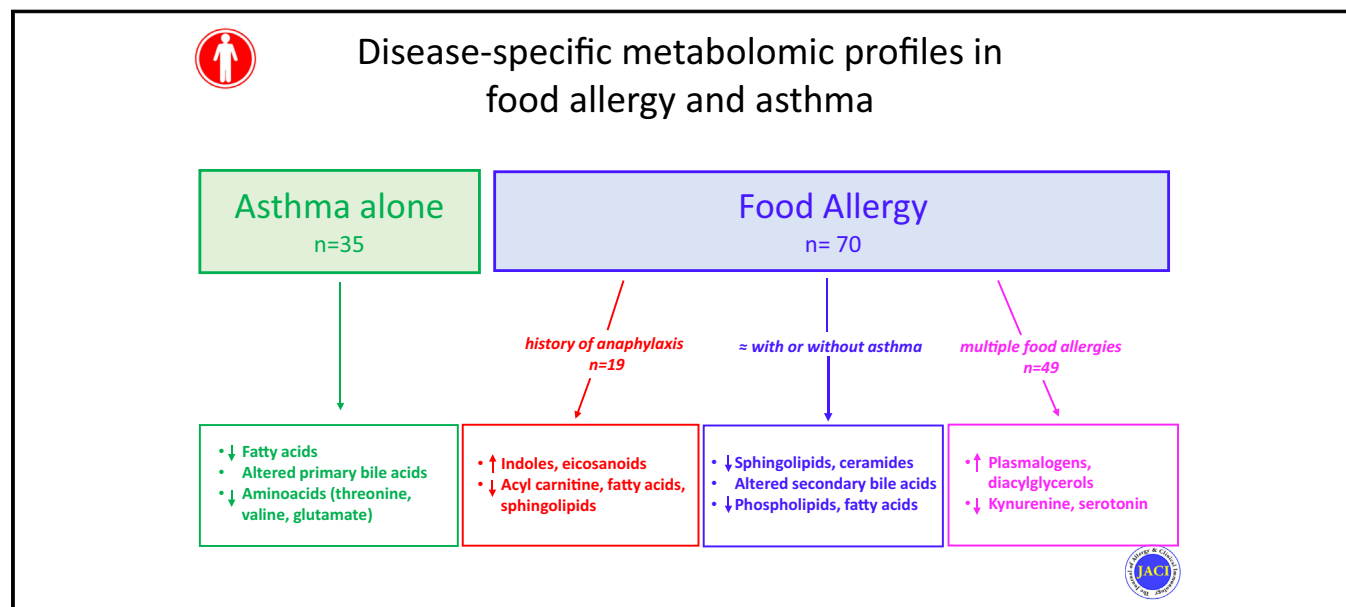


Untargeted metabolomic profiling identifies disease-specific signatures in food allergy and asthma



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GRAPHICAL ABSTRACT



Background: Food allergy (FA) affects an increasing proportion of children for reasons that remain obscure. Novel disease biomarkers and curative treatment options are strongly needed. **Objective:** We sought to apply untargeted metabolomic profiling to identify pathogenic mechanisms and candidate disease biomarkers in patients with FA.

Methods: Mass spectrometry-based untargeted metabolomic profiling was performed on serum samples of children with either FA alone, asthma alone, or both FA and asthma, as well as healthy pediatric control subjects.

Results: In this pilot study patients with FA exhibited a disease-specific metabolomic signature compared with both control subjects and asthmatic patients. In particular, FA was uniquely associated with a marked decrease in sphingolipid levels, as

well as levels of a number of other lipid metabolites, in the face of normal frequencies of circulating natural killer T cells. Specific comparison of patients with FA and asthmatic patients revealed differences in the microbiota-sensitive aromatic amino acid and secondary bile acid metabolism. Children with both FA and asthma exhibited a metabolomic profile that aligned with that of FA alone but not asthma. Among children with FA, the history of severe systemic reactions and the presence of multiple FAs were associated with changes in levels of tryptophan metabolites, eicosanoids, plasmalogens, and fatty acids.

Conclusions: Children with FA have a disease-specific metabolomic profile that is informative of disease mechanisms and severity and that dominates in the presence of asthma.

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Lower levels of sphingolipids and ceramides and other metabolomic alterations observed in children with FA might reflect the interplay between an altered microbiota and immune cell subsets in the gut. (J Allergy Clin Immunol 2020;145:897-906.)

Key words: Asthma, food allergy, invariant natural killer T cells, metabolomics, metabolites, secondary bile acids, sphingolipids, tryptophan

Allergic diseases, including food allergy (FA) and asthma, exert significant morbidity worldwide, with FA affecting up to 8% of children and 4% to 10% of adults in the United States alone.¹⁻⁴ Yet the availability of accurate biomarkers and effective treatments lags behind the ongoing FA epidemic, leaving providers with imperfect diagnostic tools and patients with food avoidance as their main treatment option. Allergic disorders are complex conditions that result from the interaction of genetic, epigenetic, and environmental influences.⁵ Although allergic disorders are defined by their shared type 2 immune mechanisms,⁶ their markedly diverse clinical manifestations suggest that additional unique pathogenic processes are involved in shaping their heterogeneous phenotypic attributes. A deeper understanding of such pathogenic mechanisms might lead to much needed improved disease biomarkers of disease, as well as to novel treatment strategies.

Untargeted metabolomic profiling has emerged as a powerful technique to dissect altered pathways contributing to complex diseases.⁷⁻¹⁰ Its ability to detect alterations that are influenced by genetic and/or environmental factors makes it particularly useful for unveiling disease mechanisms and for biomarker discovery in patients with complex atopic disorders. Several studies have applied untargeted metabolomics to the study of asthma and have identified metabolic signatures associated with severity and endotypes of asthma.¹¹⁻¹³ In comparison, very few studies have applied untargeted metabolomics to investigate FA. One murine study showed alterations in uric acid metabolism associated with peanut allergy, leading the authors to hypothesize a role for alarmins in FA pathogenesis.¹⁴ More recently, a study of fecal metabolomics suggested that intestinal *Bacteroides* species–derived sphingolipids particularly *Bacteroides fragilis*–derived α -galactosylceramide were associated with protection against clinical FA in food-sensitized children, possibly through modulation of invariant natural killer T (iNKT) cell function.¹⁵

In this pilot study we performed untargeted metabolomic profiling in children with FA with and without asthma, in children with asthma, and in nonatopic control subjects to better define the metabolic changes associated with each clinical entity. These studies aimed to identify metabolites that uniquely correlate with the presence of FA and to gain insight into the underlying mechanisms involved in disease pathogenesis. Results revealed unique metabolomic signatures associated with FA that shed light on underlying pathophysiologic mechanisms.

