Untargeted metabolomic analysis reveals different metabolites associated with response to mepolizumab and omalizumab in asthma

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Abstract

Background There is limited evidence on biomarkers associated with response to the monoclonal antibodies currently approved for asthma treatment. We sought to identify circulatory metabolites associated with response to treatment with mepolizumab or omalizumab.

Methods We conducted global metabolomic profiling of pre-treatment plasma samples from 100 patients with moderate-to-severe asthma who initiated mepolizumab (n=31) or omalizumab (n=69). The primary outcome was the change in exacerbations within 12 months of therapy. Negative binomial models were used to assess the association between each metabolite and exacerbations, adjusting for age, sex, body mass index, baseline exacerbations and inhaled corticosteroid use. Chemical similarity enrichment analysis (ChemRICH) was conducted to identify chemical subclasses associated with treatment response.

Results The mean age of the mepolizumab group was 58.7 years with on average 2.9 exacerbations over the year prior to initiation of biologic therapy. The mean age in the omalizumab group was 48.8 years with 1.5 exacerbations in the preceding year. Patients with higher levels of two tocopherol metabolites were associated with more exacerbations on mepolizumab (δ-carboxyethyl hydroxychroman (CEHC) (p=2.65E-05, false discovery rate (FDR=0.01) and δ-CEHC glucuronide (p=2.47E-06, FDR=0.003)). Higher levels of six androgenic steroids, three carnitine metabolites and two bile acid metabolites were associated with decreased exacerbations in the omalizumab group. In enrichment analyses, xanthine metabolites (cluster FDR=0.0006) and tocopherol metabolites (cluster FDR=0.02) were associated with worse mepolizumab response, while androgenic steroids (cluster FDR=1.9E-18), pregnenolone steroids (cluster p=3.2E-07, FDR=1.4E-05) and secondary bile acid metabolites (cluster p=0.0003, FDR=0.006) were the top subclasses associated with better omalizumab response.

Conclusion This study identifies distinct metabolites associated with response to mepolizumab and omalizumab, with androgenic steroids associated with response to both mepolizumab and omalizumab.

Introduction

The increase in high throughput technologies, including for metabolomics, has provided opportunities to improve clinical biomarker discovery. For patients with severe uncontrolled asthma, who bear a disproportionate amount of asthma-related healthcare burden [1, 2], metabolomics provides an opportunity to identify biomarkers to the six monoclonal antibodies currently approved for the treatment of asthma [3].

While these monoclonal antibodies target different pathways, there is a high overlap in the clinical phenotype of patients who are eligible for these therapies presenting a challenge to providers in selecting the optimal biologic therapy [3–5]. This, and the limited predictive power of currently available clinical biomarkers of response to these biologics, has limited our ability to optimise the value of these expensive therapies.

Omalizumab and mepolizumab are two of the most prescribed respiratory biologics worldwide. Omalizumab was the first respiratory biologic approved in the USA and is approved for the treatment of moderate-to-severe persistent allergic asthma [3]. Mepolizumab, an anti-interleukin-5 (IL-5) antibody, is effective in eosinophilic asthma [3]. While patients with elevated type 2 "T2" biomarkers, such as eosinophils, at baseline may be more likely to demonstrate great response to these therapies, many patients with T2 asthma who meet eligibility criteria for these therapies have poor therapeutic response. This is unsurprising given that asthma is a highly heterogeneous disease reflecting an interplay between genes, diet and environment [6–8]. Circulating metabolites could reflect this interplay, underlying asthma pathophysiology [9–13], and thus may help in evaluating factors associated with differential response to omalizumab and mepolizumab.

Metabolites can be associated with asthma onset, phenotype and/or severity as well as drug response [11, 12, 14–18]. Patients with asthma demonstrate dysregulation in multiple pathways, including the tryptophan pathway, bile acids pathway, xanthine, eicosanoids and steroid pathways, and some of these associations are independent of disease severity and/or corticosteroid use [10]. Furthermore, multiple gut microbial metabolites have been shown to be associated with asthma and/or its severity [12, 14]. However, to our knowledge, no studies have evaluated the impact of metabolites on response to these biologic treatments. We hypothesise that circulating metabolites in pathways previously shown to be associated with asthma pathophysiology will be associated with response to omalizumab and/or mepolizumab. We explored the association between the baseline metabolome and change in exacerbations after initiation of omalizumab or mepolizumab.

Methods

Study population and follow-up

We identified patients seen within the Mass General Brigham (MGB) system with a diagnosis of asthma as defined by the International Classification of Diseases (ICD) version 9 or 10 between 2015 and 2022. From this cohort, we identified patients who had at least two prescriptions for omalizumab or mepolizumab. The index date was defined as the earliest date of prescription of either of these biologics. A patient was categorised based on the first biologic they received. We excluded patients receiving omalizumab who had chronic urticaria and patients on mepolizumab with hypereosinophilic syndrome or eosinophilic granulomatosis with polyangiitis. Thereafter, we identified patients who had plasma samples drawn prior to initiation of the first biologic within the MGB Biobank. The MGB Biobank has been previously described [18, 19]. Briefly, it stores, per standard storage guidelines, serum, plasma and DNA samples from patients who received care within the MGB system and who consented to donate blood for research purposes. This was a retrospective non-experimental study approved by the MGB IRB and conformed to the guiding principles of Human Subjects Research [20].

Baseline clinical variables and outcome

We evaluated the age, sex, race, body mass index (BMI), smoking status, history of allergic rhinitis, eosinophil count, total immunoglobulin E (IgE) level and annualised exacerbation rate within 1 year of initiation of biologic therapy. In addition, we evaluated the use of inhaled corticosteroids (ICS) and other maintenance asthma medications and the burden of use, which we defined as the number of ICS prescriptions during the past 3 years prior to biologic therapy initiation [10]. Our outcome of interest was the change in exacerbations in the 12 months following biologic initiation. An exacerbation was deemed to have occurred if an individual had an emergency room visit or a hospitalisation with a primary diagnostic code of asthma or if a patient had an urgent care visit with a new prescription for oral corticosteroids for 3 or more days [21].

