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ORIGINAL ARTICLE

The impact of high-IgE levels on metabolome and microbiome in experimental allergic enteritis

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Abstract

Background: The pathological mechanism of the gastrointestinal forms of food allergies is less understood in comparison to other clinical phenotypes, such as asthma and anaphylaxis Importantly, high-IgE levels are a poor prognostic factor in gastrointestinal allergies.

Methods: This study investigated how high-IgE levels influence the development of intestinal inflammation and the metabolome in allergic enteritis (AE), using IgE knock-in (IgEki) mice expressing high levels of IgE. In addition, correlation of the altered metabolome with gut microbiome was analysed.

Results: Ovalbumin-sensitized and egg-white diet-fed (OVA/EW) BALB/c WT mice developed moderate AE, whereas OVA/EW IgEki mice induced more aggravated intestinal inflammation with enhanced eosinophil accumulation. Untargeted metabolomics detected the increased levels of N-tau-methylhistamine and 2,3-butanediol, and reduced levels of butyric acid in faeces and/or sera of OVA/EW IgEki mice, which was accompanied with reduced Clostridium and increased Lactobacillus at the genus

The first three authors equally contributed to the work, and the last two authors are both corresponding authors. In addition, the last two authors also equally contributed to the works and share the last authorship.

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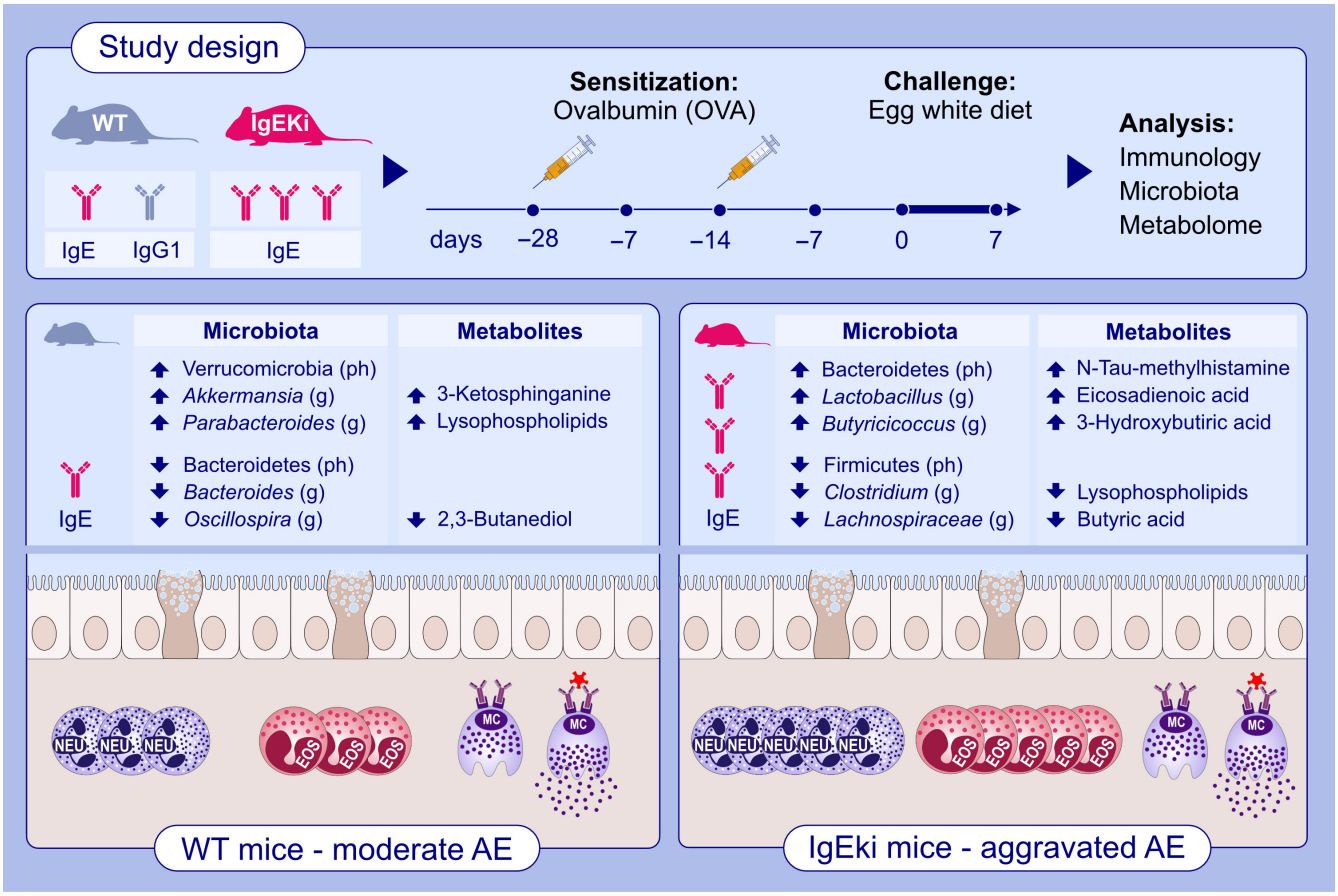
level. Non-sensitized and egg-white diet-fed (NC/EW) WT mice did not exhibit any signs of AE, whereas NC/EW IgEki mice developed marginal degrees of AE. Compared to NC/EW WT mice, enhanced levels of lysophospholipids, sphinganine and sphingosine were detected in serum and faecal samples of NC/EW IgEki mice. In addition, several associations of altered metabolome with gut microbiome—for example Akkermansia with lysophosphatidylserine—were detected.

Conclusions: Our results suggest that high-IgE levels alter intestinal and systemic levels of endogenous and microbiota-associated metabolites in experimental AE. This study contributes to deepening the knowledge of molecular mechanisms for the development of AE and provides clues to advance diagnostic and therapeutic strategies of allergic diseases.

KEYWORDS
food allergy, IgE, metabolomics, microbiome, microbiota, murine model



GRAPHICAL ABSTRACT

Distinctive metabolome and microbiome signatures were detected among different degrees of AE in WT mice and IgEki mice expressing high levels of IgE. IgEki mice exacerbated AE development with altered levels of lipids including sphingolipids, glycerophospholipids and fatty acids and histamine derivative. The exacerbated AE was accompanied with reduced *Clostridium* and increased *Lactobacillus*. These findings contribute to establishing AE diagnostics and therapies. Abbreviations: AE, allergic enteritis; g, genus; Ig, immunoglobulin; IgEki, IgE knock-in; ph, phylum; WT, wild type.

1 | INTRODUCTION

Food allergy causes clinical symptoms systemically and/or locally in cutaneous, respiratory, ocular and gastrointestinal tissues.[1–3] Allergic enteritis (AE) is a clinical manifestation of gastrointestinal food allergy.[4] AE mimics in mice some traits observed in patients with food allergy-associated intestinal inflammation characterized by thicker basal layer, crypt elongation, villous atrophy and granulocyte infiltration.[4–7] Although they are usually described as T-cell mediated allergies, patients with these pathologies often show detectable IgE to the offending food in the initial diagnosis or develop allergen-specific IgE during the course of the pathology.[6–9] Such patients, in particular those with high levels of IgE, tend to experience a prolonged and/or severe course of the disease, suggesting that IgE is a poor prognostic factor of AE.[10–14] Additionally, compared to other clinical phenotypes of food allergy such as atopic dermatitis, allergic asthma, urticaria and anaphylaxis, the pathological mechanism underlying gastrointestinal forms of allergy is not well understood, partly due to difficulty in the access to intestinal tissues.[14–17]

Notably, dysregulated metabolism has been suggested as a major factor not only in traditional metabolic syndromes but also in multiple inflammatory disorders including allergy.[18–20] Metabolomics, which studies the collection of small molecules involved in metabolism – called metabolites – has been used to identify biomarkers in the diagnosis and prediction of disease and to understand the role of metabolites as active drivers in pathological processes.[21–23] Metabolomic profiling of faeces samples collected from subjects with childhood asthma showed significant interrelationships between the intestinal metabolome and the intestinal microbiome, plasma metabolome and diet in association with childhood asthma.[24] Metabolomics of serum samples from paediatric patients with a history of severe systemic reactions and the presence of multiple type I food allergies revealed alterations in the levels of tryptophan metabolites, eicosanoids, glycerophospholipids and fatty acids.[25] However, metabolomics of gastrointestinal forms of food allergies, as well as the impact of IgE on the metabolome of intestinal tissues have not been fully investigated.

In this study, we aimed to elucidate whether and how high-IgE levels influence the development of intestinal inflammation, and their impact on the metabolome in acute AE using IgE knock-in (IgEki) mice.[26] IgEki mice express the IgE heavy chain instead of IgG1, which results in an enhanced polyclonal antigen-specific IgE response, similar to allergic patients. We show that IgEki mice induce marginal degrees of AE only by feeding an allergenic diet, whereas active sensitizations with an allergen and Alum as adjuvant and subsequent feeding with the allergenic diet aggravate AE. By means of non-targeted metabolomics using three complementary platforms—Gas Chromatography-Mass spectrometry (GC-MS), Liquid Chromatography-Mass Spectrometry (LC-MS) and Capillary Electrophoresis-Mass Spectrometry (CE-MS)—we observed remarkable alterations in systemic and gut metabolomes in the different severity stages of AE in IgEki mice. In addition, we analysed the association of altered metabolite levels with the gut microbiome profiles.

2 | MATERIALS AND METHODS

2.1 | Animals

BALB/c wild type (WT) and IgE knock-in mice (C.Ighg1tm1.1Pyu) on the BALB/c background[26] were maintained and bred under specific pathogen-free conditions in the animal facility of the Paul-Ehrlich-Institut. Animal experiments were performed in compliance with national law and approved by the local authority (Regierungspräsidium Darmstadt, Germany, license number: F107/1020).

2.2 | Induction of AE

Mice (female, 6–8 weeks old) were sensitized by an intraperitoneal injection with 10 μg of ovalbumin (OVA, Sigma-Aldrich) and 1 mg of Alum (Thermo Fisher Scientific) in 500 μL of PBS—or treated only with PBS as non-sensitized controls—twice at a 2-week interval as described previously.[4] Two weeks after the second sensitization, the mice received an egg-white diet, that contained 100% egg white as 20% of protein source (EW diet: ssniff Spezialdiäten GmbH). After 7 days of EW diet feeding, the mice were sacrificed. Intestines were harvested for histology and FACS analysis. Stool and serum samples were kept at −80°C until metabolite extraction.

2.3 | Measurement for serum levels of OVA-specific antibodies

The serum levels of OVA-specific IgE, IgG1 and IgG2a antibodies were measured by ELISA and are described in Data S1 (Table S1).

2.4 | Histology of intestinal samples

Longitudinal sections of intestinal tissue (2 cm) were taken from jejunum (9.5 cm distal to the duodenum). The tissues were fixed in 4% formalin and embedded in paraffin. Sections 5 μm thick were prepared and stained with haematoxylin and eosin (H&E) for morphologic analysis and detection of eosinophils. Histological score system is described in Table S2.

2.5 | Analysis of eosinophil and neutrophil frequency in intestinal lamina propria cells

Intestinal lamina propria cells were prepared according to a protocol described by Weigmann et al. with slight modifications.[27] A list of materials and detailed methods is described in Data S1.

2.6 | Metabolomics

The full list of reagents and standards for metabolomics is available in Data S1.