METHODS

Study population

A total of 125 subjects were included in this study: 35 children with FA alone, 35 with asthma alone, and 35 with both FA and asthma and 20 nonatopic control subjects. Children aged 12 years and younger were included, with only nonatopic control subjects including those up to 18 years of age. Participants

Abbreviations used

CDCA:	Chenodeoxycholic acid
FA:	Food allergy
iNKT:	Invariant natural killer T
NK:	Natural killer
Treg:	Regulatory T
UPLC-MS/MS:	Ultraperformance liquid chromatography coupled with high-resolution/accurate mass spectrometry

were recruited in the allergy clinic at Boston Children's Hospital and from the School Inner City Asthma Study cohort, a National Institutes of Health–funded prospective study of environmental risk factors in inner city school children with asthma (principal investigator: W. Phipatanakul).^{16,17} The study was approved by the Boston Children's Hospital Institutional Review Board.

The diagnosis of FA was made by the treating physician and was based on a combination of positive IgE test results (skin tests, specific IgE measurements, or both) and a recent history of IgE-mediated symptoms (eg, hives, angioedema, emesis, wheezing, and hypotension) developing within 2 hours of ingestion of the culprit food. Asthma diagnosis was based on validated questionnaire and American Thoracic Society criteria.^{16,17} Asthmatic patients requiring treatment with systemic steroids were excluded. Nonatopic control subjects had no personal history of IgE-mediated conditions. Exclusion criteria included a history of non-IgE-mediated or chronic inflammatory gastrointestinal disorders, use of immunosuppressive medications, and use of antibiotics within the previous 6 weeks.

Children with FA were further classified based on the number of physician-diagnosed FAs (1 vs multiple) and the history of systemic reactions requiring use of intramuscular epinephrine.

Sample preparation

Blood samples were collected in serum separator tubes, and serum was stored at -80°C within 2 hours of collection, according to recommended processing guidelines for metabolomic studies.

Metabolomic profiling

Nontargeted global metabolomic profiles were generated through Metabolon (Research Triangle, NC) using ultraperformance liquid chromatography coupled with high-resolution/accurate mass spectrometry (UPLC-MS/MS). Four platforms were used to detect a comprehensive list of metabolites: (1) UPLC-MS/MS under positive ionization, (2) UPLC-MS/MS under negative ionization, (3) UPLC-MS/MS polar platform (negative ionization), and (4) gas chromatography–mass spectrometry. Metabolites were identified by their m/z retention time and through comparison with library entities of purified known standards.

iNKT cell analyses

PBMCs were isolated by using Ficoll gradient sedimentation, according to standard protocols. Natural killer (NK), NKT, and iNKT cells were identified as $\text{CD3}^{-}\text{CD56}^{+}$, $\text{CD3}^{+}\text{CD56}^{+}$, and $\text{CD3}^{+}\text{CD56}^{+}\text{V24Ja18}^{+}$ cells, respectively (BioLegend, San Diego, Calif). iNKT cells were expressed as a percentage of PBMCs and compared between patients with FA, asthmatic patients, and nonatopic control subjects. The Mann-Whitney test was used to compare iNKT cell proportions, and the significance level was set at a P value of .05 or less.

Statistical analysis

Pairwise comparisons were performed to identify metabolites and pathways dysregulated among different groups. After log-transformation and imputation of missing values, if any, with the minimum observed value for

TABLE I. Summary characteristics of the study population

	Asthmatic children (n = 35)	Children with FA (n = 35)	Children with FA and asthma (n = 35)	Control subjects (n = 20)	P value
Age (y)	7.5*	4.1*	5.5	6.3	<.05*
Number (male/female sex)	35 (20/15)	35 (19/16)	35 (21/14)	20 (10/10)	NS
BMI (median percentile for age)	61	66.5	60.5	70	NS
Self-reported race (%)					
White	17	60	46	40	
African American	40	11	14	20	
Hispanic	37	9	28	35	
Asian	0	9	6	0	
Other/NA	6	11	6	5	
Allergic rhinitis	27/35 (77%)	23/35 (67%)	30/35 (86%)	0/35	NS
Atopic dermatitis	8/35* (23%)	21/35* (60%)	20/35* (57%)	0/35	<.05*
Total IgE (geometric mean, kU/L)	NA	219†	547†	26.5†	<.005†
Number of food allergies (%)					
1		29	31		
≥2		71	69		
Anaphylaxis (%)		23	29		

BMI, Body mass index; NA, not available; NS, not significant.

* $P < .05$.

† $P < .005$.

each compound, each biochemical was rescaled to set the median equal to 1 (scaled value). The Welch t test, the Mann-Whitney test, and ANOVA contrasts were used to identify biochemicals that differed significantly between experimental groups. A stringent level of significance at a P value of less than .005 (equivalent to a false discovery rate of less than 0.15 with Benjamini-Hochberg correction) was applied to account for multiple comparisons.¹⁸ Pairwise unadjusted contrasts were also applied to compare groups of children with FA with differential severity phenotypes based on the number of FAs and history of anaphylaxis. Statistical analyses were carried out with GraphPad Prism software (version 7a; GraphPad Software, La Jolla, Calif) and SPSS software (version 24; SPSS, Chicago, Ill).

Random forest analysis was performed to identify the most differentially expressed metabolites in pairwise group comparisons. Using a 50,000-tree forest size, the importance score was used to identify the top 30 ranking metabolites. Pathway enrichment analysis, mapping to the Metabolon proprietary database, was applied to identify metabolic pathways with metabolites that are overrepresented and are therefore targets of significant perturbations under different disease conditions. A pathway enrichment value of greater than 1 indicates that the pathway contains more experimentally regulated compounds relative to the study overall. Pathway enrichment was determined as follows:

(Number of significant metabolites in pathway [k]/Total number of detected metabolites in pathway [m])/(Total number of significant metabolites [n]/Total number of detected metabolites [N]) or (k/m)/(n/N).

Regression analyses were performed to adjust for characteristics showing statistically different distributions among study groups, in particular atopic dermatitis given its known association with FA. Adjusted analyses resulted in a smaller number of significant metabolites, although all metabolomic pathways detected in unadjusted analyses were confirmed to be dysregulated in adjusted comparisons.

RESULTS

Characteristics of study subjects

Population demographics and disease attributes are summarized in Table I. Detailed patient information is provided in Table E1 in this article's Online Repository at www.jacionline.org. A slightly higher but not statistically significant proportion of male participants was present in all groups except for healthy control subjects. The median age was 4.1 years for children with FA alone, 5.5 years for children with FA and asthma, 7.5 years for

children with asthma alone, and 6.3 years for control subjects. A marginally significant ($P < .05$) age difference was present only between children with FA alone and those with asthma alone. Differences in race distributions were observed, likely resulting from underlying recruitment strategies, with a higher proportion of nonwhite children in the asthma-only group. A slightly higher rate of allergic rhinitis prevalence was observed in children with asthma with and without FA compared with those with FA alone. Atopic dermatitis was significantly more frequent in children with FA with or without asthma compared with those with asthma alone. Total serum IgE concentrations were available for the majority of children with FA and were significantly higher in those with concomitant asthma. Total IgE concentrations were significantly lower in nonatopic control subjects. Among children with FA, about 30% of subjects had 1 FA, whereas the remainder had allergies to 2 or more foods. The history of anaphylactic reactions treated with epinephrine was similar in children with FA with and without asthma.