Metabolomic profiling

Metabolomic profiling was conducted by Metabolon Inc. (Morrisville, NC, USA) using four complementary non-targeted liquid chromatography mass spectroscopy (LC-MS) platforms and a targeted platform. Metabolite peaks are quantified using area-under-the-curve. Raw area counts for each metabolite in each sample are normalised to correct for variation resulting from instrument inter-day tuning differences by the median value for each run-day, therefore, setting the medians to 1.0 for each run. Metabolites are identified by automated comparison of the ion features in the experimental samples to a reference library of ~8000 chemical standard entries that include retention time, molecular weight (m/z), preferred adducts and in-source fragments as well as associated MS spectra, and curated by visual

inspection for quality control using software developed at Metabolon, Inc. [22]. These platforms extensively cover amines, amino acids and acylcarnitines, polar and non-polar lipids, free fatty acids and bile acids. Quality assurance and control processes were conducted as per standard operating procedures and guidelines for metabolomics profiling [23]. Metabolites were identified by matching to a reference database and confirmed by a targeted assay or by mass spectroscopy. We excluded metabolites with ⩾75% missingness and subsequently imputed missing values for the remaining metabolites using half-minimum approach, log-10 transformed and pareto-scaled.

Statistical analysis

We modelled the association between metabolites and the number of exacerbations in the 12 months after biologic initiation using a negative binomial regression for each biologic. The baseline metabolite value was an independent variable and we adjusted for age, sex, BMI, ICS prescription count and baseline annualised exacerbation rate, as well as time from sample collection to biologic initiation. We restricted the analysis to identifiable metabolites only (n=1150). Then, we conducted ChemRICH set enrichment analysis to identify pathways associated with response [24]. The ChemRICH analysis utilised the one-sided Kolmogorov–Smirnov test on the uncorrected p-value distribution of metabolites in the same set based on chemical similarity. Only metabolites with known simplified molecular-input line-entry system (SMILES) were included in the ChemRICH analysis (n=1119) [24]. Multiple-hypothesis testing control was performed using Benjamini–Hochberg false discovery rate (FDR) correction [25]. Metabolites with uncorrected p-value of <0.05 were evaluated in exploratory analyses. Hierarchical clustering was performed using the *hclust* function in R based on the Euclidean distance and the Ward clustering of log10-transformed and pareto-scaled metabolite abundance.

Results

Clinical phenotype and characterisation of biologic initiators

100 individuals with asthma met the eligibility criteria. Of the 100 patients, 31 initiated mepolizumab and 69 initiated omalizumab. There were more females than males in both groups: mepolizumab (71.0%) and omalizumab (87.0%) and the BMI was on average obese in both groups. The mepolizumab group had a mean age of 58.7 years, median eosinophil count of 346 cells·μL^{-1} and median IgE of 204 IU μL^{-1}. The omalizumab group had a mean age of 48.8 years, median eosinophil count of 127 cells·μL^{-1} and median IgE of 203 IU·μL^{-1}. In the year prior to treatment initiation, patients in the omalizumab group had on average 1.5 exacerbations and those in the mepolizumab group had 2.9 exacerbations (table 1).

Response (change in exacerbations) to mepolizumab and its association with baseline metabolite levels

Over the first year following the initiation of mepolizumab, the exacerbation rate reduced from 2.9 to 2.2 (rate ratio 0.79, 95% confidence interval (CI) 0.58–1.09; p=0.15), while the number of patients who

TABLE 1 Baseline characteristics of participants		
Characteristics	**Mepolizumab**	**Omalizumab**
Patients n	31	69
Age years, mean±SD	58.7±11.5	48.8±16.5
Female, n (%)	22 (71.0)	60 (87.0)
White race, n (%)	26 (83.9)	52 (75.4)
Current smoker, n (%)	1 (3.2)	5 (7.2)
Former smoker, n (%)	10 (32.3)	10 (14.5)
Body mass index kg·m^{-2}, mean±SD	29.0±7.1	31.4±8.7
Exacerbation rate in the prior year, mean±SD	2.9±2.1	1.5±1.5
Inhaled corticosteroid use, n (%)	31 (100.0)	69 (100.0)
Long-acting β-agonist use, n (%)	16 (51.6)	32 (46.4)
Long-acting muscarinic antagonist use, n (%)	12 (38.7)	11 (15.9)
Leukotriene receptor antagonist use, n (%)	14 (45.2)	34 (49.3)
Oral corticosteroid prescription ⩾6 months in the prior year, n (%)	3 (9.7)	0 (0.0)
Blood eosinophil count cells·μL^{-1}, median (IQR)	346 (176–436)	127 (100–186)
Immunoglobulin E IU·μL^{-1}, median (IQR)	204 (47–390)	203 (69–327)
Allergic rhinitis, n (%)	26 (83.9)	64 (92.8)[#]

IQR: interquartile range. [#]: all patients had physician-reported allergic rhinitis though diagnostic code of allergic rhinitis not included in chart.