2.6.1 | Metabolite extraction

Faecal samples were lyophilized overnight at −80°C (LyoQest −85, Telstar). The dried samples were treated (i) with MeOH:MTBE (4:1, v/v) and (ii) with MeOH:H_2O (4:1, v/v). After centrifugation, equal volumes (300 μL) of the supernatants from (i) and (ii) were mixed and aliquoted for subsequent analysis. Serum samples (100 μL) were deproteinized by mixing with cold acetonitrile (300 μL: 1:3 v/v) for 15 min in a vortex. After cooling on an ice bath for 10 min, the mixtures were centrifuged and stored at −20°C until the start of sample treatment.[28,29] The detailed extraction procedure is available in the Data S1.

2.6.2 | Preparation of metabolite samples and quality controls (QCs)

Treatment of metabolic extracts and preparation of individual QC samples for MS analysis are described in the Data S1.

2.6.3 | Metabolomics data acquisition

Samples were analysed using three analytical platforms (LC-MS positive and negative ionization modes, CE-MS positive and GC-MS) in order to maximize the metabolite coverage. LC–MS analysis was performed on an Agilent HPLC system (1290 infinity II series, Agilent Technologies, Waldbronn, Germany), coupled to a quadrupole-time of flight analyser mass spectrometer (QTOF-MS) series 6545 (Agilent Technologies).[28] CE-MS was performed on a CE system (7100 Agilent) coupled to a TOF analyser (6224 Agilent).[30,31] In GC-MS, the samples were measured in a GC system (Agilent Technologies 7890A) equipped with an autosampler (Agilent 7693) and coupled to an MS with an inert MSD quadrupole detector (Mass Selective Detector, Agilent 5975C).[32] Both matrices were analysed in the same analytical sequence in two batches: first faeces and then serum, and in each one of them, the samples were measured in a randomized order.

Further details about the methodology for data acquisition can be found in the Data S1.

2.6.4 | Data treatment, metabolite annotation and statistical analysis

After the acquisition of raw data by the MS system, they were processed and subsequently filtered following the Quality Assurance procedure (QA) adapted from Godzien et al.[33,34] In LC–MS, the metabolite annotation was carried out for statistically significant features by CEU Mass Mediator (http://ceumass.eps.uspceu.es/).[35] The identity of those compounds with relevant annotations was confirmed with LC–MS/MS. In CE-MS, the compound annotation was performed using an in-house library of standards.[30,31] In GC-MS, the annotation was performed by using Fiehn RTL (Retention Time Locked)[36] and NIST v.14 libraries (https://chemdata.nist.gov/).[37] For the annotation of the biological class of each metabolite, Human Metabolome Database (HMDB, http://www.hmdb.ca/)[38] and Kyoto Encyclopedia of Genes and Genomes (KEGG, www.genome.jp/kegg/)[39] were mainly applied.

For the immunological analysis, comparisons of mean values between different groups were performed by ANOVA in GraphPad Prism 7 (San Diego, USA). *p*-Values <.05 and <.01 were designated with * and **, respectively, and considered significant. For metabolomics, after QA, the data were used to build multivariate statistic models in SIMCA (v.14.1, Umetrics®, Umeå, Sweden). In parallel, a univariate analysis was performed in MATLAB (v.R2018b, MathWorks®, Natick, Massachusetts, USA) to obtain the corrected *p*-value for each metabolite. Compounds were considered significant if the corrected $p < .05$ for both two-factor Kruskal–Wallis (KW) with a Benjamini–Hochberg correction and Mann–Whitney *U* test (MWU) with a post hoc Bonferroni correction.

For the analysis of metabolic pathways, significant pathways ($p < .05$) were selected with MetaboAnalyst 5.0 (https://www.metaboanalyst.ca)[40] and IMPaLA (http://impala.molgen.mpg.de).[41] Data were plotted by using KEGG database and the Metscape tool (http://metscape.ncibi.org/) from Cytoscape software (https://cytoscape.org/).

Detailed information about this section can be found in Data S1.

2.7 | Microbiome analysis

2.7.1 | Metagenomics sequencing

DNA was extracted from faeces using Qiagen QIAamp DNA Stool Mini Kit (QIAGEN GmbH), following manufacturer instructions. Specifically, the V3–V4 region of the bacterial 16S ribosomal RNA (rRNA) genes were then amplified using primers on a MiSeq platform (Illumina) with the MiSeq reagent kit v2 (500 cycles) as previously described.[42,43] Demultiplexed sequences were processed with the DADA2 pipeline (q2-dada2 plugin) available in Quantitative Insights into Microbial Ecology 2 (QIIME 2, version 2021.2).[44] Unique amplicon sequence variants (ASVs) were assigned a taxonomy using a pre-trained Naive Bayes classifier and the q2-feature-classifier plugin against the Greengenes 13_8 99% OTUs (https://greengenes.lbl.gov/).[45] The diversity indices (alpha and beta) were calculated by qiime diversity core-metrics-phylogenetic method using q2-diversity plugin on QIIME2. Furthermore, the 16S read counts obtained from QIIME2 were further uploaded to the web-based

MicrobiomeAnalyst 2.0[46] to obtain the abundance profiling value of the taxonomic composition.

2.7.2 | Statistics

The MicrobiomeAnalyst online tool (v. 2.0)[46] was used for data analysis and marker profiling. For the cladogram of differences in species abundance between groups, we applied the linear discriminant analysis (LDA) effect size (LEfSe) tool,[47] via the interactive webpage (https://huttenhower.sph.harvard.edu/galaxy/). This high-dimensional data analysis tool for biological indicators compares various groups, focuses on biological correlations and statistical significance, and identifies biological indicators that differ significantly between groups.

GraphPad Prism (v.10.0.3) was used to perform the multivariate analysis and to represent the correlation matrices by means of heatmaps. Pearson's correlation was used. All tests were two-tailed.

3 | RESULTS

3.1 | IgE promotes development of clinical signs and intestinal inflammation in a murine AE model

To assess the impact of high-IgE on the development of AE, WT and IgEki mice were sensitized with OVA and Alum and challenged with an EW diet (see immunization schedule in Figure S1A). OVA-sensitized and EW diet-fed (OVA/EW) IgEki mice developed significantly stronger clinical signs, that is reduction in body weight and body temperature during 7 days of EW diet, compared to OVA/EW WT mice (Figure 1A). Their controls, non-sensitized and EW diet-fed (NC/EW) WT and NC/EW IgEki mice did not develop clinical signs (Figure 1A). OVA/EW IgEki mice produced significantly higher levels of OVA-specific IgE antibodies than OVA/EW WT mice, due to the expression of the IgE heavy chain, instead of IgG1 as consequence of the knock-in modification (Figure 1A and Figure S1B). IgG2a production was maintained in IgEki mice (Figure S1B). NC/EW IgEki mice, but not NC/EW WT mice, produced detectable, but marginal levels of OVA-specific IgE antibodies (Figure 1A).

OVA/EW WT mice developed moderate inflammatory features of AE, for example elongated crypts, abundant hypertrophic goblet cells and villi atrophy with oedema in the lamina propria, whereas OVA/EW IgEki mice aggravated AE (Figure 1B and Figure S2A). FACS analysis showed that the frequencies of eosinophils ($SiglecF^+CD11b^+$ cells) and neutrophils ($Ly6G^+CD11b^+$ cells) in the lamina propria $CD45^+$ cells were significantly higher in IgEki OVA/EW mice, compared to OVA/EW WT mice (Figure S2B). Such inflammatory features could not be detected in NC/EW WT mice, whereas marginal inflammation was observed in NC/EW IgEki mice (Figure 1B and Figure S2A). The results indicate that IgE enhances development of AE in a murine model. Overall, OVA sensitization and/or EW diet feeding of WT mice and IgEki mice induce four groups with different degrees of AE severity: allergic mice with **aggravated AE** (OVA/EW IgEki mice), allergic mice with **moderate AE** (OVA/EW WT mice), allergic mice with **marginal AE** mirroring sensitization stage (NC/EW IgEki mice) and **non-allergic mice** (NC/EW WT mice).

3.2 | Metabolic characterization of serum and faeces points to alterations of metabolome in different degrees of AE severity

To get insight into the metabolome at different inflammatory degrees of AE, faeces and serum samples of the four mouse groups were analysed by multiplatform metabolomics. We detected a total of 5155 metabolic features—3059 in faeces and 2096 in serum samples. For comparisons between the four groups, discriminant multivariate analysis, univariate analysis and annotation of significant metabolites were performed. The numbers of metabolites after each step of the data treatment are presented in Table S4. Non-supervised PCA models show an overview of the differences among the four groups (Figure S3), which are commented in Results in Data S1. Annotation of the significant metabolites in all comparisons resulted in a total of 215 identified metabolites in both serum and faeces samples (Figure 2 and Tables S5–S8).

3.3 | Marginal and moderate AE alters the local metabolome to a greater extent than the systemic metabolome

To investigate the metabolic profile in marginal AE, the levels of metabolites were compared between NC/EW WT mice and NC/EW IgEki mice. In this comparison, clear differences of metabolite levels in the faeces samples were observed in all analytical platforms, since all the PLS-DA models showed a perfect separation in the first component, with a percentage of prediction (Q^2) up to 96%. PLS-DA and PCA-X models for this comparison are depicted in Figure S4. Regarding serum samples, no significant models could be obtained in multivariant statistics between the two NC/EW groups. Metabolites in serum and faeces samples reflect systemic and local metabolomes, respectively. The results suggest that the systemic metabolome is less influenced than the local metabolome in marginal degrees of AE.

After both multivariate and univariate statistics in the comparison between NC/EW IgEki and NC/EW WT groups, 56 and 8 metabolites were annotated in faeces and serum samples, respectively (Figure 2 and Table S5). The heatmap analysis of faeces showed clear metabolic signatures and a perfect clustering of the two NC/EW groups (Figure S5). Significantly higher levels of sphingolipids (sphingosine and sphinganine) and lysophospholipids (LysoPC (14:0), LysoPC (16:0) and LysoPG (16:0)), and significantly lower levels of acetylcarnitine, carnitine and amino acids (lysine, aspartic and glutamic acids) were detected in faeces samples of NC/EW IgEki mice (Figure 3A and Table S5). Metabolites derived from gut bacteria, such