Global metabolomic profiling

A total of 1165 biochemicals, 868 compounds of known identity (named biochemicals) and 297 compounds of unknown structural identity (unnamed biochemicals), were detected in this study. After removal of drug metabolites and xenometabolites, 733 compounds were included in the analyses. Levels of gonadal steroid metabolites strongly correlated with age. Differences in this category of steroids are not reported because they are considered to reflect age effects rather than true pathophysiologic characteristics of the study groups.

Comparisons of children with FA, children with asthma, and control subjects

Pairwise comparison of children with FA and nonatopic control subjects revealed 53 metabolites that differed significantly at a stringent cutoff P value of less than .005 (Fig 1 and see Table E2 in this article's Online Repository at www.jacionline.org).

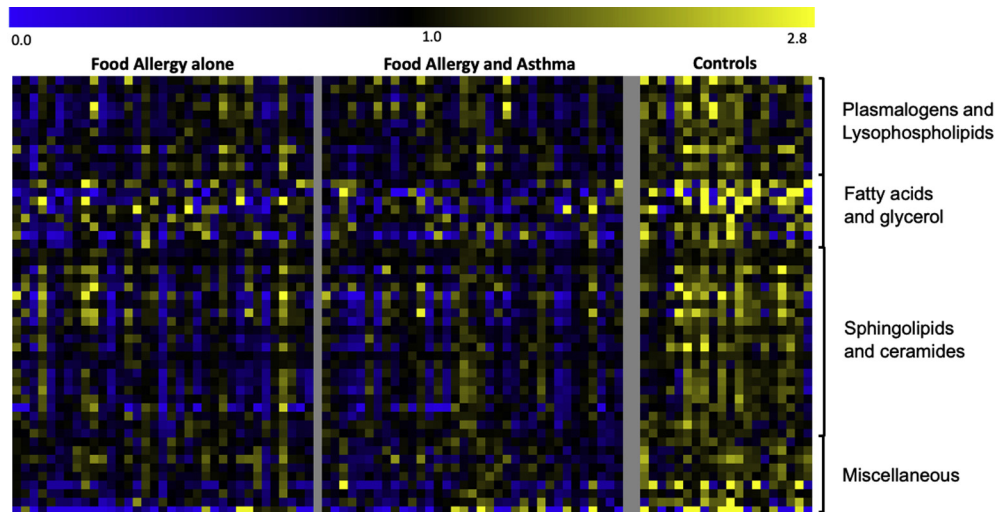


FIG 1. Heat map of metabolites significantly different ($P < .005$) between children with FA and nonatopic control subjects. For each metabolite, a colorimetric representation of relative expression in each sample is shown according to the scale depicted at the top. Metabolites are grouped into main dysregulated pathways and a miscellaneous category.

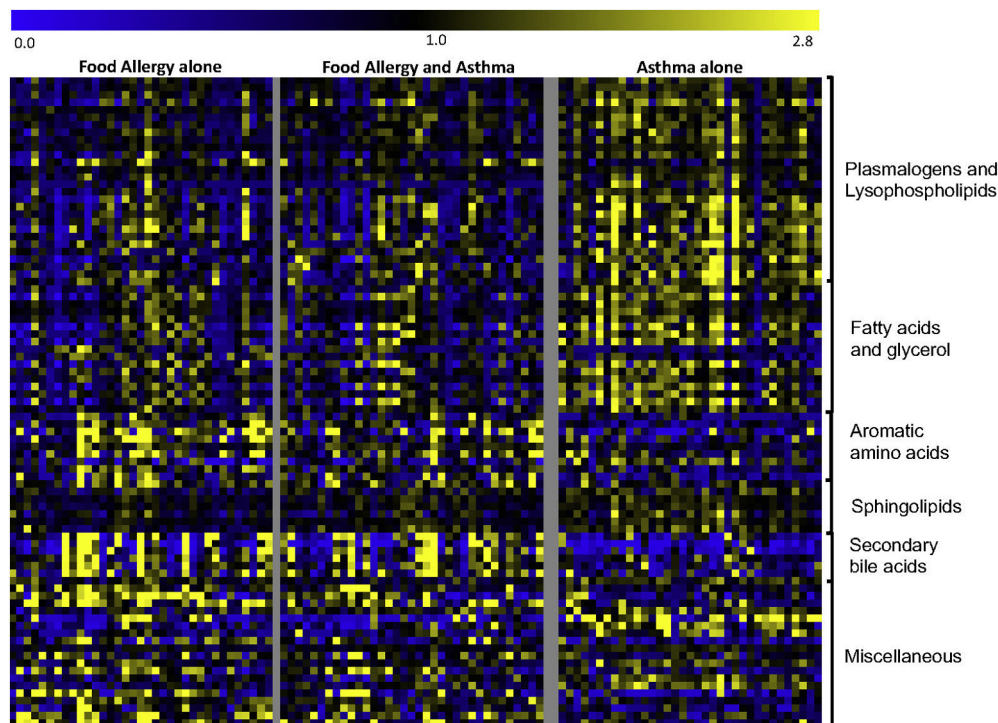


FIG 2. Heat map of metabolites significantly different ($P < .005$) between children with FA and children with asthma. For each metabolite, a colorimetric representation of relative expression in each sample is shown according to the scale depicted at the top. Metabolites are grouped into main dysregulated pathways and a miscellaneous category.

Prominent among these are lipid metabolites, most notably sphingolipids and ceramides, lysophospholipids, lysoplasmalogens, diacylglycerol, and fatty acids. Other metabolites included those of the amino acid lysine, threonine, and valine metabolism, and the β/γ isoforms of tocopherol. There were no differences in levels of these 53 metabolites between children with FA with and without eczema, and most remained significantly associated

with FA when analysis was restricted to only children with FA without eczema (see Table E7 in this article's Online Repository at www.jacionline.org). In comparison with children with asthma, patients with FA showed changes in 81 metabolites in pairwise comparisons, involving the metabolisms of sphingolipids, lysophospholipids, monoacylglycerol and diacylglycerol, fatty acids (monohydroxy and acylcholines), amino acids (tyrosine,