experienced at least one exacerbation reduced from 27 to 20 (risk ratio 0.74, 95% CI 0.55–0.99; p=0.04). Of 1150 metabolites, 123 metabolites were associated with exacerbation response (uncorrected p<0.05): 50 were associated with decreased number of exacerbations and 73 with increased exacerbations (figure 1a and supplementary table S1). Four metabolites remained statistically significant after FDR correction. These included two tocopherol metabolites, δ-carboxyethyl hydroxychroman (CEHC) (FDR=0.01) and δ-CEHC glucuronide (FDR=0.0028), which were associated with a poorer response to mepolizumab (higher exacerbations while on mepolizumab) and two partially characterised bilirubin degradation products, $C_{16}H_{18}N_2O_5$ (1) and C16H18N2O5 (2) (FDR of 0.005 and 0.04, respectively), which were associated with better response to mepolizumab (fewer exacerbations while on mepolizumab). In exploratory analyses, the top 30 significant metabolites were from different chemical classes including amino acids, cofactors and vitamins, energy-related metabolites, lipids, nucleotides, peptides, xenobiotics and partially characterised molecules. Among the top five metabolites, cyclo(pro-val), a diketopiperazine, was associated with increased on-treatment exacerbations (uncorrected p<0.001) but did not meet the FDR threshold (FDR=0.18). Kynurenate, a tryptophan metabolite (uncorrected p=0.002), and 3-methylxanthine, a xanthine metabolite (uncorrected p=0.009), were associated with higher exacerbations on mepolizumab, but not after FDR correction (figure 1b). Hierarchical clustering of the top 30 metabolites associated with on-treatment exacerbations showed a distinct pattern of clustering and distinguished patients who had increased post-mepolizumab exacerbations from those who had decreased exacerbations on treatment (supplementary figure S1). For four metabolites with FDR<0.05, there was no substantial difference between sexes (supplementary figure S2), while the direction of association when categorised by number of post-treatment exacerbations was consistent with the main analysis (supplementary figure S3).

There were 10 chemical subclasses associated with mepolizumab response in ChemRICH analysis (cluster uncorrected p<0.05). After FDR correction, five subclasses remained significant including two subclasses associated with increased exacerbations, xanthine metabolites with a ChemRICH cluster uncorrected p=7.1×10^{-6} (FDR=6.1×10^{-4}) and tocopherol metabolites with uncorrected p=7.9×10^{-4} (FDR=0.017), two subclasses associated with decreased exacerbations, androgenic steroids with uncorrected p=1.9×10^{-4} (FDR=0.0083) and pregnenolone steroids with uncorrected p=4.7×10^{-4} (FDR=0.013), and one subclass with mixed association, phosphatidylcholines (PC) with uncorrected p=0.0016 (FDR=0.028). In addition, polyamine metabolites, glutamate metabolites and γ-glutamyl amino acids were associated with increased exacerbations. Meanwhile, methionine, cysteine, S-adenosylmethionine (SAM), and taurine metabolites and tryptophan metabolites showed a mixed association (figure 2 and supplementary table S2).

Response (change in exacerbations) to omalizumab and its association with baseline metabolite levels

Over the first year following the initiation of omalizumab, the exacerbation rate reduced from 1.5 to 1.4 (rate ratio 0.92, 95% CI 0.70–1.22; p=0.58), and the number of patients who experienced at least one exacerbation reduced from 44 to 35 (risk ratio 0.80, 95% CI 0.59–1.07; p=0.12). For omalizumab, most of the significant metabolites with uncorrected p<0.05 (95 out of 117) were associated with decreased on-treatment exacerbations, while 22 were associated with increased exacerbations (figure 3a and supplementary table S3). 17 metabolites remained significant after FDR correction. All were associated with decreased exacerbations, and of these, 15 were lipids: six androgenic steroids (androstenediol (3β,17β) disulfate, 5α-androstan-3β,17β-diol disulfate, androstenediol (3β,17β) monosulfate, androstenediol (3α, 17α) monosulfate, androsterone sulfate and dehydroepiandrosterone sulfate (DHEA-S)), three pregnenolone steroids (pregnenetriol disulfate, pregnen-diol disulfate and 21-hydroxypregnenolone disulfate), two carnitine metabolites (deoxycarnitine and carnitine), two secondary bile acid metabolites (deoxycholic acid 12-sulfate and tauroursodeoxycholic acid sulfate), one primary bile acid metabolite (glycochenodeoxycholate glucuronide) and one short chain acyl carnitine metabolite (acetylcarnitine (C2)). The other two were cysteine s-sulfate, a cysteine metabolite and 4-acetylcatechol sulfate, a xenobiotic. Among the top 30 metabolites, 25 (83%) were lipids and 10 (33%) were androgenic steroids. Only α-tocopherol, 1-palmitoyl-2-stearoyl-GPC (16:0/18:0) and 1-(1-enyl-palmitoyl)-GPC (p-16:0) were associated with increased post-omalizumab exacerbations but neither met the FDR-corrected threshold of <0.05 (figure 3b and supplementary table S3). Hierarchical clustering of the top 30 metabolites showed a distinct, yet imperfect, pattern comparing those with higher *versus* fewer exacerbations (supplementary figure S4). Among the top five metabolites with FDR <0.05, only 5α-androstan-3β,17β-diol disulfate, an androgenic steroid, was higher in males than females (supplementary figure S5), while the direction of association when categorised by post-omalizumab exacerbation counts was consistent with the main analysis (supplementary figure S6).