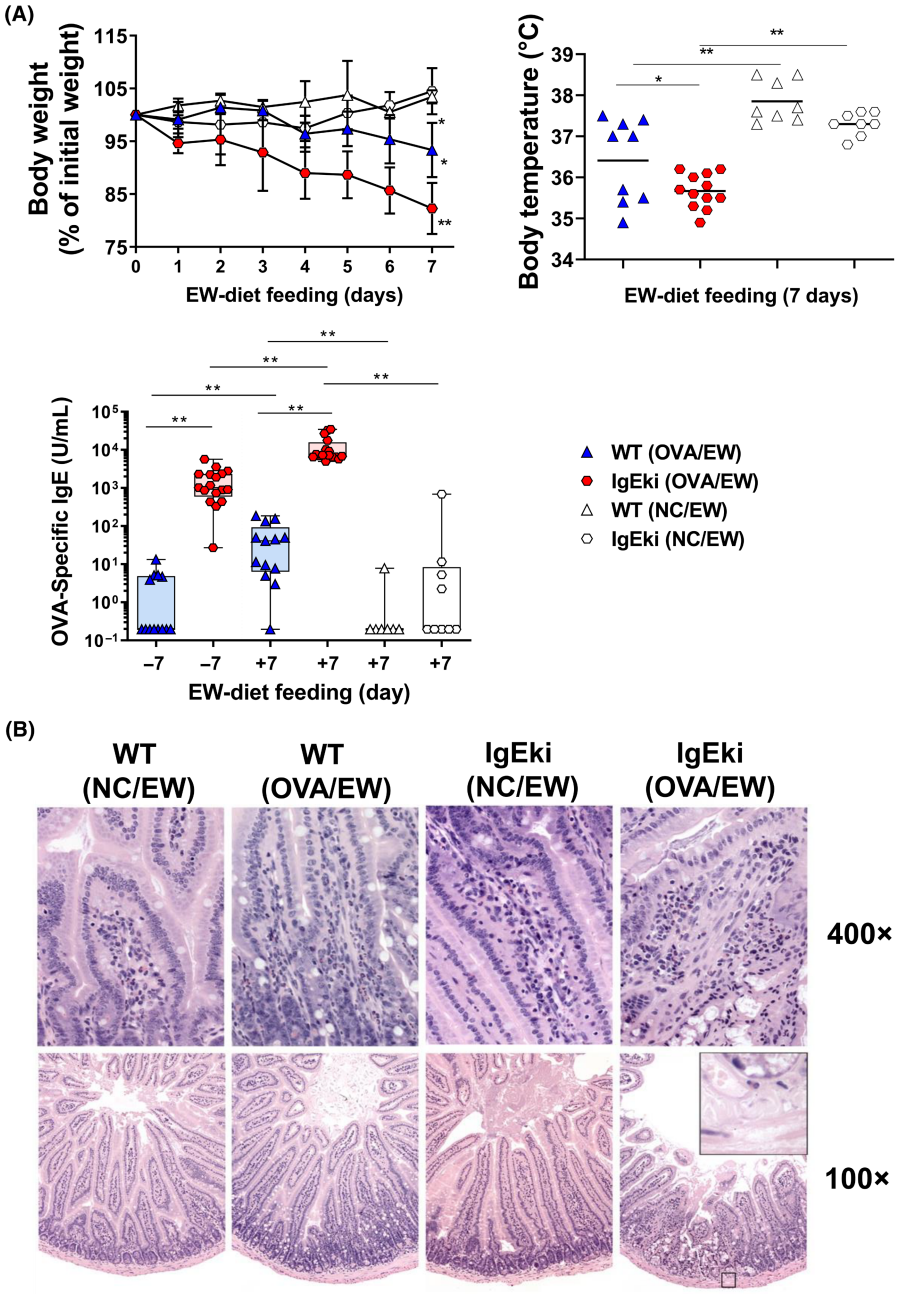


FIGURE 1 Induction of allergic enteritis in wild type and IgEki mice. WT and IgEki mice were i.p. sensitized with OVA plus ALUM and fed EW diet for 7 days. As controls, mice were fed EW diet without sensitization. (A) During EW diet feeding, the weight of the mice was monitored daily. Body temperature and the serum levels of OVA-specific IgE levels of each mouse on day 7 of EW diet were measured. (B) Jejunum tissues were harvested from mice and stained with H&E. The data is representative for three independent experiments. NC/EW, Non-sensitized and EW diet-fed mice; OVA/EW, OVA-sensitized and EW diet-fed mice. $^{*}p < .05$, $^{**}p < .01$.

as valeric and shikimic acids, were also increased in faeces samples of NC/EW IgEki mice (Table S5). Pathway analysis showed that the urea cycle and glycerophospholipid-, glycosphingolipid- and lysine-related metabolisms were significantly altered in intestines of NC/EW IgEki mice ($p < .05$) (Figure 3B). Regarding serum samples, higher levels of lysophospholipids—LysoPC (14:0), LysoPC (18:2), LysoPE (18:1) and LysoPG (16:0)—were detected in NC/EW IgEki mice, compared to NC/EW WT mice (Figure 3A and Table S5).

Next, we compared the metabolite levels between NC/EW WT mice and OVA/EW WT mice to know the metabolic profile of moderate AE. In this comparison, 28 metabolites were annotated in faeces (Figure 2, Table S7). Among them, most of the changes affecting the metabolism of carbohydrates, lysophospholipids, amines (e.g. spermidine and putrescine), sphingolipids (e.g. 3-ketosphinganine) and amino acids (lysine, pipecolic acid and proline) were increased in OVA/EW WT (Figure 4A,B). The detailed data of the OVA/EW

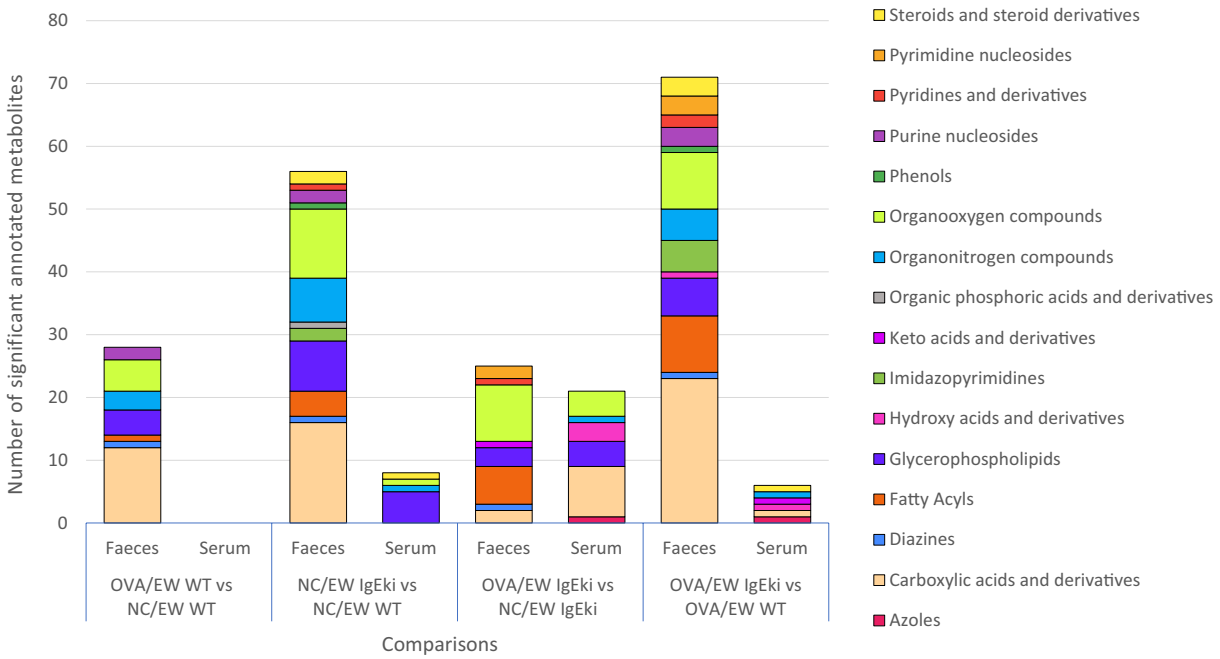


FIGURE 2 The number of significantly annotated metabolites in NC/EW or OVA/EW WT and IgEki mice. Stacked bar chart of biochemical classes of the significant annotated metabolites in the four comparisons of faeces and serum samples. The biochemical class was taken from HMDB website. NC/EW, Non-sensitized and EW diet-fed mice; OVA/EW, OVA-sensitized and EW diet-fed mice.

WT versus NC/EW WT comparison are included in the Data S1 (Figures S6 and S7, Results in Data S1). These results suggest that OVA/EW WT and NC/EW IgEki mice show a similar trend to increase the levels of sphingolipids and lysophospholipids in intestinal tissues.

3.4 | Aggravated AE alters both systemic and local metabolomes

Next, the influence of aggravated AE on metabolomes was analysed by comparing OVA/EW IgEki mice and OVA/EW WT mice. Clear differences between OVA/EW groups were observed in both faeces and serum samples in all analytical platforms by means of PLS-DA models: a percentage of prediction (Q^2) up to 95% and 89%, respectively. PLS-DA and PCA-X models for this comparison are shown in Figure S8.

After all statistical analyses in the comparison of two OVA/EW groups, 71 and 6 annotations were obtained in faeces and serum samples, respectively (Figure 2, Table S6). The heatmap for faeces samples showed a blind separation of the two OVA/EW groups and a decrease in most of the significant metabolites in OVA/EW IgEki mice (Figure S9). The levels of lysophospholipids (LysoPE (15:0), LysoPE (17:0), LysoPE (17:1) and LysoPE (14:0)) and a sphingolipid (3-ketosphinganine) were lower in faecal samples of OVA/EW IgEki mice (Figure 5A). In addition, a decrease of butyric acid and an increase of 2,3-butanediol in OVA/EW IgEki mice were detected (Figure 5A). Metabolic pathway analysis suggests alterations of several pathways in OVA/EW IgEki mice that include butanoate-, lysine and other amino acid-, urea cycle, vitamin B3-, purine- and pyrimidine-related pathways (Figure 5B).

In serum samples, increased levels of N-tau-methylhistamine, cholesterol, ketoleucine and 3-hydroxbutanoate were found in OVA/EW IgEki mice, compared to OVA/EW WT mice (Figure 6A). Pathway analysis showed that butanoate, histidine, valine degradation and cholesterol-related pathways are enhanced in OVA/EW IgEki mice (Figure 6B).

The comparison of metabolite levels between NC/EW IgEki mice and OVA/EW IgEki mice was also performed. In this comparison, lower levels of lysophospholipids, sphingolipids and butyric acid in faecal samples, and higher levels of N-tau-methylhistamine and 3-hydroxbutanoate in serum samples were detected in OVA/EW IgEki mice (Figures S10 and S11), as seen in the comparison between OVA/EW IgEki mice and OVA/EW WT mice (Figures 5 and 6). The detailed data in OVA/EW IgEki versus NC/EW IgEki comparisons are included in the Data S1 (Table S8, Figures S12 and S13). These results suggest that high-IgE levels in AE may alter the levels of both gut microbiota-derived and endogenous metabolites significantly.

3.5 | Microbiome characterization in different degrees of AE

Next, to assess the impact of high levels of IgE on gut microbiome and its association with metabolome, 16S rRNA gene sequencing analysis was performed using feacal samples of WT and IgEki mice. Analysis of α-diversity and richness revealed a statistically significant increase in NC/EW IgEki mice, compared to the other groups ($p < .05$) (Figure S14). In comparison to NC/EW groups, OVA/EW groups increased Verrucomicrobiota and Actinobacteria at the phylum level and *Akkermansia* at the genus level, and decreased Bacteroidetes at