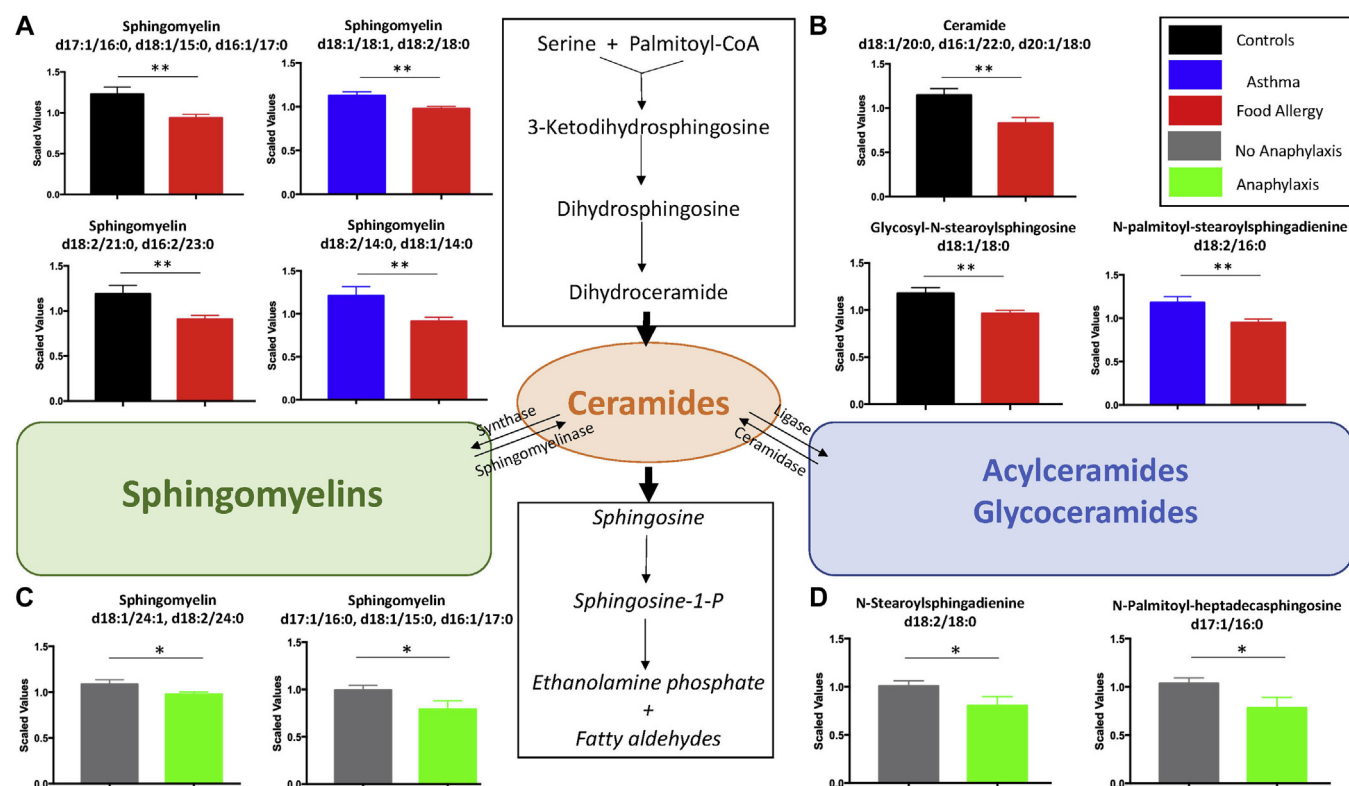


FIG 3. Sphingolipid alterations are prominent in patients with FA. Pairwise comparison of sphingomyelin (A and C) and ceramide (B and D) metabolites between children with FA and either asthmatic or nonatopic children, as well as between children with FA with and without a history of anaphylaxis treated with epinephrine, are shown. Means and SEMs are shown. * $P \leq .05$ and ** $P \leq .005$.

tryptophan, and histidine), bile acids, and tocopherol (Fig 2 and see Table E3 in this article's Online Repository at www.jacionline.org). Interestingly, no differences in levels of these 81 metabolites were present when comparing children with FA either with or without asthma, suggesting that children with these overlapping allergic phenotypes acquire a metabolomic signature concordant with that of FA alone rather than asthma alone. Nine metabolites were uniquely dysregulated in children with FA in comparison with both control subjects and asthmatic children, including the lysoplasmalogen metabolite 1-(1-enyl-palmitoyl)-GPC, lysophospholipids (1-arachidonoyl-GPC, 1-linoleoyl-GPC, 1-palmitoleoyl-GPC, 1-palmitoyl-GPC), N2-acetyllysine, β/γ tocopherol, and sphingomyelins. Alterations uniquely detected in the comparison of children with FA and those with asthma alone included changes in secondary bile acids and aromatic amino acid (histidine, tyrosine, and tryptophan) metabolisms.

Comparisons of children with asthma and control subjects

Pairwise contrasts of children with asthma versus nonatopic control subjects identified 51 metabolites that differed significantly ($P < .005$) between the 2 groups (see Fig E1 and Table E4 in this article's Online Repository at www.jacionline.org), with changes affecting the metabolism of plasmalogens, phospholipids, fatty acids (monohydroxy, medium chain, and dicarboxylate), sphingolipids, and amino acids (lysine, leucine, valine, and threonine). Among these are metabolites previously reported to

be associated with asthma phenotypes and pathogenesis, including amino acids (threonine,¹⁹⁻²¹ valine,²² isoleucine,²² and glutamate²³), primary bile acids (taurocholate),²⁴ fatty acids (caproate),²⁵ and phosphatidylcholine.²⁶

A highly pronounced sphingolipid signature in children with FA

A particularly prominent pathway affected in patients with FA is sphingolipid metabolism. The steps involved in ceramides and sphingolipid metabolism are schematically shown in Fig 3. Key to this pathway is the synthesis of ceramides and their further metabolisms to sphingomyelins and conjugated ceramides. Several metabolites along this pathway were decreased in children with FA compared with control subjects and children with asthma (Fig 3 and see Fig E2 in this article's Online Repository at www.jacionline.org). Most of the changes detected in patients with FA appeared to center on the conversion of ceramides to sphingomyelin and acylceramide and glucosylceramide, which were decreased in patients with FA (Fig 3, A and B). Alterations in this pathway were also associated with characteristics of FA severity, as described below (Fig 3, C and D).

FA is not associated with alterations in circulating iNKT cells

iNKT cells represent a small population of circulating NKT cells that carry an invariant T-cell receptor able to recognize lipid moieties, including sphingolipids produced by gut microbial