Of the 10 chemical subclasses associated with exacerbations (cluster uncorrected p<0.05), seven subclasses including androgenic steroids, pregnenolone steroids, secondary bile acid metabolites, leucine, isoleucine and valine metabolites, acetylated peptides, urea cycle-related arginine and proline metabolites, and
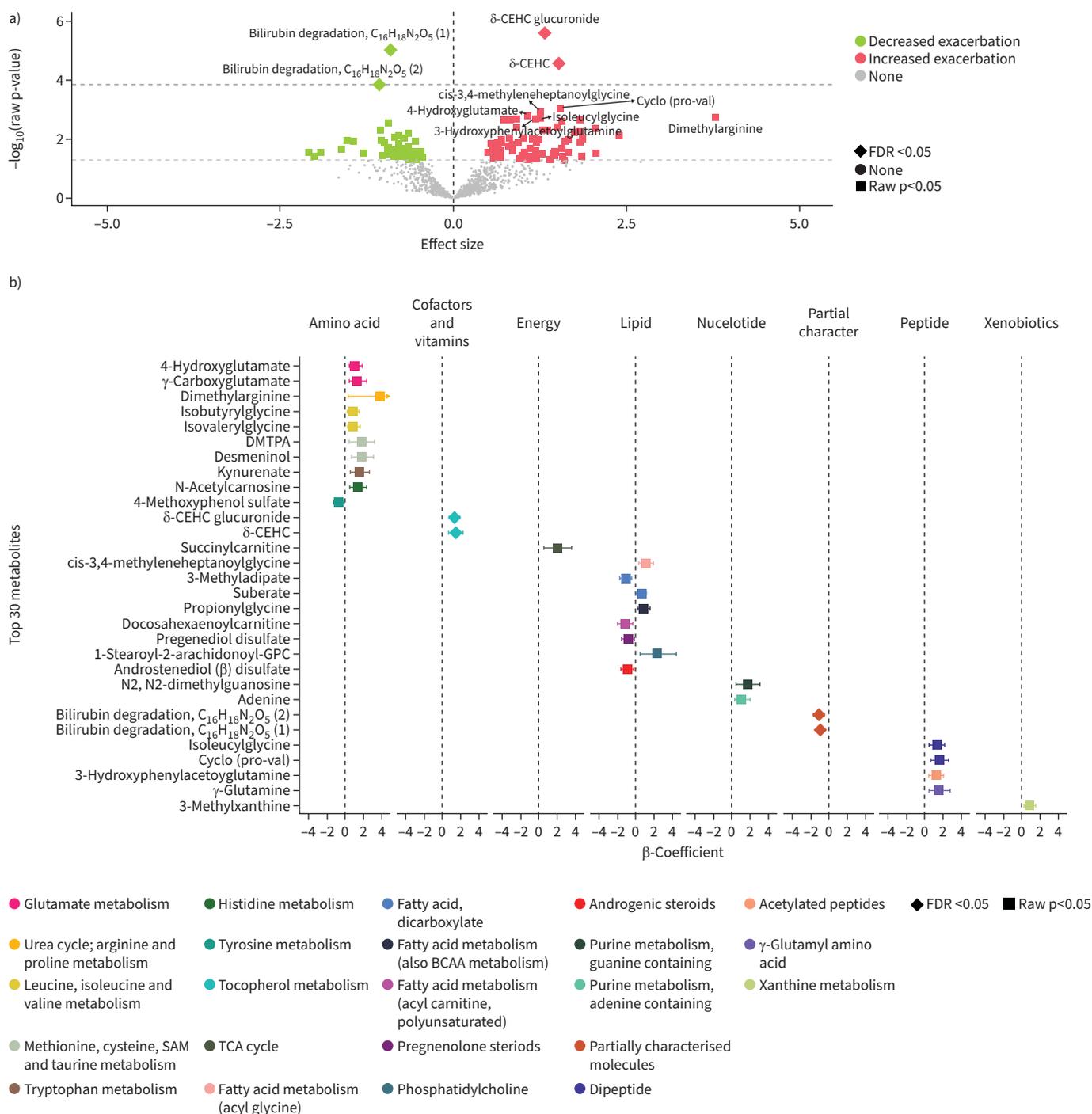
FIGURE 1 Metabolites associated with change in exacerbations on mepolizumab. a) Volcano plots showing the association of each metabolite with change in exacerbations on mepolizumab adjusted by age, sex, body mass index, inhaled corticosteroid prescription counts, pre-mepolizumab annualised exacerbation rate and time from sample collection to mepolizumab initiation. Only known metabolites (n=1150) were included in this analysis. Effect sizes were presented as β-coefficients, where positive/negative values indicate association with increased/decreased post-omalizumab exacerbation. The vertical dashed lines represent no difference. The grey horizontal dashed line represents raw p-value <0.05. The black horizontal dashed line represents a false discovery rate (FDR) of <0.05. b) Forest plots showing the top 30 metabolites associated with change in exacerbations on mepolizumab with metabolites ranked within each chemical class by p-value. Diamonds indicate metabolites with significant association after FDR correction. Squares indicate metabolites with significant association before, but not after, FDR correction. CEHC: carboxyethyl hydroxychroman; SAM: S-adenosylmethionine.
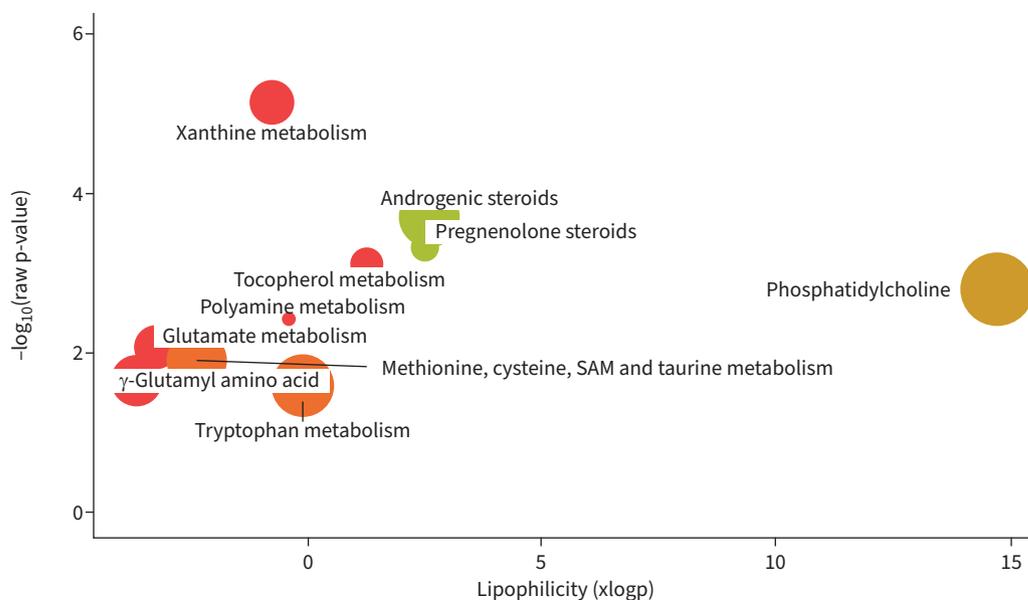
FIGURE 2 Chemical subclasses associated with change in exacerbations on mepolizumab. Chemical similarity enrichment analysis shows statistically significant baseline chemical classes associated with post-mepolizumab annualised exacerbation rate. The chemical classes are ranked by chemical class significance level and chemical lipophilicity. Only known metabolites with identified simplified molecular-input line-entry system are included in this analysis (n=1119). Green or red circles indicate the chemical class associated with decreased or increased exacerbations respectively, and orange circles indicate the chemical class with mixed association. Circle size indicates the chemical class size. SAM: S-adenosylmethionine.