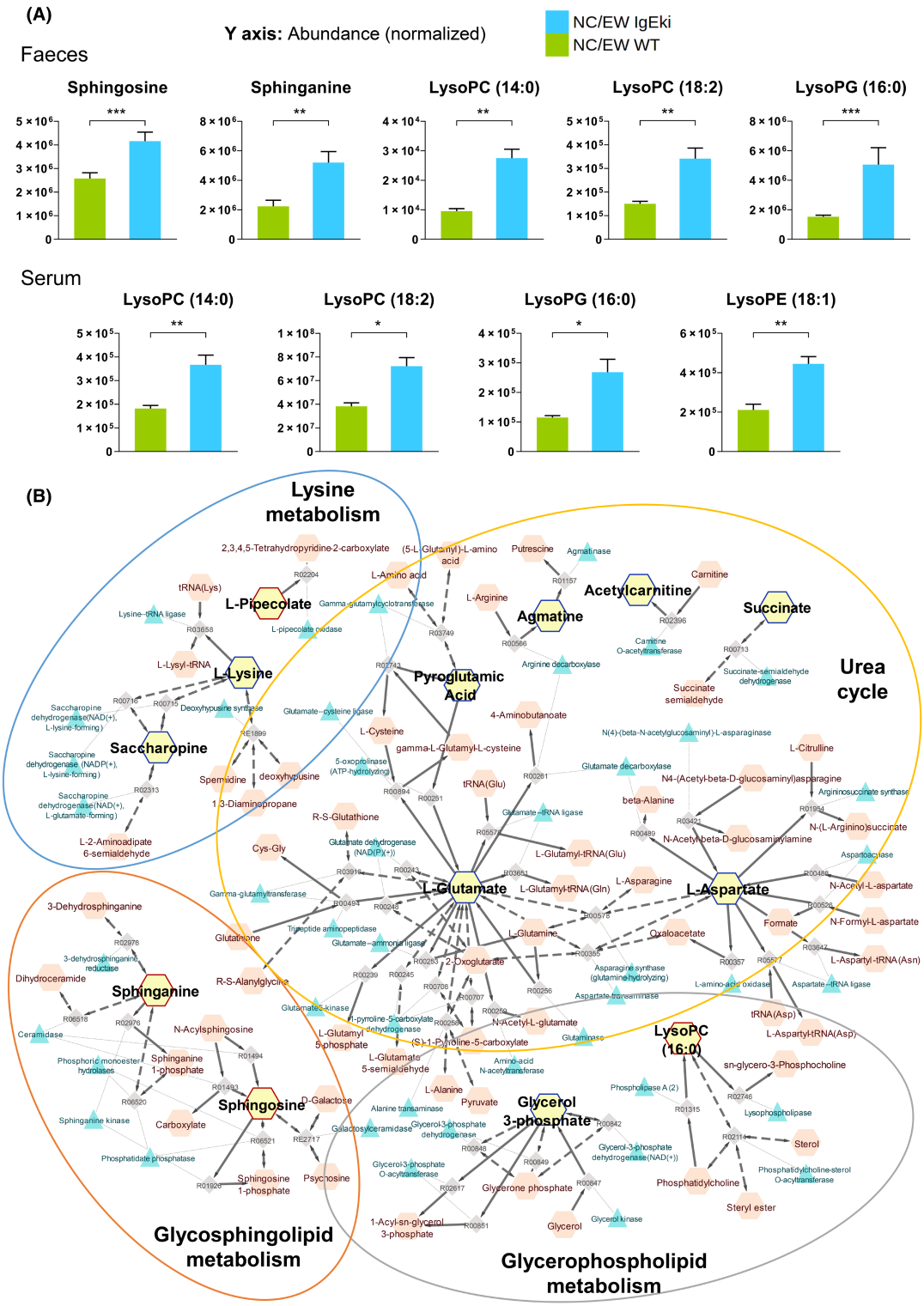


FIGURE 3 Characterization of key faecal and serum metabolites in NC/EW IgEki versus NC/EW WT comparison. (A) Trajectories of significant annotated metabolites in faeces and serum between NC/EW IgEki and NC/EW WT groups. Asterisks denote significance of the MWU test with a post hoc Bonferroni correction: $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$. (B) Significant metabolic pathways ($p < .05$) detected using faeces samples. Yellow hexagons are the metabolites significantly altered. The blue border means that it is decreased in that comparison and the red one that it is increased. Orange hexagons are the metabolites that participate in the route. Grey diamonds are the reactions that take place. Reactions are described in Table S9. Blue triangles are the enzymes that participate in each of the reactions. NC/EW, Non-sensitized and Non-sensitized and EW diet-fed mice.

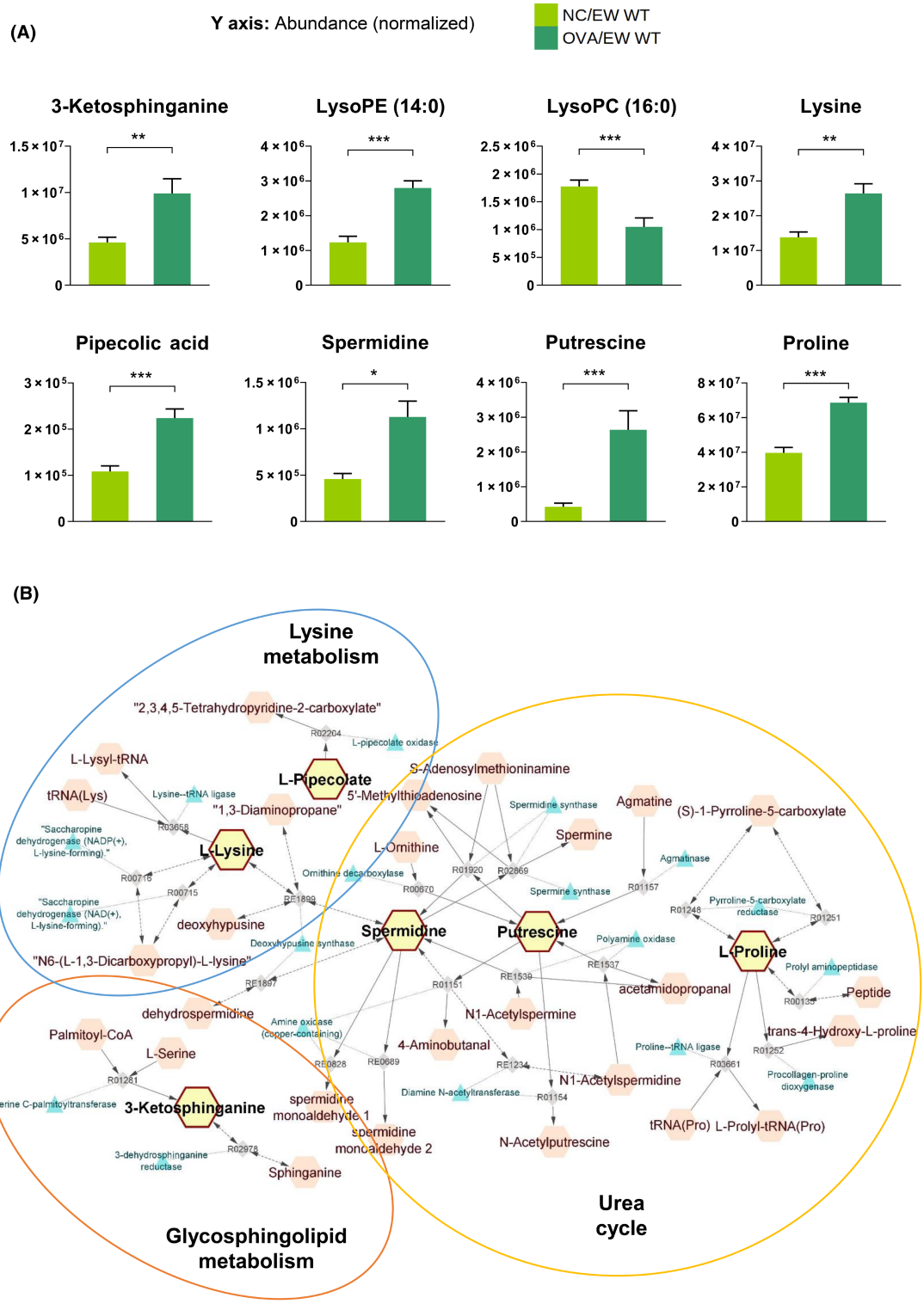


FIGURE 4 Characterization of key faecal metabolites in e OVA/EW WT versus NC/EW WT comparison. (A) Trajectories of significant annotated metabolites in faeces between OVA/EW WT versus NC/EW WT groups. Asterisks denote significance of the MWU test with a post hoc Bonferroni correction: $*p < .05$; $**p < .01$; $***p < .001$. (B) Significant metabolic pathways ($p < .05$) detected using the faeces samples. Yellow hexagons are the metabolites significantly altered. The blue border means that it is decreased in that comparison and the red one that it is increased. Orange hexagons are the metabolites that participate in the route. Grey diamonds are the reactions that take place. Reactions are described in Table S12. Blue triangles are the enzymes that participate in each of the reactions. NC/EW, Non-sensitized and EW diet-fed mice; OVA/EW, OVA-sensitized and EW diet-ed mice.

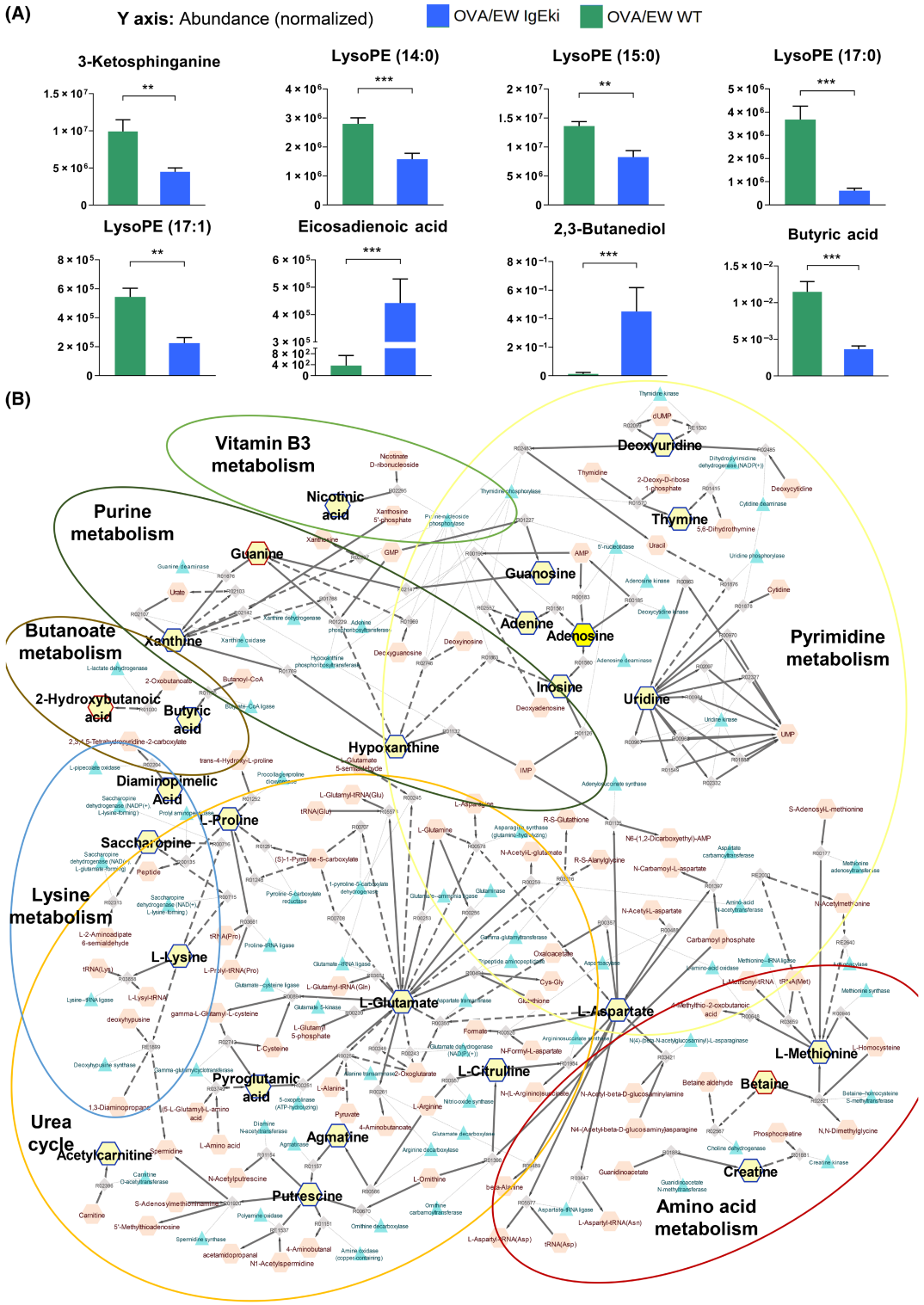


FIGURE 5 Characterization of key faecal metabolites in OVA/EW IgEki versus OVA/EW WT comparison. (A) Trajectories of significant annotated metabolites in faeces between OVA/EW IgEki versus OVA/EW WT groups. Asterisks denote significance of the MWU test with a post hoc Bonferroni correction: $^{**}p < .01$; $^{***}p < .001$. (B) Significant metabolic pathways ($p < .05$) detected using faeces. Yellow hexagons are the metabolites significantly altered. The blue border means that it is decreased in that comparison and the red one that it is increased. Orange hexagons are the metabolites that participate in the route. Grey diamonds are the reactions that take place. Reactions are described in Table S10. Blue triangles are the enzymes that participate in each of the reactions. OVA/EW, OVA-sensitized and EW diet-ed mice.