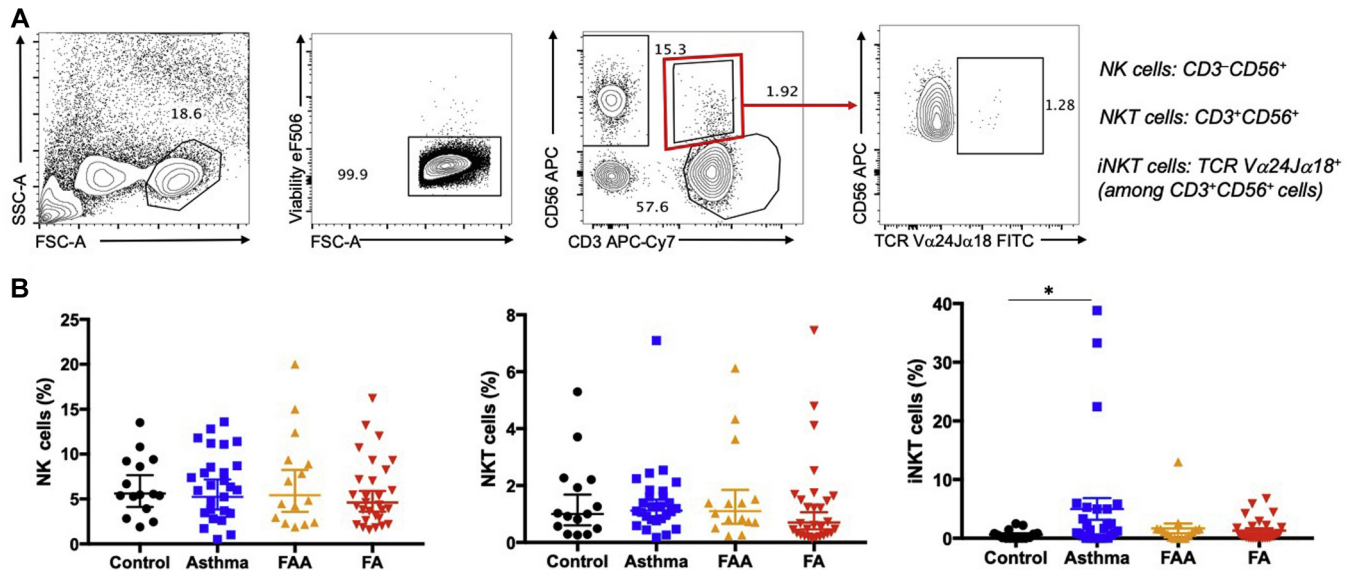


FIG 4. Circulating iNKT cells are unchanged in patients with FA. **A**, Gating strategy for NK, NKT, and iNKT cells. FSC, Forward scatter; SSC, side scatter. **B**, Proportions of NK, NKT, and iNKT cells in children with FA, children with asthma, children with FA and asthma (FAA), and nonatopic control subjects. * $P \leq .05$

species, such as *Bacteroidetes* and *B fragilis* in particular.^{27,28} A decrease in peripheral iNKT cells has been previously reported in association with FA.^{29,30} We sought to investigate whether changes in iNKT cells are present in children with FA compared with those in children with asthma and control subjects. The proportion of iNKT cells in PBMCs was measured in 28 patients with FA, 28 patients with asthma, 15 patients with both FA and asthma, and 15 nonatopic control subjects. No differences in the proportions of NK and NKT cells were detected among the different groups. The proportion of iNKT cells was not different between children with FA and control subjects, although an increased proportion of iNKT cells was observed in children with asthma, which is consistent with previous reports (Fig 4).^{31,32}

Metabolomic associations of FA severity phenotypes

Children with FA were further stratified based on the number of diagnosed FAs and on the history of systemic reactions requiring intramuscular epinephrine injection, as documented by a physician.

A total of 41 metabolites in diverse pathways were found to differ between children with FA to 1 food item ($n = 21$) and children with 2 or more FAs ($n = 49$, see Table E5 in this article's Online Repository at www.jacionline.org). Of interest, children with reactivity to more than 1 food showed significantly higher levels of plasmalogens and diacylglycerols and lower levels of tryptophan metabolites in the kynurenine and serotonin pathways and of acyl carnitine fatty acids (Fig 5 and see Fig E3 and Table E5).

A total of 19 metabolites were significantly different when comparing children with FA with ($n = 19$) and without ($n = 51$) a history of anaphylaxis requiring treatment with intramuscular epinephrine. A positive history of anaphylaxis was associated with higher levels of indole tryptophan metabolites and of eicosanoids and lower levels of branched and acyl carnitine fatty

acids and sphingolipids (Fig 5 and see Fig E3 and Table E6 in this article's Online Repository at www.jacionline.org).

Interestingly, both characteristics of FA severity were found to be associated with alterations in tryptophan metabolism. Tryptophan is an essential aromatic amino acid that in the gut is under the direct influence of the microbiota and is metabolized through the 3 main pathways depicted in Fig 5.³³ Although children with more than 1 FA exhibit a decrease in metabolites of the kynurenine and serotonin pathways, children with a positive history of anaphylaxis show a unique increase in the indole pathway, as reflected by significantly higher levels of the metabolite indole propionate.

Secondary bile acid metabolism

Changes in bile acid metabolism were observed in the pairwise comparison of children with FA and asthma (Fig 2 and see Fig E4 in this article's Online Repository at www.jacionline.org). Secondary bile acids are derived from primary bile acids through the action of the bacterial metabolism and they in turn modulate the composition of the gut microbiome.^{34,35}

To establish whether the observed differences in bile acid composition attributable to FA might be secondary to altered bacterial metabolism, we compared ratios of different bile acid species that reflect enzymatic conversion steps in the liver and gut.³⁶ A schematic illustration of bile acid metabolism is shown in Fig 6. No differences in cholesterol level and metabolism of primary bile acids, including changes between the classic and alternative pathways, were observed between asthmatic children with or without FA. When comparing ratios of microbiome-derived secondary bile acids with those of liver-generated primary bile acids, children with FA showed increased production of the alternative pathway secondary bile acids ursodeoxycholic acid (UDCA/chenodeoxycholic acid [CDCA] ratio), glyoursodeoxycholic acid (GUDCA/CDCA ratio), tauroursodeoxycholic acid (TUDCA/CDCA ratio), and hyocholic acid (HCA/GLCA ratio)