phenylalanine metabolites were solely associated with fewer exacerbations while dihydroxy fatty acid metabolites, adenine-containing purine metabolites, and nicotinate and nicotinamide metabolites were mixed. After FDR correction, androgenic steroids, pregnenolone steroids, dihydroxy fatty acid metabolites, secondary bile acid metabolites, and leucine, isoleucine and valine metabolites remained statistically significant (figure 4 and supplementary table S4).

Metabolites associated with both mepolizumab and omalizumab response

There were 20 overlapping metabolites which were associated (uncorrected $p<0.05$) with exacerbations in both the mepolizumab and omalizumab groups (figure 5). Of the 20 overlapping metabolites, 10 were lipid metabolites and had the same direction of association for both the mepolizumab and the omalizumab groups. This included multiple androgenic steroids, such as androstenediol monosulfate, androstenediol disulfate and DHEA-S which were associated with fewer on-treatment exacerbations and remained significant in the omalizumab group after FDR correction (figure 5). The dipeptide isoleucyl-glycine, 1-palmitoyl-2-stearoyl-GPC (16:0/18:0), propionylglycine and 2-palmitoleoyl-GPC (16:1) were associated with more exacerbations in both groups. Nine of the overlapping metabolites were associated with higher exacerbations in the mepolizumab group. These were mostly amino acid metabolites, including the tryptophan metabolite, N-acetylkynurenine, but included other metabolites, including α-ketoglutarate, a key regulator in the Krebs cycle (figure 5) [26].

Discussion

In this single centre metabolomics study, we evaluated the association between the baseline levels of 1150 metabolites and reductions in asthma exacerbations in 100 patients with moderate-to-severe asthma who received mepolizumab or omalizumab. After FDR correction, we found two tocopherol metabolites, δ-CEHC and δ-CEHC glucuronide, to be associated with a higher rate of exacerbations on mepolizumab. In enrichment analyses, higher levels of xanthine and tocopherol metabolites were associated with higher on-treatment exacerbations in the mepolizumab group. Higher levels of cysteine s-sulfate, multiple androgenic steroids, pregnenolone steroids and carnitine metabolites were significantly associated with fewer exacerbations on omalizumab. In enrichment analysis, androgenic steroids showed significant association with lower on-treatment exacerbation rate for both mepolizumab and omalizumab initiators.
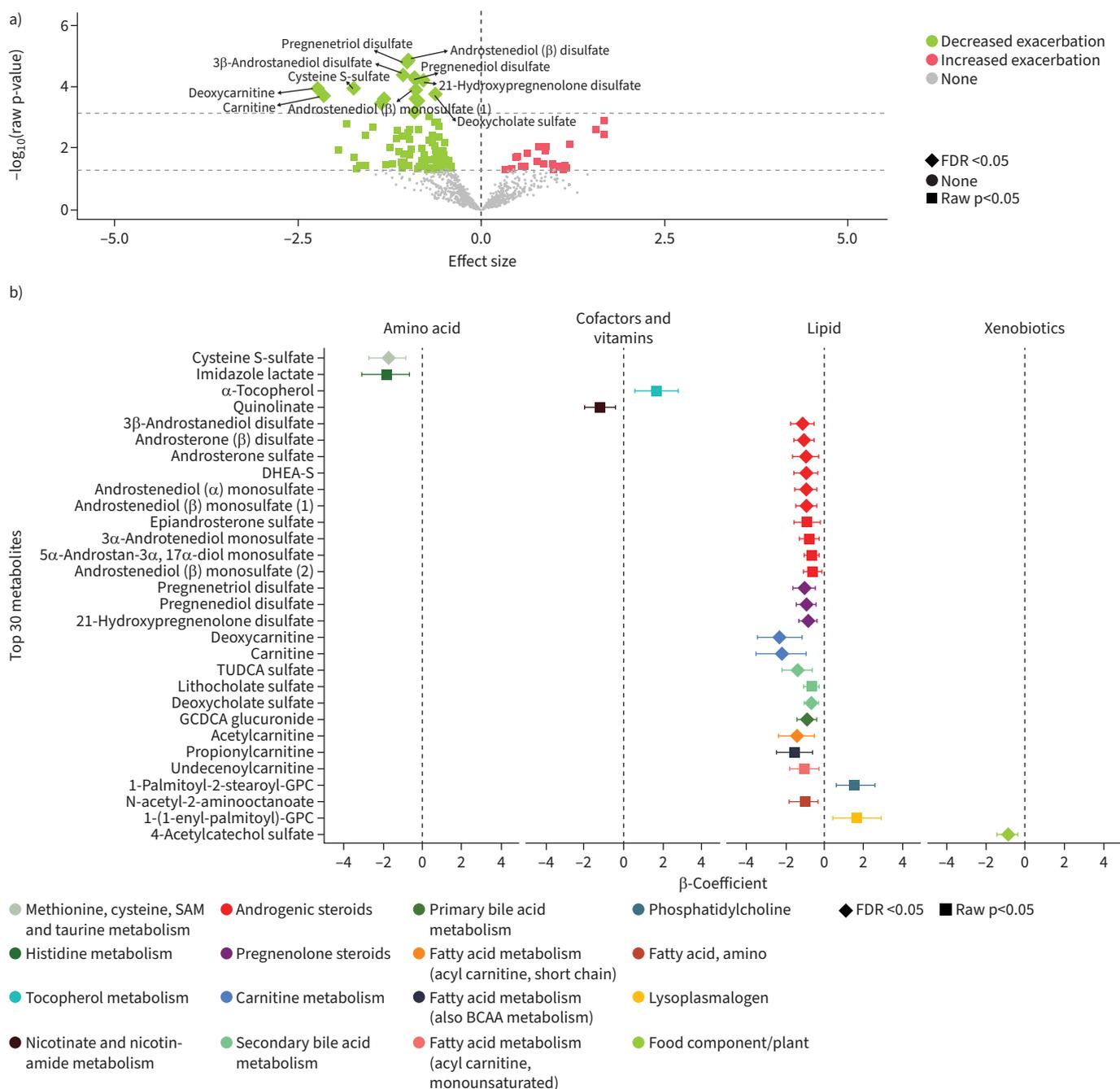
FIGURE 3 Metabolites associated with change in exacerbations on omalizumab. a) Volcano plots showing the association of each metabolite with change in exacerbations on omalizumab adjusted by age, sex, body mass index, inhaled corticosteroid prescription counts, pre-omalizumab annualised exacerbation rate and time from sample collection to omalizumab initiation. Only known metabolites (n=1150) were included in this analysis. Effect sizes were presented as β-coefficients where positive/negative values indicate an association with increased/decreased post-omalizumab exacerbation. The vertical dashed lines represent no difference. The grey horizontal dashed line represents raw $p < 0.05$. The black horizontal dashed line represents false discovery rate (FDR) <0.05. b) Forest plots showing the top 30 metabolites associated with change in exacerbations on omalizumab with metabolites ranked within each chemical class by p-value. Diamonds indicate metabolites with significant association after FDR correction. Squares indicate metabolites with significant association before, but not after FDR correction. DHEA: dehydroepiandrosterone sulfate; TUDCA: tauroursodeoxycholic acid; SAM: S-adenosylmethionine.