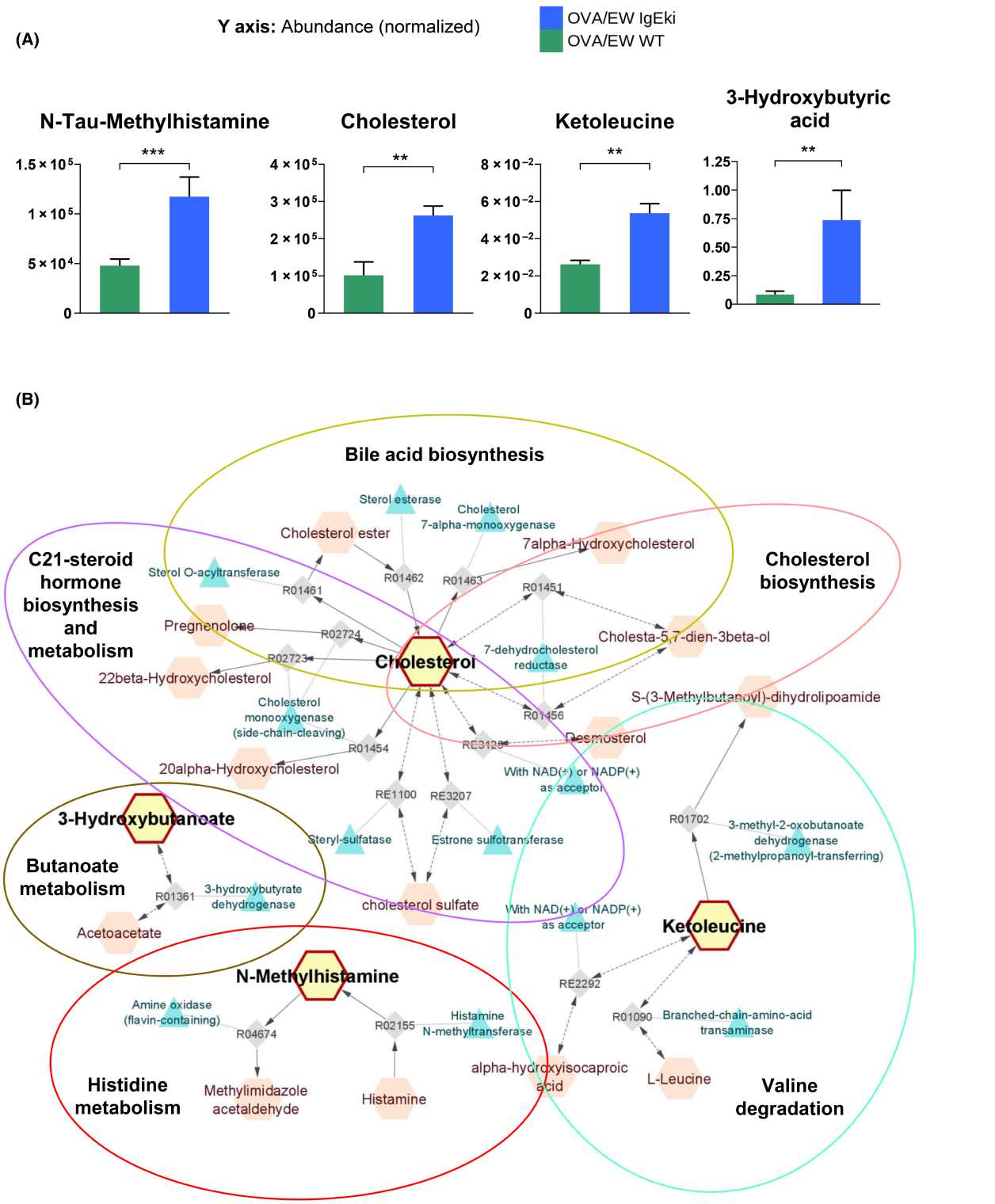


FIGURE 6 Characterization of key serum metabolites in OVA/EW IgEki versus OVA/EW WT comparison. (A) Trajectories of significant annotated metabolites in sera between OVA/EW IgEki versus OVA/EW WT groups. Asterisks denote significance of the MWU test with a post hoc Bonferroni correction: $^{**}p < .01$; $^{***}p < .001$. (B) Significant metabolic pathways ($p < .05$) detected using serum samples. Yellow hexagons are the metabolites significantly altered. The blue border means that it is decreased in that comparison and the red one that it is increased. Orange hexagons are the metabolites that participate in the route. Grey diamonds are the reactions that take place. Reactions are described in Table S11. Blue triangles are the enzymes that participate in each of the reactions. OVA/EW, OVA-sensitized and EW diet-ed mice.

the phylum level and *Lachnospiraceae* at the family level in both WT and IgEki mice (Figure 7A). In comparison to WT mice, IgEki mice increased *Bacteroides* and *Butyricicoccus*, and decreased *Clostridium*, *Desulfovibrio*, *Enterococcus*, *Lachnospiraceae* and *Blautia* at the genus level (Figure 7A). Linear discriminant analysis (LDA) of effect size (LEfSe) showed that the main genus enriched in the microbiota of OVA/EW IgEki mice with the severe AE is *Lactobacillus*, while NC/EW WT mice, the non-allergic control, is enriched in *Clostridium* genus (Figure 7B).

Association analysis of between the levels of OVA-specific IgE, the metabolome and the microbiome by combining data of all four groups was performed. The levels of OVA-specific IgE were positively associated with those of N-tau-methylhistamine, and 2-aminooctanoic acid in the serum samples and the ratio of *Lactobacillus* in the faecal samples, but adversely associated with those of several purine- and pyrimidine-related pathways, that is uridine, 2-deoxythyidine, thymidine and xanthine in the faecal samples (Figure S15). Among the associated metabolites with IgE levels, N-tau-methylhistamine was highly detected in OVA/EW IgEki mice (Figure 6A).

Association analysis of metabolome and microbiome showed potential links of metabolites and bacteria (Figure 8). Within the metabolites detected in the faecal samples, sphinganine was associated with *Clostridium*, *Desulfovibrio* and *Enterococcus* at the genera level, which was highly detected in NC/EW WT mice. LysoPE (14:0) and LysoPE (15:0) were associated with *Akkermansia*, *Parabacteroides* and/or *Adlercreutzia*. The levels of LysoPE (17:0) were associated with *Anaerotruncus*, *Desulfovibrio* and *Enterococcus*. Another lipid compound, 3-ketosphinganine, displayed association with *Adlercreutzia*, *Blautia* and *Parabacteroides*, which were observed in NC/EW IgEki mice. Eicosadienoic acid and 2-3-butanediol were associated with *Butyricicoccus*, which was detected in OVA/EW IgEki group. Aminobutyric acid was associated with *Bacteroides*, which was reduced in OVA/EW IgEki group. Within the metabolites detected in serum samples of OVA/EW IgEki mice, 2-hydroxybutyric acid was associated with *Lactobacillus*. The results suggested a complex network of potential associations between the microbial environment and metabolites in the AE.

4 | DISCUSSION

In the present study, using IgEki mice, we provide evidence that high levels of IgE enhance the accumulation of granulocytes, the development of AE and alterations in systemic and local metabolomes. The enhanced inflammatory features in OVA/EW IgEki mice—with intensive granulocyte accumulation and aggravated weight loss—are similar to those in patients with intestinal allergy.[7,48] On the other hand, NC/EW IgEki mice could be a model of the initial stage of the disease, presenting a marginal degree of inflammation without detectable clinical signs. Untargeted metabolomics identified distinctive metabolomic signatures in WT and IgEki mice with different degrees of AE. In the comparisons of all tested four groups, the levels of lipids—including sphingolipids, glycerophospholipids and fatty acids—were significantly altered. Analyses of gut microbiome also showed differential abundance of microbial communities among the groups.

We found that the levels of 3-ketosphinganine (a precursor of sphinganine) and lysoPE (14:0) or (15:0) (lysophospholipids, a class of glycerophospholipids) were higher in faeces of OVA/EW WT mice, a model of moderate AE, compared to NC/EW WT mice, a non-allergic control. The increased levels of lysoPE (14:0) was also found in faeces of NC/EW IgEki mice with marginal AE. Lee-Sarwar K et al. showed that 3-ketosphinganine is significantly increased in faeces samples of subjects with food sensitization.[24] Their study also detected an association of impaired respiratory responses with plasma levels of sphingolipids (e.g. sphinganine and ceramide) in patients with asthma. Other studies observed elevations of lysophospholipids in bronchoalveolar lavage fluids of patients with asthma and animal models with acute lung injury.[49–51] The observations, including ours, suggest that the elevation of sphinganines or lysophospholipids might be an indication of sensitization or the development of allergic inflammation.

Several studies showed that lysophospholipids exhibit pro-inflammatory properties, for example by induction of inflammatory cytokine/chemokine expression in human monocytes and bronchial epithelial cell lines.[52,53] Nishiyama et al. showed that LysoPC induces eosinophil infiltration and resistance in the airways of a murine asthmatic model.[54] It has also been suggested that the levels of sphingolipids are increased by dysbiosis.[55–57] In our study, the levels of LysoPE (14:0) and LysoPE (15:0) were associated with the abundances of *Akkermansia* and *Parabacteroides*, mucin-degrading genera. Involvement of *Akkermansia* in sphingolipid metabolism was detected in DSS-induced colitis.[58] In addition, *Akkermansia muciniphila* promoted food allergic responses by mucosal barrier breakdown in fibre-deprived mice.[59] Sphingolipids are the major components of cell membranes.[60,61] The lipids might be released by damaged cells at inflammatory sites and trigger the development of inflammation. However, it also needs to be noticed that the abundance of *Akkermansia* was lower in OVA/EW IgEki mice, compared to OVA/EW WT mice. The result suggests that the elevation of *Akkermansia* and lysoPE may be involved in the development of AE, but not be directly associated with the severity of AE.

The most visibly elevated fatty acid in faeces samples of OVA/EW IgEki mice was eicosadienoic acid, a naturally occurring n-6 polyunsaturated fatty acid (PUFA) derived from linoleic acid.[62] It is mainly detected in animal tissues when the activity of Δ6-fatty acid desaturase—the key enzyme in the biosynthesis of polyunsaturated fatty acids—is inhibited. Reduced desaturase activity was observed in bronchial epithelial cells from asthma patients,[63] suggesting that inflammation influences the expression of desaturase and thereby alters lipid metabolism. High levels of eicosadienoic acid, along with other PUFAs in breast-fed infants (from 6 to 12 months of age) have been associated with asthma-like symptoms.[64] Eicosadienoic acid is a precursor for the biosynthesis of eicosanoids providing pro-inflammatory signals[62] and could be involved in the development and aggravation of AE.