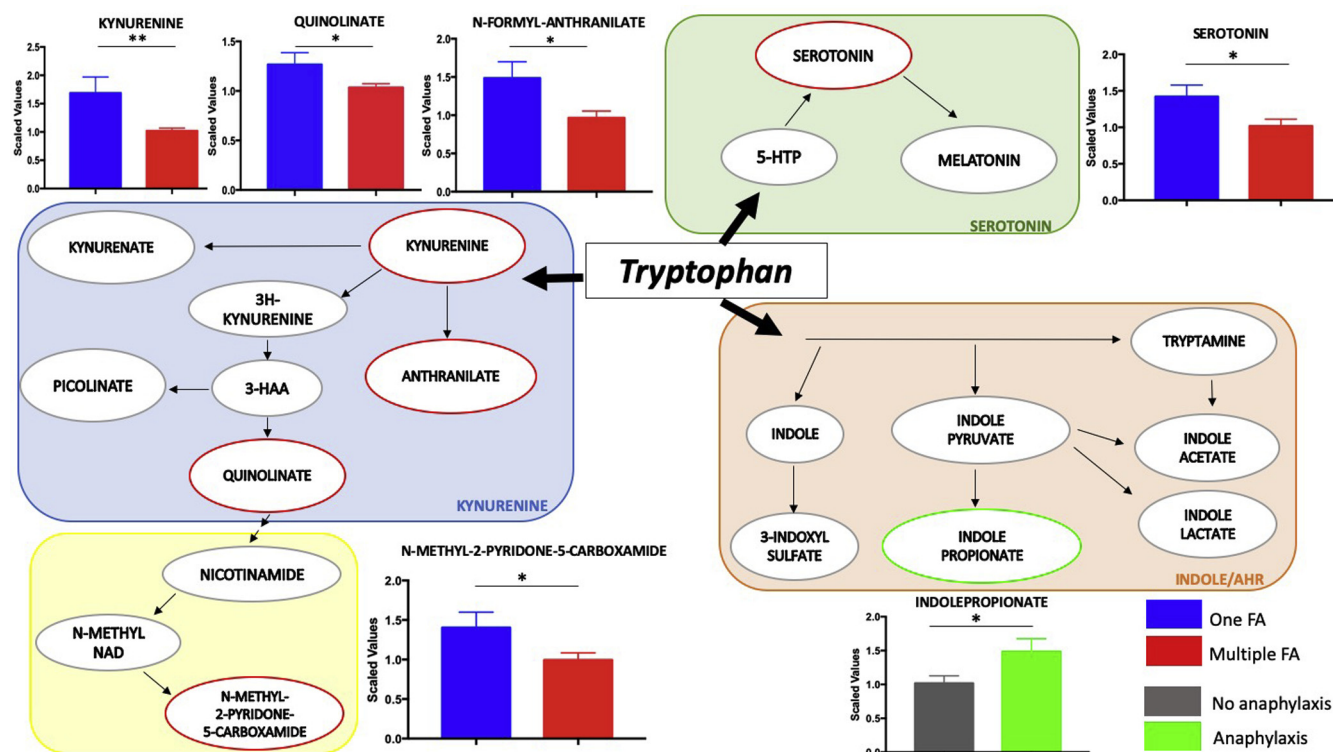


FIG 5. Tryptophan metabolism is dysregulated in children with multiple FAs and a history of anaphylaxis. The 3 branches of tryptophan metabolism are depicted together with pairwise comparisons of metabolites that are significantly different in children with multiple FAs or a history of anaphylaxis. Means and SEMs are shown. * $P \leq .05$ and ** $P \leq .005$.

and decreased production of glycolithocholic acid (GLCA/CDCA). When testing enzymatic activities related to the conjugation of bile acids to glycine or taurine, the presence of FA was associated with decreased glycine conjugation in secondary bile acids of the classic pathway (GDCA/DCA ratio) and increased taurine conjugation in the alternative pathway (THCA/HCA ratio), suggesting alterations in the processing of primary bile acids by the microbiota.

Pathway analyses

As shown in Fig E5, A, in this article's Online Repository at www.jacionline.org, the most dysregulated pathways associated with the presence of FA in comparison with control subjects include dihydrosphingomyelins, lactosylceramides, lysoplasmalogen, oxidative phosphorylation, sphingomyelins, and hexosylceramides. When compared with asthma (see Fig E5, A, in this article's Online Repository at www.jacionline.org), the most dysregulated pathways associated with FA included creatine metabolism, pantothenate and coenzyme A metabolism, and fatty acid (acyl choline) and secondary bile acid metabolism.

Random forest analysis

Random forest binning of FA versus control samples showed moderate accuracy (70%). Binning of asthma versus control samples and of FA versus asthma samples resulted in overall predictive accuracies of 79% and 87%, which were substantially better than the 50% that would occur by chance alone. Among

children with FA, random forest binning of samples with and without asthma was not predictive, suggesting strong similarity between children with FA alone and those with overlapping FA and asthma. The biochemical importance plot, which displays the top 30 differentiating biochemical metabolites in the comparison between children with FA and control subjects (see Fig E5, B), suggests key differences in membrane lipids (glycerophosphoinositol and sphingomyelin being the most discriminating), vitamins (γ - and β -tocopherol), and amino acids (glutamine, lysine, proline). 1-Arachidonoyl-GPC, a plasmalogen and precursor in the metabolism of platelet-activating factor, is the highest predictive metabolite of FA versus control samples. In the comparison of children with FA alone and those with asthma alone (see Fig E6, B), the most discriminating metabolites are represented by vitamins/cofactors (γ - and β -tocopherol and pantothenate), lipids (glycerophosphoinositol), and amino acids/peptides (leucylalanine and cysteine sulfate).

DISCUSSION

In this study untargeted serum metabolomic profiling of children with distinct atopic phenotypes led to identification of a unique metabolomic signature associated with FA. This signature was dominant in patients with both FA and asthma, likely reflecting unique pathogenic mechanisms active in FA that are different from those involved in asthma and, potentially, other atopic disorders. Given the pilot nature of this study, the observed signatures will need to be explored and validated in larger and/or more focused cohorts to tease out the contributions of other

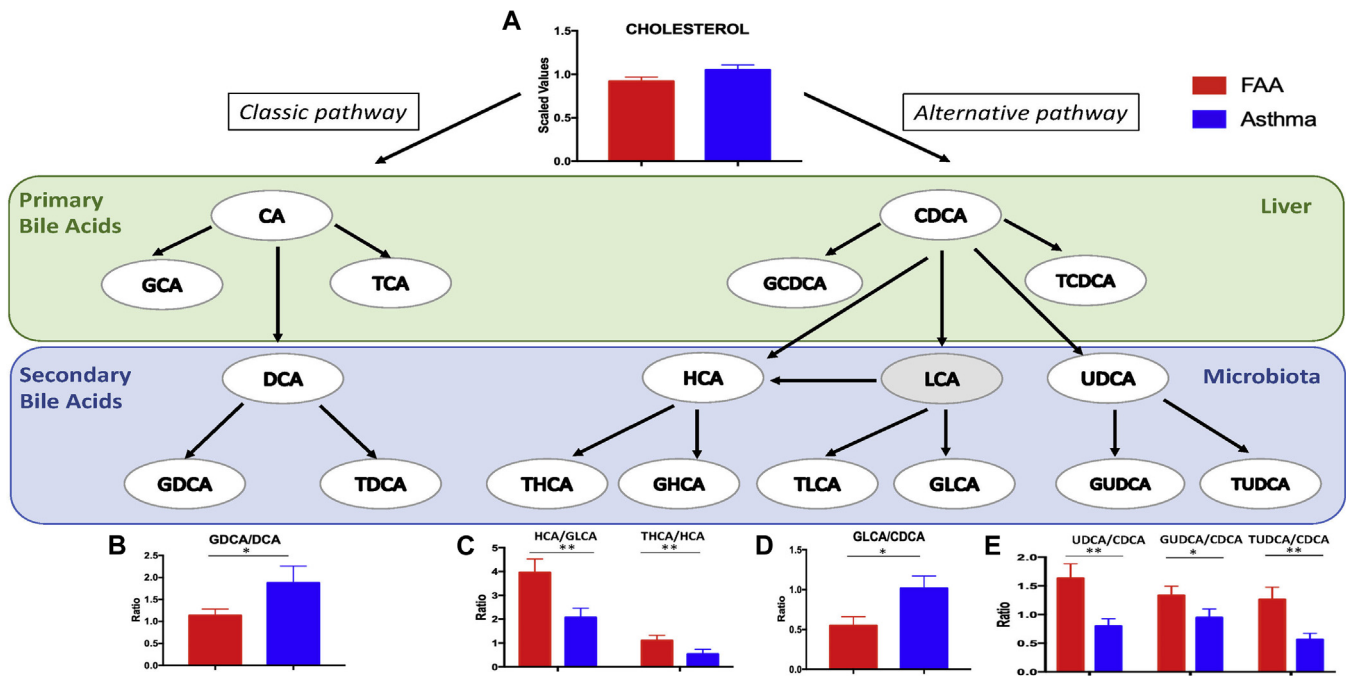


FIG 6. A-E, Differences in bile acid ratios between children with FA and those with asthma. A schematic representation of bile acid metabolism in the liver and gut is depicted, followed by pairwise comparison of differentially altered bile acid ratios between asthmatic children and children with FA. Means and SEMs are shown. * $P \leq .05$ and ** $P \leq .005$.

factors, such as ethnicity, diet, the microbiome, the presence of specific FAs, and others.