Some of the metabolite associations trended in opposite directions between the mepolizumab and omalizumab groups. These included N-acetylkynurenine and cyclo(pro-val), which were associated with higher exacerbations on mepolizumab and lower exacerbations on omalizumab.
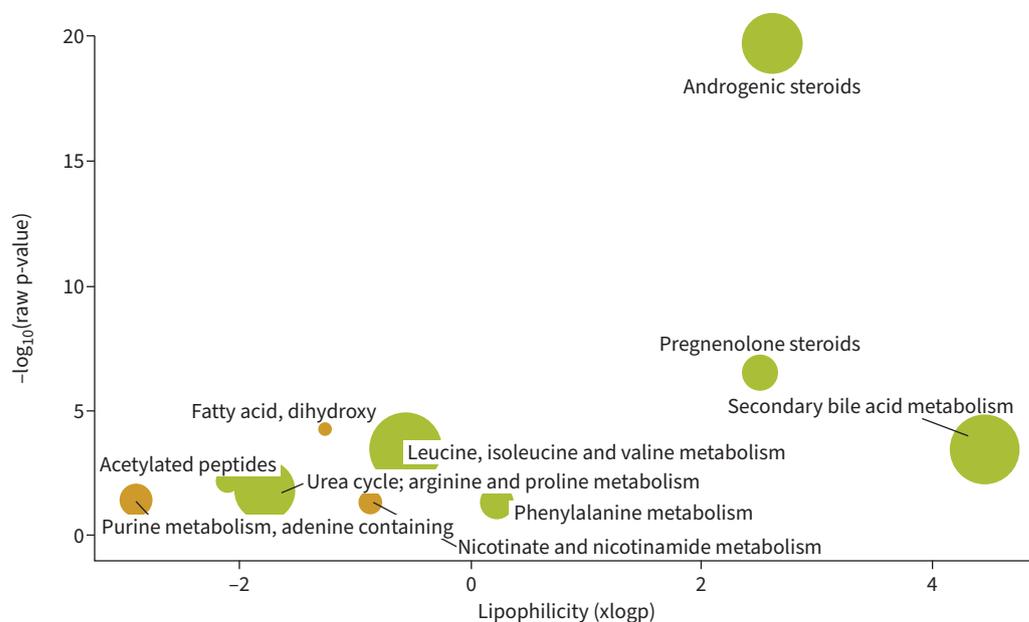
FIGURE 4 Chemical subclasses associated with change in exacerbations on omalizumab. Chemical similarity enrichment analysis shows statistically significant baseline chemical classes associated with post-omalizumab annualised exacerbation rate. The chemical classes are ranked by chemical class significance level and chemical lipophilicity. Only known metabolites with identified simplified molecular-input line-entry system are included in this analysis (n=1119). Green or red circles indicate the chemical class associated with decreased or increased exacerbations respectively, and orange circles indicate the chemical class with mixed association. Note: for this, no class was associated with increased exacerbations (red). Circle size indicates the chemical class size.