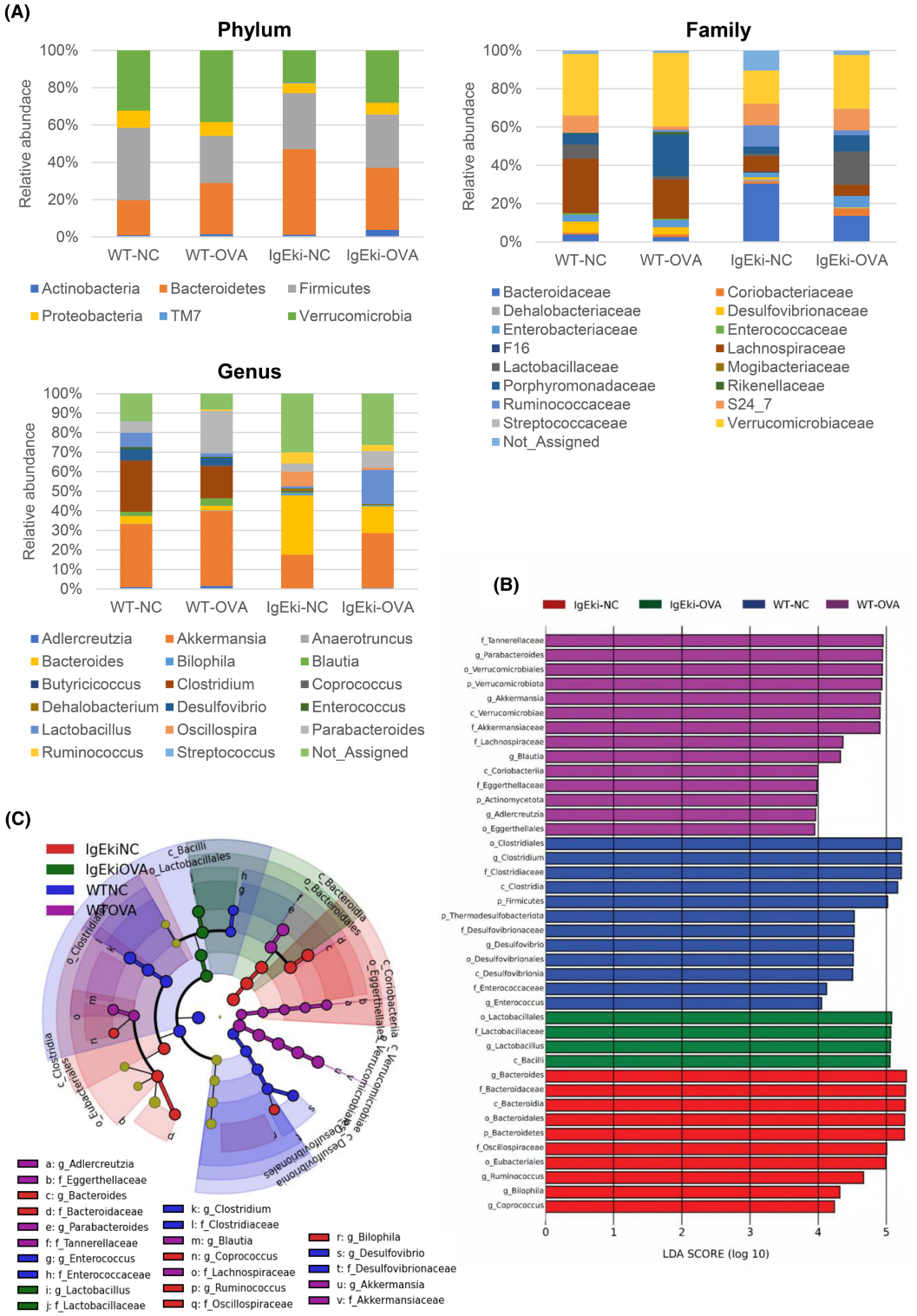


FIGURE 7 Analysis of gut microbiome in NC/EW or OVA/EW WT and IgEki mice. (A) Relative abundance of the main phyla, family and genus in the mouse groups. (B) Linear discriminant analysis (LDA) scores resulting from the LDA-effect size (LEfSe) tool in the Galaxy/Hutlab webpage. (C) Cladogram of the LEfSe analysis. NC/EW, Non-sensitized and EW diet-fed mice; OVA/EW, OVA-sensitized and EW diet-ed mice.

The abundance of *Butyricicoccus*, a genus belonging to the *Clostridiales* order, was correlated with the levels of 2-3-butanediol, which were significantly higher in OVA/EW IgEki mice. Significant taxonomic differences in atopic dermatitis (AD) and allergic rhinitis (AR) including higher abundance of *Butyricicoccus* have been described.[65–67] Meanwhile, 2,3-butanediol is produced by the gut bacteria genera *Klebsiella*, *Enterobacter* and *Serratia* genera, which are reportedly linked to an increased risk of allergy.[68–71] In contrast, reduced levels of butyric

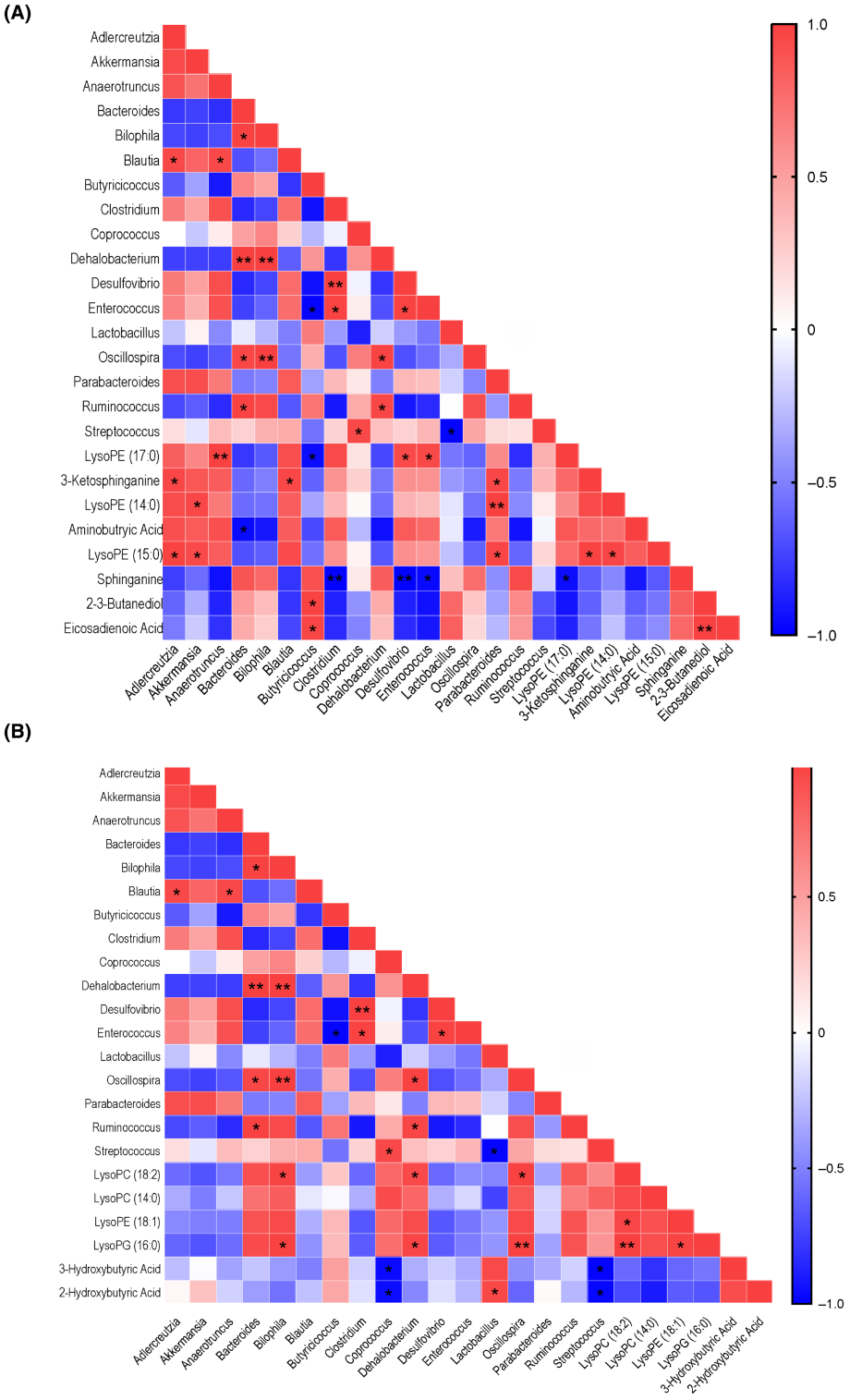


FIGURE 8 Correlation matrices between metabolites and gut microbiome at the levels of genera. Pearson correlation index: **p*-value <.05; ***p*-value <.01. (A) Correlation matrix identified in faecal samples. (B) Correlation matrix identified in serum samples.

acid, valeric acid and 2-hydroxyisocaproic acid were also detected in OVA/EW IgEki mice. The abundance of the genera *Lachnospiraceae* and *Clostridium*, that cover butyrate-producing species, was also reduced in the mice. This is consistent with previous studies showing a reduction in butyrate-producing gut bacteria in allergic patients.[72–74] Butyrate is an anti-inflammatory bacteria-derived short-chain fatty acid (SCFA) that has the potential to induce resolution from allergy by developing regulatory T cells and enhancing intestinal epithelial barrier integrity.[74–77] In addition, 2-hydroxyisocaproic acid is known as a metabolite of *Lactobacillus* species.

Lactobacillus was highly abundant in OVA/EW IgEki mice. We had also observed increased abundance of *Lactobacillus* in food allergic mice with severe symptoms previously.[78] A low relative abundance of *Lactobacillus* was reported to be associated with asthma development early in life and inflammatory bowel disease.[79-81] Conversely, intestinal abundance of Lactobacillus was positively correlated with Crohn's disease (CD) patients.[82] Notably, the genus Lactobacillus comprises more than 180 species.[82] Further analysis is required to determine whether certain Lactobacillus species or genotypes are induced in high-IgE environments and are involved in the development of allergies.

It is worth noticing that serum levels of N-tau-methylhistamine were significantly elevated in OVA/EW IgEki mice with severe inflammation, and positively associated with the levels of OVA-specific IgE antibodies. N-tau-methylhistamine is a major metabolite of histamine produced by the enzyme histamine N-methyltransferase[83] and constitutes a reliable parameter for histamine release from mast cells in serum.[84,85] Previous studies described that histamine N-methyltransferase is responsible for 70%–80% of histamine biotransformation to N-tau-methylhistamine.[86,87] Increased N-tau-methylhistamine has been detected in serum and urine samples in mastocytosis.[88,89] Taken together, these studies suggest that high levels of allergen-specific IgE increase histamine release from mast cells and thereby promote the serum levels of N-tau-methylhistamine in OVA/EW IgEki mice. Thus, N-tau-methylhistamine could constitute a marker of mast cell activation and disease severity in AE.