A hallmark of the FA metabolomic signature is the presence of distinct alterations in lipid metabolites, most prominently a marked decrease in levels of sphingolipids, including sphingomyelins and ceramides. Sphingolipids are a major class of lipids in eukaryotes.³⁷ *De novo* synthesized sphingolipids are major components of cellular membranes and participate in multiple cellular functions.³⁷ Sphingolipids are also derived from dietary sources and can be produced by *Bacteroides* species in the gut.³⁸ Changes in plasma sphingolipids have been reported in association with severe allergic respiratory responses.³⁹ In a recent report higher levels of stool sphingolipids were detected in food-sensitized children compared with patients with clinical FA or control subjects.¹⁵ iNKT cells recognize lipid ligands presented by the atypical MHC class I molecule CD1d, including *Bacteroides* species-derived sphingolipids. In the latter study *in vitro* iNKT cell activation by fecal sphingolipids was increased in children with food sensitization but not those with FA. A role for iNKT cells in patients with FA has also been suggested by reports of decreased iNKT cell numbers in children with cow's milk allergy²⁹ and their increase has been reported after milk desensitization.³⁰ Nevertheless, we did not find differences in the frequency of iNKT cells in children with FA versus nonatopic control subjects, whereas a small and significant increase in the proportion of iNKT cells was observed in asthmatic children, which is consistent with previous observations.^{31,32}

Sphingolipid changes observed in patients with FA suggest decreased generation of ceramides and also attenuation of additional distal steps in the conversion of ceramides into sphingomyelins, possibly at the level of the enzymes sphingomyelin synthase and sphingomyelinase. This step in

sphingomyelin metabolism has been noted to control the differentiation of T_H17 cells,⁴⁰ suggesting an immune-regulatory role for this pathway in modulating regulatory T (Treg) cell/T_H17 cell differentiation relevant to FA.⁴¹ Furthermore, the protein product of *ORMDL3*, a gene highly associated with asthma susceptibility,⁴² modulates sphingolipid metabolism by inhibiting *de novo* sphingolipid synthesis.⁴³⁻⁴⁵ This observation suggests a key role for sphingolipid homeostasis in lung allergic inflammation, offering candidate disease biomarkers and potential targets for therapeutic intervention.⁴⁶ The results of our studies similarly suggest an unexpected and potentially important role for sphingolipid metabolism in patients with FA and also the potential use of sphingolipid metabolites as disease biomarkers.

Patients with FA manifested reductions in other classes of lipids, including lysophospholipids and fatty acids, such as monoacylglycerols and diacylglycerols. We have recently observed a similar reduction in levels of lysophospholipids and especially in levels of plasmalogens in a mouse model of FA (data not shown). Of note, the strong inverse association of the platelet-activating factor metabolite precursor 1-arachidonoyl-GPC with FA is in line with recent reports of a possible role of altered platelet function in patients with severe food-induced respiratory allergic reactions.³⁹ These observations strongly suggest a key role for lipid alterations in the development of FA that is conserved across species and warrants further investigation. Dysregulation in metabolism of selected amino acids (lysine, valine, threonine) and vitamin E was also associated with FA.

Changes in the microbiome have been linked to allergic disorders, including FA⁴⁷ and asthma.⁴⁸ We have recently shown a critical role for dysbiosis in the pathogenesis of FA in both mice and human subjects.⁴¹ Microbiota can affect the host with various mechanisms, including through production of secreted

metabolites.⁴⁹ In our study children with FA and asthma showed significant differences in ratios of secondary bile acids, which are generated in the gut through the action of anaerobic bacteria. Specifically, we observed increased production of the alternative bile acid ursodeoxycholic acid and its conjugates, as well as differences in the rate of taurine/glycine conjugation between the classical and alternative pathways. Our findings not only support a role for dysbiosis in patients with FA and asthma but also suggest that differential signatures of microbially induced metabolites can be detected in distinct allergic phenotypes and serve as markers of disease. Furthermore, secondary bile acids activate bile acid receptors expressed both in the intestine and other tissues to modulate local and systemic responses, including inflammatory and innate immune responses.⁵⁰

Changes in bile acids have been implicated in the pathogenesis of inflammatory disorders,^{36,51} and long-ranging effects of dysregulated secondary bile acid metabolism might be present in children with FA.

When comparing children with FA and those with asthma, we observed that children with both conditions have a metabolomic profile that overlapped with that of children with FA alone. This observation suggests that the presence of FA imparts a metabolomic signature reflective of pathogenic mechanisms in FA that dominate in the copresence of asthma. Also consistent with distinct disease mechanisms are reports that FA is an independent risk factor for worse asthma outcomes, including the frequency and severity of symptoms.^{52,53} These results argue for synergistic interactions between mechanisms operative in different atopic disorders that can influence disease manifestations.

Patients with FA can exhibit severe phenotypes characterized by multiple FAs, life-threatening systemic reactions, or both. Changes in sphingolipid metabolites were observed in children with a positive history of anaphylaxis (Fig 3). Sphingolipids exert important regulatory effects in mast cells, with sphingosine-1-phosphate acting as a positive regulator that is secreted by mast cells after antigen-specific cross-linking of the high-affinity IgE receptor and sphingomyelins and ceramide metabolites acting as negative regulators of mast cell activation.^{54,55} The marked decrease in sphingomyelin and ceramide levels observed in children with FA might reflect an altered mast cell responsiveness toward a hyperresponsive state that predisposes to severe anaphylactic reactions. The presence of multiple FAs and of a positive history of anaphylaxis were associated with specific metabolomic alterations, including those that contribute to oral tolerance, most prominently the essential aromatic amino acid tryptophan and short-chain fatty acids. Tryptophan is metabolized through 3 different pathways: (1) direct transformation by the gut microbiota into indole metabolites, which can activate the aryl hydrocarbon receptor; (2) the kynurenine pathway in both immune and epithelial cells through the action of 2,3-dioxygenase-1; and (3) the production of serotonin in enterochromaffin cells through Trp hydroxylase-1. We found decreased levels of metabolites in the kynurenine and serotonin pathways in children with multiple FAs and a significant increase in the aryl hydrocarbon receptor ligand indole propionate in children with a history of anaphylaxis. Both the kynurenine and indole pathways are important in immune responses at mucosal barriers, including Treg cells, and contribute to intestinal homeostasis. Similarly, short-chain fatty acids affect Treg cell homing and stability at the barrier interface. Thus our findings

indicate that abnormalities in tolerogenic metabolic pathways might be a determinant of disease severity in children with FA.

A key strength of our study is the recruitment of carefully phenotyped patients with either 1 or overlapping atopic diseases (FA, asthma, and FA and asthma). A physician's diagnosis of FA was used based on the combination of recent occurrence of IgE-mediated symptoms and positive IgE-based test results to the culprit food. This approach eliminates known biases related to self-reporting of FA symptoms, which have been shown to not adequately correlate with a physician's diagnosis of FA.⁵⁶

Another strength is the availability of information on disease severity, which allowed correlation between the severity of FA and changes in specific metabolomic pathways for the first time.