Androgens and androgen receptor expression have been postulated as underlying sex differences in asthma incidence [27–29]. Androgenic steroids inhibit the differentiation and reduce the number of ILC2 cells [30, 31], which play a key role in asthma pathogenesis by releasing type 2 cytokines, IL-5 and IL-13, following activation by alarmins [32]. Likewise, testosterone, a main androgenic steroid, could alleviate airway inflammation in asthma by reducing IL-4, IL-13 and IL-17A *via* androgen receptor signalling [33]. Additionally, low androgen receptor expression is associated with increased asthma severity [28], and children prone to respiratory infections, a common trigger for asthma exacerbations, demonstrate lower levels of androgenic steroids [34]. Thus, patients with higher androgens may represent individuals with particularly active type-2 inflammation in the airways who would benefit from T2-targeted therapies. However, this might also reflect patients with more severe disease with increased adrenal suppression from exogenous corticosteroid use. A previous study had shown that even at low doses, ICS use in patients with asthma could lead to widespread reductions in blood endogenous steroid metabolites and clinical features suggestive of adrenal suppression [10]. Similarly, in the U-BIOPRED study, high doses of ICS were also associated with greater reductions of urinary endogenous steroid metabolites compared to low ICS doses [35]. Other studies of metabolome changes in children and adults with asthma have also shown reductions in pregnenolone, a precursor for androgens, [36] and reduced endogenous steroids as an indicator of ongoing adrenal suppression [37].

Our findings corroborate prior studies. Decreased urinary carnitine levels were previously shown to have the strongest association with asthma severity and reflect mitochondrial dysfunction in the U-BIOPRED study [38]. Although, in the same study, urinary carnitine levels were unchanged in the omalizumab group compared to patients with severe asthma not on biologics. However, blood carnitine levels may play a role in omalizumab response. In our study, omalizumab users with higher baseline carnitine levels experienced fewer exacerbations on omalizumab. This might reflect individuals with lower inherent asthma severity due to milder disruption of mitochondrial function. A similar direction of association was also observed in mepolizumab users, though this was not statistically significant after FDR correction. Consistent with prior studies evaluating metabolites' associations with asthma that have found associations with bile acids, we found tauroursodeoxycholic acid (TUDCA) sulfate, a secondary bile acid metabolite, to be associated with exacerbation reduction in the omalizumab group. TUDCA alleviates allergen-induced airway inflammation
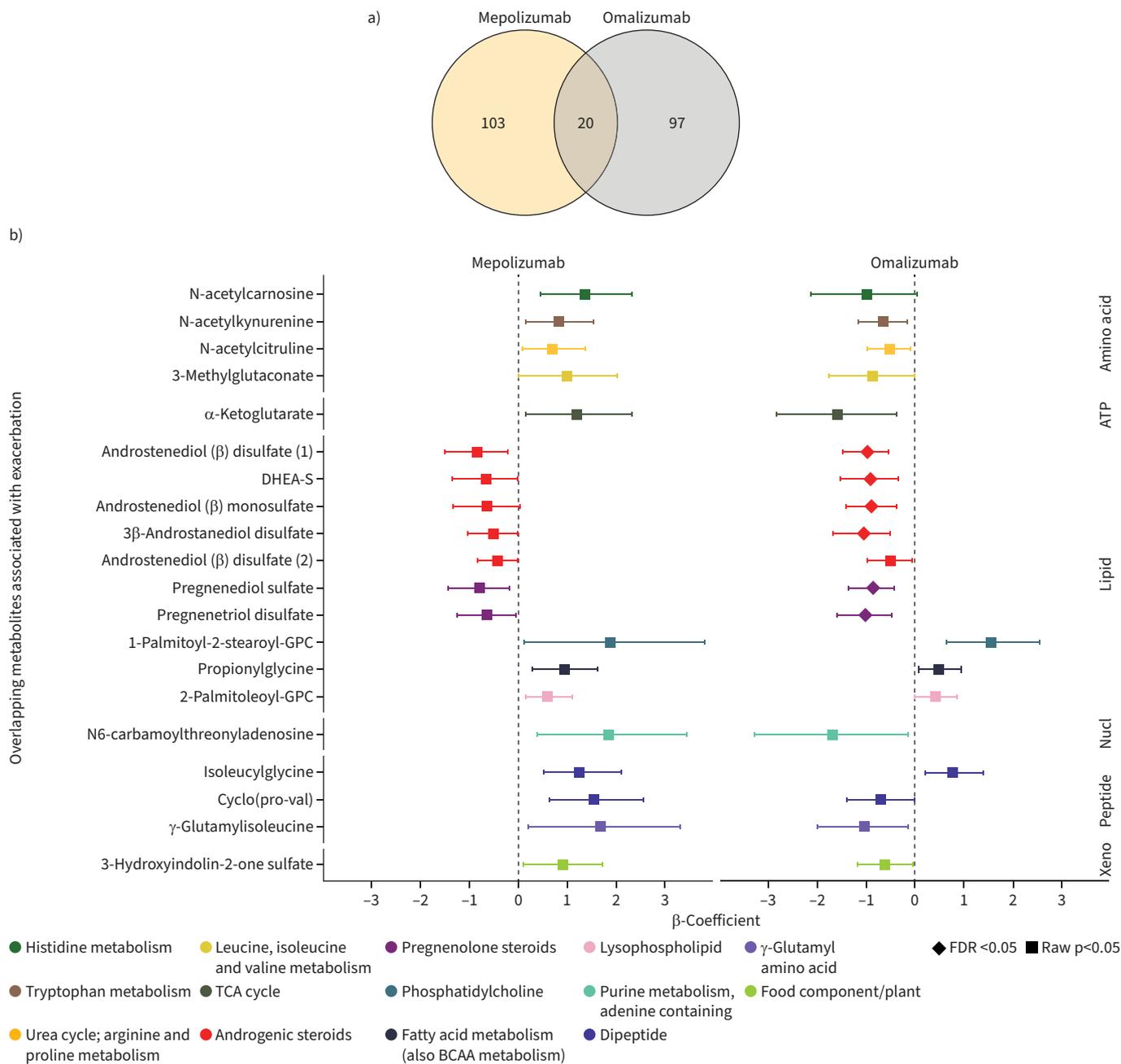
FIGURE 5 Overlapping metabolites associated with change in exacerbations in the mepolizumab and omalizumab groups. a) Venn diagram indicates the numbers of significant metabolites and overlapping metabolites. For each biologic initiator, the metabolites were ranked by p-value from negative binomial regression. Metabolites with raw $p < 0.05$ were considered statistically significant. Only known metabolites (n=1150) were included in this analysis. b) Forest plot of overlapping metabolites associated with change in exacerbations in both the mepolizumab and omalizumab groups. Effect sizes are presented as β-coefficients with 95% confidence intervals where positive/negative values indicate association with increased/decreased post-biologic exacerbation. Squares indicate metabolites with significant association before, but not after, false discovery rate (FDR) correction. The vertical dashed lines represent no difference. ATP: energy; Nucl: nucleotide; Xeno: xenobiotics; DHEA: dehydroepiandrosterone sulfate.

