo-10E,12Z-octadecadienoic acid	LA	NA	0.014	11	0.028	0	0	—	—	—	None
Carb-TBX A2	9α,11α-methylene-15S-hydroxy-11a-deoxy-11a-methylene-thromba-5Z,13E-dien-1-oic acid	AA	COX	0.007	11	0.007	0	0	—	—	—	None
LTB4	5S,12R-dihydroxy-6Z,8E,10E,14Z-eicosatetraenoic acid	AA	5-LOX	0.028	10	0.028	0	1	0.756	0.756	0.756	None
LTD4	5S-hydroxy-6R-(S-cysteinylglycinyl)-7E,9E,11Z,14Z-eicosatetraenoic acid	AA	5-LOX/GST	0.001	10	0.028	0	1	0.134	0.134	0.134	None
LTE4	5S-hydroxy-6R-(S-cysteinyl)-7E,9E,11Z,14Z-eicosatetraenoic acid	AA	5-LOX/GST	0.003	10	0.028	1	0	—	—	—	None

(Continued)

TABLE E2. *(Continued)*

Compound	Name	Precursor fatty acid	Pathway	LOD		LOQ		No. samples >LOQ	Median*	Min*	Max*	Analysis type†
				pg/μg protein	No. samples	pg/μg protein	No. samples		pg/μg protein			
LXA4 isomers	5S,6R,15S-trihydroxy-7E,9E,11Z,13E-eicosatetraenoic acid and 5(S),6(R),15(R)-trihydroxy-7E,9E,11Z,13E-eicosatetraenoic acid	AA	5-LOX	0.001	1	0.001	0	10	0.465	0.119	3.222	Quantitative
LXB4	5S,14R,15S-trihydroxy-6E,8Z,10E,12E-eicosatetraenoic acid	AA	5-LOX	0.014	1	0.028	0	10	4.356	0.978	28.343	Quantitative
PGD2	9α,15S-dihydroxy-11-oxo-prosta-5Z,13E-dien-1-oic acid	AA	COX	0.001	7	0.001	0	4	0.338	0.155	1.734	Present/absent
PGE2	9-oxo-11α,15S-dihydroxy-prosta-5Z,13E-dien-1-oic acid	AA	COX	0.007	0	0.028	0	11	0.676	0.279	1.960	Complete
$PGF_2\alpha$ isomers	9α,11α,15S-trihydroxy-prosta-5Z,13E-dien-1-oic acid and 9α,11α,15R-trihydroxy-prosta-5Z,13E-dien-1-oic acid	AA	COX	0.001	0	0.001	0	11	0.332	0.146	0.899	Complete
RvD1	7S,8R,17S-trihydroxy-4Z,9E,11E,13Z,15E,19Z-docosahexaenoic acid	DHA	15-LOX	0.001	1	0.071	8	2	0.320	0.093	0.547	Quantitative
RvD2	7S,16R,17S-trihydroxy-4Z,8E,10Z,12E,14E,19Z-docosahexaenoic acid	DHA	15-LOX	0.014	1	0.028	0	10	0.936	0.341	3.947	Quantitative
RvD3	4S,11R,17S-trihydroxy-5Z,7E,9E,13Z,15E,19Z-docosahexaenoic acid	DHA	15-LOX	0.007	11	0.014	0	0	—	—	—	None
RvD5	7S,17S-dihydroxy-4Z,8E,10Z,13Z,15E,19Z-docosahexaenoic acid	DHA	15-LOX	0.014	0	0.028	1	10	5.096	2.384	14.380	Complete
RvE1	5S,12R,18R-trihydroxy-6Z,8E,10E,14Z,16E-eicosapentaenoic acid	EPA	CYP/5-LOX	0.007	11	0.014	0	0	—	—	—	None
TXB2	9α,11,15S-trihydroxythromba-5Z,13E-dien-1-oic acid	AA	COX	0.014	2	0.028	0	9	0.235	0.079	1.340	Quantitative

LOD, Limit of detection; *LOQ,* limit of quantification.

*Median, minimum, and maximum reported for samples with analyte levels detectable over the LOQ.

†Analysis type determined by number of samples at or above the LOQ: complete = 11, quantitative = 8-10, presence/absence = 4-7, none = 0-3.