Pathway analysis showed that multiple energy metabolism pathways were dysregulated in OVA/EW IgEKi mice: the urea cycle, pyrimidine and purine metabolisms. OVA/EW IgEKi mice exhibited severe inflammation and weight loss. Weight loss is one of the clinical signs often caused by severe intestinal allergy.[7,11,14,48,90] We have also noticed that many of the metabolite differences in OVA/EW WT mice and OVA/EW IgEki mice may be induced by changes in nutrient absorption due to weight loss and inflammation. The absorption of certain micronutrients is hindered during inflammation, that would influence on the levels of metabolites.[91] Cellular metabolism of immune cells and profile of gut microbiome are also altered in inflammation.[92,93] Weight loss alone could also drive changes in metabolism and microbiota.[94] AE could alter the absorption levels of micronutrients, that would also alter intestinal and systemic metabolome. This could be considered as a potential limitation of our study since metabolites differences might be due to changes in nutrient absorption. Further studies are necessary to elucidate whether and how the absorption of micronutrients influences on the intestinal and systemic metabolome in AE condition.

In addition, the use of IgEki mice to induce aggravated AE is a limitation of this study. IgEki mice express high total IgE and do not express IgG1 antibodies, since the exons encoding the soluble form of IgE are preceded by the IgG1 class switch region and downstream by the membrane exons of IgG in the animals.[26] NC/EW IgEki mice exhibited a greater number of significant metabolite changes than OVA/EW WT mice, when those were compared to NC/EW WT mice. Although IgA antibodies play the main role in intestinal immunity and homeostasis, IgG antibodies are partly involved in the maintenance of commensal bacteria and the induction of oral tolerance in gut-associated lymphoid tissues.[95] In addition to the high levels of IgE antibodies, the defect of IgG1 antibodies might influence the gut microbiome profile and induction of AE, thereby altering metabolome in NC/EW and OVA/EW IgEki mice. In conclusion, our study demonstrated remarkable shifts in metabolism in experimental AE. Furthermore, high-IgE levels alter broader metabolisms at the severe inflammation stage, including those related to the gut microbiota. However, to determine the direct influence of IgE on the gut microbiome, it may be necessary to perform IgE-seq to identify IgE-coated gut bacteria and verify whether the bacteria are involved in the onset and development of allergies. Although this study could be valuable in understanding the molecular mechanisms of AE, further studies in humans are needed to analyse and validate the proposed panel of biomarkers. Our results also showed the impact of high-IgE levels on the gut microbiome. Association studies between gut metabolome and bacterial composition are challenging. The gut metabolome is influenced by diet, nutrient absorption, host-derived and microbial-derived metabolites, and the effects of specific metabolites on the gut microbiota remain largely unknown. Hence the value of our study, whose findings reiterate the complexity of AE and guide our approaches to the prevention, treatment and control of this disease.

AUTHOR CONTRIBUTIONS

The conceptualization of the study was carried out by MT, AV, YP, MP-G and DB.; Metabolome analysis was performed by EZ-V and TCB-T, supervised by DR, AV and MP-G. Animal works were done by FB-P and HS.; FACS analysis was carried out by FB-P.; Immunological assays were performed by FB-P, JL and MK.; YP designed the animal model (IgEki mice); MK worked for design of the animal breeding; Histological analysis were made by IG-M, MM and LQ-M.; Gut microbiome analysis was made by JI and TN.; EZ-V and MP-G performed the gut microbiome and metabolome association analysis; Data visualization was made by EZ-V (Metabolome analysis and association analysis with microbiome), FB-P (Immunological analysis), JI (Microbiome analysis) and IG-M and MM (Histological analysis).; The writing of the manuscript was performed by EZ-V, FB-P, TCB-T, MT and MP-G.; Supervision was carried out by CB, SV, DB, MT and MP-G. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in NIH Common Fund's National Metabolomics Data Repository at https://www.metabolomicsworkbench.org, reference number DOI: 10.21228/M8D42R.

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REFERENCES

1. Waserman S, Bégin P, Watson W. IgE-mediated food allergy. *Allergy Asthma Clin Immunol*. 2018;14:55.
2. Anvari S, Miller J, Yeh C-Y, Davis CM. IgE-mediated food allergy. *Clin Rev Allergy Immunol*. 2019;57:244-260.
3. Ho MH-K, Wong WH-S, Chang C. Clinical spectrum of food allergies. A comprehensive review. *Clin Rev Allergy Immunol*. 2014;46:225-240.
4. Burggraf M, Nakajima-Adachi H, Hachimura S, et al. Oral tolerance induction does not resolve gastrointestinal inflammation in a mouse model of food allergy. *Mol Nutr Food Res*. 2011;55:1475-1483.
5. Zubeldia-Varela E, Barker-Tejeda TC, Blanco-Pérez F, Infante S, Zubeldia JM, Pérez-Gordo M. Non-IgE-mediated gastrointestinal food protein-induced allergic disorders. Clinical perspectives and analytical approaches. *Foods*. 2021;10:2662.
6. Ruffner MA, Ruymann K, Barni S, Cianferoni A, Brown-Whitehorn T, Spergel JM. Food protein-induced enterocolitis syndrome. Insights from review of a large referral population. *J Allergy Clin Immunol Pract*. 2013;1:343-349.
7. Caubet JC, Ford LS, Sickles L, et al. Clinical features and resolution of food protein-induced enterocolitis syndrome. 10-year experience. *J Allergy Clin Immunol*. 2014;134:382-389.
8. Hwang J-B, Sohn SM, Kim AS. Prospective follow-up oral food challenge in food protein-induced enterocolitis syndrome. *Arch Dis Child*. 2009;94:425-428.
9. Ishige T, Yagi H, Tatsuki M, et al. Endoscopic findings in the acute phase of food protein-induced enterocolitis syndromae. *Pediatr Allergy Immunol*. 2015;26:90-91.
10. Savage J, Johns CB. Food allergy. Epidemiology and natural history. *Immunol Allergy Clin N Am*. 2015;35:45-59.
11. Nowak-Wegrzyn A, Sampson HA, Wood RA, Sicherer SH. Food protein-induced enterocolitis syndrome caused by solid food proteins. *Pediatrics*. 2003;111:829-835.
12. Nowak-Węgrzyn A, Katz Y, Mehr SS, Koletzko S. Non-IgE-mediated gastrointestinal food allergy. *J Allergy Clin Immunol*. 2015;135:1114-1124.
13. Caubet J-C, Szajewska H, Shamir R, Nowak-Węgrzyn A. Non-IgE-mediated gastrointestinal food allergies in children. *Pediatr Allergy Immunol*. 2017;28:6-17.
14. Czerwionka-Szaflarska M, Łoś-Rycharska E, Gawryjołek J. Allergic enteritis in children. *Prz Gastroenterol*. 2017;12:1-5.
15. Pineton de Chambrun G, Desreumaux P, Cortot A. Eosinophilic enteritis. *Dig Dis*. 2015;33:183-189.
16. Bischoff SC. Food allergy and eosinophilic gastroenteritis and colitis. *Curr Opin Allergy Clin Immunol*. 2010;10:238-245.
17. Prussin C. Eosinophilic gastroenteritis and related eosinophilic disorders. *Gastroenterol Clin N Am*. 2014;43:317-327.
18. Quinn KD, Schedel M, Nkrumah-Elie Y, et al. Dysregulation of metabolic pathways in a mouse model of allergic asthma. *Allergy*. 2017;72:1327-1337.
19. Queener AM, Chiarella SE, Cuervo-Pardo L, Coden ME, Abdala-Valencia H, Berdnikovs S. Metabolism of epithelial cells in health and allergic disease: collegium Internationale Allergologicum update 2021. *Int Arch Allergy Immunol*. 2021;182:663-678.
20. Park S, Choi N-K, Kim S, Lee C-H. The relationship between metabolic syndrome and asthma in the elderly. *Sci Rep*. 2018;8:9378.
21. Obeso D, Zubeldia-Varela E, Villaseñor A. Uncovering the influence of diet and gut microbiota in human serum metabolome. *Allergy*. 2021;76:2306-2308.
22. López-López Á, López-Gonzálvez Á, Barker-Tejeda TC, Barbas C. A review of validated biomarkers obtained through metabolomics. *Expert Rev Mol Diagn*. 2018;18:557-575.
23. Villaseñor A, Rosace D, Obeso D, et al. Allergic asthma: an overview of metabolomic strategies leading to the identification of biomarkers in the field. *Clin Exp Allergy*. 2017;47:442-456.
24. Lee-Sarwar KA, Kelly RS, Lasky-Su J, et al. Integrative analysis of the intestinal metabolome of childhood asthma. *J Allergy Clin Immunol*. 2019;144:442-454.
25. Crestani E, Harb H, Charbonnier L-M, et al. Untargeted metabolomic profiling identifies disease-specific signatures in food allergy and asthma. *J Allergy Clin Immunol*. 2020;145:897-906.
26. Lubben W, Turqueti-Neves A, Okhrimenko A, et al. IgE knock-in mice suggest a role for high levels of IgE in basophil-mediated active systemic anaphylaxis. *Eur J Immunol*. 2013;43:1231-1242.
27. Weigmann B, Tubbe I, Seidel D, Nicolaev A, Becker C, Neurath MF. Isolation and subsequent analysis of murine lamina propria mononuclear cells from colonic tissue. *Nat Protoc*. 2007;2:2307-2311.
28. Binek A, Rojo D, Godzien J, et al. Flow cytometry has a significant impact on the cellular metabolome. *J Proteome Res*. 2019;18:169-181.
29. Zubeldia-Violeta E, Raczkowska BA, Ferrer M, Perez-Gordo M, Rojo D. Chapter 4 Techniques for phenotyping the gut microbiota metabolome. In: Faintuch J, Faintuch S, eds. *Microbiome and Metabolome in Diagnosis, Therapy, and Other Strategic Applications*. Academic Press; 2019:33-41.
30. Mamani-Huanca M, de La Fuente AG, Otero A, et al. Enhancing confidence of metabolite annotation in capillary electrophoresis-mass spectrometry untargeted metabolomics with relative migration time and in-source fragmentation. *J Chromatogr A*. 2021;1635:461758.