and hyperresponsiveness [39, 40], and its anti-inflammatory effects extend beyond airway inflammation to other inflammatory processes, with a prior study showing that TUDCA attenuates intestinal inflammation in NAFLD [41].

For mepolizumab, two tocopherol metabolites, δ- and γ-CEHC, which have been shown to block the neutrophil oxidative burst *via* protein kinase C inhibition, were associated with worse mepolizumab

response [42]. In murine models, γ-tocopherol, a metabolite of the antioxidant, vitamin E, has been shown to reduce allergen-induced inflammation [43, 44]. Thus, elevated δ- and γ-CEHC in the serum might reflect patients with concomitant non-atopic and neutrophilic asthma, a more severe asthma endotype [45], despite meeting criteria for eosinophilic asthma based on peripheral blood eosinophil counts. Unsurprisingly, these patients will not respond well to the anti-eosinophilic agent, mepolizumab, which would improve eosinophilic but not neutrophilic inflammation. This might also explain our finding that high levels of cyclo(pro-val), an anti-inflammatory diketopiperazine associated with reduced IL-1β, IL-6 and tumour necrosis factor-α level, was associated with poorer response to mepolizumab, though this was not significant after FDR correction [46]. High levels of cyclo(pro-val) at baseline might be reactive to ongoing non-T2 inflammation, or presence of T2-low asthma [47].

Elevated xanthine metabolites, which were present in patients with more exacerbations while on mepolizumab in ChemRICH analysis after FDR correction, might suggest higher baseline asthma severity. Prior studies have shown that xanthine metabolites are elevated following coffee, tea or chocolate intake [48], and patients with poorly controlled asthma may self-medicate with coffee for its temporary bronchodilatory effect [49]. Likewise, while not significant after FDR correction for each individual metabolite, we found the xanthine metabolites theobromine and 3-methylxanthine to be associated with worse improvement in exacerbations on mepolizumab. As theophylline and theobromine have a bronchodilatory effect in asthma patients [50], patients might self-medicate with coffee, tea or with dark chocolate, which is particularly high in theobromine [51]. Similarly, cyclo (pro-val), which we found to be associated with poorer response to mepolizumab, increases with habitual coffee intake [48]. This might also suggest confounding by disease severity.

We found contrasting relationships between N-acetylkynurenine and response to mepolizumab and omalizumab, though its association was not significant after FDR correction. It was associated with better response to omalizumab but worse mepolizumab response. This is consistent with a previous study in children with severe asthma which reported increased L-kynurenine, a tryptophan metabolite, in the urine of omalizumab responders [52]. Children with asthma, which is mostly of the allergic phenotype, have increased urinary kynurenate [53], and in a murine model, kynurenate had a synergistic effect with allergen immunotherapy leading to reduced eosinophil counts and Th2 cytokine levels [54]. Thus, N-acetylkynurenine and tryptophan metabolism as a whole may reflect the underlying asthma endotype and/or therapy and may be helpful in differentiating potential responders to omalizumab from responders to mepolizumab.

The strengths of this study include the use of a well-characterised clinical cohort and the evaluation of a question that is important to multiple stakeholders including patients, providers, payers and the health system. However, the results should be interpreted with caution. Patients prescribed these biologics may differ in ways not captured by clinical variables. Thus, there is the possibility of confounding by indication and some of the associations may reflect the different clinical phenotypes and disease severity, which might explain some of the associations particularly with steroid and xanthine metabolites, and underlying pathophysiology rather than the effect of these metabolites on therapeutic response. Secondly, this retrospective cohort was from a single healthcare system in the USA, had a small sample size and lacked information on several environmental variables that can affect metabolism, such as diet. Thus, we had limited power, and our results may not be generalisable, prompting the need for replication in larger cohorts and of a prospective cohort with comprehensive evaluation of patients' characteristics and exposures. Third, these associations might reflect medication use. We controlled for ICS use, the mainstay of treatment for persistent asthma, but patients might not be compliant with prescribed therapy. Moreover, there might be residual confounding by other asthma medications. Lastly, metabolites are dynamic, and patients might have been on different trajectories of their asthma when samples were collected. To address this last issue, we included time from sample collection to biologic initiation in our model and measures of disease severity but there might still be residual confounding. Also, this study aimed to evaluate the association between baseline metabolite levels and asthma-related exacerbations. Thus, we were unable to assess longitudinal changes in the metabolome due to the use of omalizumab or mepolizumab.

In conclusion, we identified unique and shared metabolites associated with the change in exacerbation rates in patients with moderate-to-severe asthma who received mepolizumab or omalizumab. Androgenic steroids were associated with fewer on-treatment exacerbations in both groups. Xanthine and tocopherol metabolites were associated with poorer response to mepolizumab, while secondary bile acid and carnitine metabolites were associated with better response to omalizumab. N-acetylkynurenine and cyclo(pro-val) were associated with poorer mepolizumab response, but greater response to omalizumab. This result presents potential biomarkers that might help biologic selection for asthma.

Ethics statement: This study was considered exempt by the institutional review board.

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