31. Mamani-Huanca M, Gradillas A, de La Fuente AG, López-Gonzálvez Á, Barbas C. Unveiling the fragmentation mechanisms of modified amino acids as the key for their targeted identification. *Anal Chem*. 2020;92:4848-4857.
32. Garcia A, Barbas C. Gas chromatography-mass spectrometry (GC-MS)-based metabolomics. *Methods Mol Biol*. 2011;708:191-204.
33. Godzien J, Alonso-Herranz V, Barbas C, Armitage EG. Controlling the quality of metabolomics data: new strategies to get the best out of the QC sample. *Metabolomics*. 2015;11:518-528.
34. Dudzik D, Barbas-Bernardos C, García A, Barbas C. Quality assurance procedures for mass spectrometry untargeted metabolomics. A review. *J Pharm Biomed Anal*. 2018;147:149-173.
35. Gil-de-la-Fuente A, Godzien J, Saugar S, et al. CEU mass mediator 3.0: a metabolite annotation tool. *J Proteome Res*. 2019;18:797-802.
36. Kind T, Wohlgemuth G, Lee DY, et al. FiehnLib: mass spectral and retention index libraries for metabolomics based on quadrupole and time-of-flight gas chromatography/mass spectrometry. *Anal Chem*. 2009;81:10038-10048.
37. Stein SE. *NIST/EPA/NIH Mass Spectral Library-PC Version, NIST Standard Reference Database 1A*. NIST; 1977.
38. Wishart DS, Feunang YD, Marcu A, et al. HMDB 4.0: the human metabolome database for 2018. *Nucleic Acids Res*. 2018;46:D608-D617.
39. Kanehisa M, Sato Y, Kawashima M. KEGG mapping tools for uncovering hidden features in biological data. *Protein Sci*. 2022;31:47-53.
40. Chong J, Wishart DS, Xia J. Using MetaboAnalyst 4.0 for comprehensive and integrative metabolomics data analysis. *Curr Protoc Bioinformatics*. 2019;68:e86.
41. Kamburov A, Cavill R, Ebbels TMD, Herwig R, Keun HC. Integrated pathway-level analysis of transcriptomics and metabolomics data with IMPaLA. *Bioinformatics*. 2011;27:2917-2918.
42. Usami K, Niimi K, Matsuo A, et al. The gut microbiota induces Peyer's-patch-dependent secretion of maternal IgA into milk. *Cell Rep*. 2021;36:109655.
43. Islam J, Tanimizu M, Shimizu Y, et al. Development of a rational framework for the therapeutic efficacy of fecal microbiota transplantation for calf diarrhea treatment. *Microbiome*. 2022;10:31.
44. Bolyen E, Rideout JR, Dillon MR, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat Biotechnol*. 2019;37:852-857.
45. McDonald D, Price MN, Goodrich J, et al. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *ISME J*. 2012;6:610-618.
46. Lu Y, Zhou G, Ewald J, Pang Z, Shiri T, Xia J. MicrobiomeAnalyst 2.0: comprehensive statistical, functional and integrative analysis of microbiome data. *Nucleic Acids Res*. 2023;51:W310-W318.
47. Segata N, Izard J, Waldron L, et al. Metagenomic biomarker discovery and explanation. *Genome Biol*. 2011;12:R60.
48. Sicherer SH, Sampson HA. Food allergy. A review and update on epidemiology, pathogenesis, diagnosis, prevention, and management. *J Allergy Clin Immunol*. 2018;141:41-58.
49. Chilton FH, Averill FJ, Hubbard WC, Fonteh AN, Triggiani M, Liu MC. Antigen-induced generation of lyso-phospholipids in human airways. *J Exp Med*. 1996;183:2235-2245.
50. Yoder M, Zhuge Y, Yuan Y, et al. Bioactive lysophosphatidylcholine 16:0 and 18:0 are elevated in lungs of asthmatic subjects. *Allergy Asthma Immunol Res*. 2014;6:61-65.
51. Arbibe L, Koumanov K, Vial D, et al. Generation of lyso-phospholipids from surfactant in acute lung injury is mediated by type-II phospholipase A2 and inhibited by a direct surfactant protein A-phospholipase A2 protein interaction. *J Clin Invest*. 1998;102:1152-1160.
52. Vladykovskaya E, Ozhegov E, Hoetker JD, et al. Reductive metabolism increases the proinflammatory activity of aldehyde phospholipids. *J Lipid Res*. 2011;52:2209-2225.
53. Zhuge Y, Yuan Y, van Breemen R, et al. Stimulated bronchial epithelial cells release bioactive lysophosphatidylcholine 16:0, 18:0, and 18:1. *Allergy Asthma Immunol Res*. 2014;6:66-74.
54. Nishiyama O, Kume H, Kondo M, Ito Y, Ito M, Yamaki K. Role of lysophosphatidylcholine in eosinophil infiltration and resistance in airways. *Clin Exp Pharmacol Physiol*. 2004;31:179-184.
55. Rohrhofer J, Zwirzitz B, Selberherr E, Untersmayr E. The impact of dietary sphingolipids on intestinal microbiota and gastrointestinal immune homeostasis. *Front Immunol*. 2021;12:635704.
56. Bryan P-F, Karla C, Edgar Alejandro M-T, Sara Elva E-P, Gemma F, Luz C. Sphingolipids as mediators in the crosstalk between microbiota and intestinal cells: implications for inflammatory bowel disease. *Mediat Inflamm*. 2016;2016:9890141.
57. Kayser BD, Prifti E, Lhomme M, et al. Elevated serum ceramides are linked with obesity-associated gut dysbiosis and impaired glucose metabolism. *Metabolomics*. 2019;15:140.
58. Xue L, Zhao Y, Wang H, et al. The effects of live and pasteurized *Akkermansia muciniphila* on DSS-induced ulcerative colitis, gut microbiota, and metabolomics in mice. *Food Funct*. 2023;14:4632-4646.
59. Parrish A, Boudaud M, Grant ET, et al. *Akkermansia muciniphila* exacerbates food allergy in fibre-deprived mice. *Nat Microbiol*. 2023;8:1863-1879.
60. Kihara A, Igarashi Y. Cross talk between sphingolipids and glycerophospholipids in the establishment of plasma membrane asymmetry. *Mol Biol Cell*. 2004;15:4949-4959.
61. Castro-Gómez P, Garcia-Serrano A, Visioli F, Fontecha J. Relevance of dietary glycerophospholipids and sphingolipids to human health. *Prostaglandins Leukot Essent Fatty Acids*. 2015;101:41-51.
62. Huang Y-S, Huang W-C, Li C-W, Chuang L-T. Eicosadienoic acid differentially modulates production of pro-inflammatory modulators in murine macrophages. *Mol Cell Biochem*. 2011;358:85-94.
63. Rodriguez-Perez N, Schiavi E, Frei R, et al. Altered fatty acid metabolism and reduced stearoyl-coenzyme a desaturase activity in asthma. *Allergy*. 2017;72:1744-1752.
64. Soto-Ramírez N, Karmaus W, Zhang H, et al. Fatty acids in breast milk associated with asthma-like symptoms and atopy in infancy: a longitudinal study. *J Asthma*. 2012;49:926-934.
65. Patumcharoenpol P, Kingkaw A, Nakphaichit M, et al. Exploring longitudinal gut microbiome towards metabolic functional changes associated in atopic dermatitis in early childhood. *Biology*. 2023;12:1262.
66. Wang Y, Hou J, Tsui JC-C, et al. Unique gut microbiome signatures among adult patients with moderate to severe atopic dermatitis in southern Chinese. *IJMS*. 2023;24:12856.
67. Zhu L, Xu F, Wan W, et al. Gut microbial characteristics of adult patients with allergy rhinitis. *Microb Cell Factories*. 2020;19:171.
68. Nguyen M, Sharma A, Wu W, et al. The fermentation product 2,3-butanediol alters *P. aeruginosa* clearance, cytokine response and the lung microbiome. *ISME J*. 2016;10:2978-2983.
69. Syu MJ. Biological production of 2,3-butanediol. *Appl Microbiol Biotechnol*. 2001;55:10-18.
70. Le Ta DH, Chan JCY, Yap GC, et al. A compromised developmental trajectory of the infant gut microbiome and metabolome in atopic eczema. *Gut Microbes*. 2020;12:1-22.
71. Song CW, Park JM, Chung SC, Lee SY, Song H. Microbial production of 2,3-butanediol for industrial applications. *J Ind Microbiol Biotechnol*. 2019;46:1583-1601.
72. Di Costanzo M, de Paulis N, Biasucci G. Butyrate: a link between early life nutrition and gut microbiome in the development of food allergy. *Life (Basel)*. 2021;11:384.
73. Watts AM, West NP, Zhang P, Smith PK, Cripps AW, Cox AJ. The gut microbiome of adults with allergic rhinitis is characterised by reduced diversity and an altered abundance of key microbial taxa compared to controls. *Int Arch Allergy Immunol*. 2021;182:94-105.

74. Cait A, Cardenas E, Dimitriu PA, et al. Reduced genetic potential for butyrate fermentation in the gut microbiome of infants who develop allergic sensitization. *J Allergy Clin Immunol*. 2019;144:1638-1647.e3.
75. Corrêa-Oliveira R, Fachi JL, Vieira A, Sato FT, Vinolo MAR. Regulation of immune cell function by short-chain fatty acids. *Clin Transl Immunology*. 2016;5:e73.
76. Arpaia N, Campbell C, Fan X, et al. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature*. 2013;504:451-455.
77. Furusawa Y, Obata Y, Fukuda S, et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature*. 2013;504:446-450.
78. He C, Liu Y, Schülke S, et al. β-1,3-glucan, but not β-1,3/1,6-glucan, exacerbates experimental food allergy, while both increase IgA induction. *Allergy*. 2024;79:503-506.
79. White Z, Cabrera I, Kapustka I, Sano T. Microbiota as key factors in inflammatory bowel disease. *Front Microbiol*. 2023;14:1155388.
80. Durack J, Kimes NE, Lin DL, et al. Delayed gut microbiota development in high-risk for asthma infants is temporarily modifiable by lactobacillus supplementation. *Nat Commun*. 2018;9:707.
81. Rosas-Salazar C, Shilts MH, Tovchigrechko A, et al. Nasopharyngeal lactobacillus is associated with a reduced risk of childhood wheezing illnesses following acute respiratory syncytial virus infection in infancy. *J Allergy Clin Immunol*. 2018;142:1447-1456.e9.
82. Heeney DD, Gareau MG, Marco ML. Intestinal lactobacillus in health and disease, a driver or just along for the ride. *Curr Opin Biotechnol*. 2017;49:140-147.
83. Herman KS, Bowsher RR, Henry DP. Synthesis of N pi-methylhistamine and N alpha-methylhistamine by purified rabbit lung indolethylamine N-methyltransferase. *J Biol Chem*. 1985;260:12336-12340.
84. Steiner JM. Review of commonly used clinical pathology parameters for general gastrointestinal disease with emphasis on small animals. *Toxicol Pathol*. 2014;42:189-194.
85. Simpson KW, Fyfe J, Cornetta A, et al. Subnormal concentrations of serum cobalamin (vitamin B12) in cats with gastrointestinal disease. *J Vet Intern Med*. 2001;15:26-32.
86. Smolinska S, Jutel M, Crameri R, O'Mahony L. Histamine and gut mucosal immune regulation. *Allergy*. 2014;69:273-281.
87. Lieberman P. The basics of histamine biology. *Ann Allergy Asthma Immunol*. 2011;106:S2-S5.
88. Voelker D, Pongdee T. Urine mast cell mediators in the evaluation and diagnosis of mast cell activation syndrome. *Curr Allergy Asthma Rep*. 2024;24:33-38.
89. Butterfield J, Weiler CR. The utility of measuring urinary metabolites of mast cell mediators in systemic mastocytosis and mast cell activation syndrome. *J Allergy Clin Immunol Pract*. 2020;8:2533-2541.
90. Biermé P, Nowak-Wegrzyn A, Caubet J-C. Non-IgE-mediated gastrointestinal food allergies. *Curr Opin Pediatr*. 2017;29:697-703.
91. Roth-Walter F, Berni Canani R, O'Mahony L, et al. Nutrition in chronic inflammatory conditions: bypassing the mucosal block for micronutrients. *Allergy*. 2024;79:353-383.
92. Roth-Walter F, Adcock IM, Benito-Villalvilla C, et al. Metabolic pathways in immune senescence and inflammaging: novel therapeutic strategy for chronic inflammatory lung diseases. An EAACI Position Paper from the Task Force for Immunopharmacology. *Allergy*. 2023;79:1089-1122.
93. Caruso R, Lo BC, Núñez G. Host-microbiota interactions in inflammatory bowel disease. *Nat Rev Immunol*. 2020;20:411-426.
94. Liu R, Hong J, Xu X, et al. Gut microbiome and serum metabolome alterations in obesity and after weight-loss intervention. *Nat Med*. 2017;23:859-868.
95. Castro-Dopico T, Clatworthy MR. IgG and Fcγ receptors in intestinal immunity and inflammation. *Front Immunol*. 2019;10:805.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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