There are also limitations in our study that stem from its pilot nature. Sample sizes were relatively limited. We have addressed this limitation by applying a more stringent significance level ($P \leq .005$) to report differences among study groups. Future studies with larger sample sizes will be needed to validate our findings, as well as to perform subgroup analyses.

Children with FA have restricted diets, which might in turn affect their metabolomic profiling. To address this concern, children with different FAs were included to counteract the effects of individual food avoidance. Although it is possible that dietary practices might still have affected the metabolomic alterations observed in children with FA, many of the changes detected in our study involved metabolites that are not directly related to dietary intake or are contained in a variety of foods that were not differentially avoided by patients with FA. Future studies looking at larger groups of children with individual FAs, as well as careful dietary recall, will be needed to determine the extent of dietary restrictions on the metabolomic profile in children with FA.

In summary, our studies established the presence of unique metabolomic signatures associated with FA and disease severity. They also revealed the alignment of the metabolomic signatures of children with FA with and without asthma to be indicative of distinct disease mechanisms operative in patients with FA, regardless of the presence of asthma. Our observations suggest that metabolomic profiling can be a powerful approach in advancing the knowledge into FA pathogenesis and a useful tool in identifying candidate biomarkers and targets for therapeutic intervention.

Key messages

- FA is associated with disease-specific metabolomic signatures, especially in sphingolipid and phospholipid metabolism, that distinguish it from asthma.
- The metabolomic profile of patients with both FA and asthma aligns with those with FA alone but not those with asthma as well.
- Distinct signatures were associated with disease severity in children with FA, reflecting perturbations in tolerogenic pathways.

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Untargeted Metabolomic Profiling Identifies Disease-specific

Signatures in Food Allergy and Asthma

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Supplementary Figure and Table Legends

Fig E1. Heat-map of metabolites significantly different ($p < 0.005$) between asthmatic children and non-atopic controls. For each metabolite, a colorimetric representation of relative expression in each sample is shown according to the scale depicted on top. Metabolites are grouped into main dysregulated pathways and a miscellaneous category.

Fig. E2. Comparison of sphingomyelin and ceramide metabolites of interest displayed in Figure 3 across all study groups. Mean and SEM are shown. * $p \leq 0.05$, ** $p \leq 0.005$, *** $p \leq 0.001$

Fig. E3. Comparison of tryptophan metabolites of interest displayed in Figure 5 across all study groups. Mean and SEM are shown. * $p \leq 0.05$, ** $p \leq 0.005$

Fig. E4. Comparison of bile acid ratios of interest displayed in Figure 6 across all study groups. Mean and SEM are shown. * $p \leq 0.05$

Fig. E5. Pathway analysis and Random Forrest Analysis of metabolites significantly dysregulated between FA children and controls. Panel **A** shows the fold increase over controls of the most dysregulated pathways in children with FA. Panel **B** shows the top thirty metabolites that most strongly discriminate between children with FA and controls.

Fig. E6. Pathway analysis and Random Forrest Analysis of metabolites significantly dysregulated between FA and asthmatic children. Panel **A** shows the fold increase over controls of the most dysregulated pathways in children with FA. Panel **B** shows the top thirty metabolites that most strongly discriminate between children with FA and asthma.

Table E1. Patient Demographics and clinical attributes.

Table E2. List of metabolites significantly different ($p \leq 0.005$) in pairwise comparisons of children with FA and non-atopic controls. Metabolite name, pathway, unadjusted and Atopic Dermatitis (AD) adjusted p-values are listed.

Table E3. List of metabolites significantly different ($p \leq 0.005$) in pairwise comparisons of children with FA and asthma. Metabolite name, pathway, unadjusted and Atopic Dermatitis (AD) adjusted p-values are listed.

Table E4. List of metabolites significantly different ($p \leq 0.005$) in pairwise comparisons of children with asthma and non-atopic controls. Metabolite name, pathway, unadjusted and Atopic Dermatitis (AD) adjusted p-values are listed.

Table E5. List of metabolites statistically different ($p \leq 0.05$) in pairwise comparisons between children with FA allergy to one and multiple food items. Metabolite name, pathway, and p-values are listed.

Table E6. List of metabolites significantly different ($p \leq 0.05$) in pairwise comparisons between food allergic children with and without history of anaphylaxis. Metabolite name, pathway, and p-values are listed.

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76 **Table E7.** List of metabolites significantly different ($p \leq 0.05$) in pairwise comparisons
77 between food allergic children without Atopic Dermatitis and non-atopic controls.
78 Metabolite name, pathway, and p-values are listed.

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Supplementary Table 1. Individual patient characteristics.

Group	Sample	Age	Gender	Race	BMI percentile	Food Allergies	Asthma	Asthma Severity	Environmental	Eczema	Total IgE	Epinephrine	Food allergic reaction	Specific IgE (kU/L)	Skin testing (mm)
FA	1	6	M	AA	93%	sesame	no		no	no	152	yes	hives, facial swelling, emesis, cough (sesame)	sesame 3.58	sesame 14
FA	2	1	F	other/mixed	91%	egg, peanut, tree nuts	no		no	no	236	yes	hives, facial swelling, emesis (peanut), flushing, itching, emesis (baked egg challenge)	egg white 22., peanut 11.7, almond 8.82, hazelnut 5.45, cashew 6.78, pistachio 7.84, walnut 9.08, pecan 2.71	egg 15, peanut 13, walnut 7, cashew 4
FA	3	2	M	AA	11%	egg, peanut, fish, shellfish	no		yes	yes	351	no	hives (egg)	egg white 13.3, peanut 27.1, cod 14.8, salmon 10.4, tilapia 16.5, shrimp 66.4, crab 54.4, lobster 51.1	egg 6, peanut 11
FA	4	6	F	White	50%	peanut, tree nuts, sesame	no		yes	no	405	no	hives (peanut)	peanut >100, hazelnut 14.6, cashew 16, pistachio 15.8, walnut 10.3, pecan 8.78, sesame 2.31	peanut 24, hazelnut 24, cashew 25, pistachio 9, walnut 13, pecan 14, sesame 17
FA	5	7	M	White	63%	tree nuts	no		yes	no	2,828	no	hives (pistachio food challenge), hives, emesis (cashew)	hazelnut >100, cashew 1.73, pistachio 6.21, walnut 3.02	hazelnut 14, cashew 13, pistachio 7, walnut 11
FA	6	6	F	White	98%	peanut, tree nuts	no		no	no	37	no	hives (peanut food challenge)	peanut 2.04, walnut 5.68, pecan 1.03	peanut 9, walnut 14, pecan 3
FA	7	1	F	Asian	n.a.	egg, peanut, tree nuts, flaxseed	no		yes	yes	491	no	hives (flaxseed), facial rash (egg)	egg white 27.3, peanut 21.2, almond 5.96, hazelnut 2.77, cashew 1.92, pistachio 2.91, flaxseed 16.7	n.a.
FA	8	3	F	Asian	78%	egg	no		yes	no	64	no	hives	egg white 2.88	egg 24
FA	9	1	M	White	50%	egg	no		no	no	89	no	hives, face swelling	egg white 2.45	egg 13
FA	10	1	M	White	88%	peanut, egg	no		yes	yes	92	no	hives (egg)	egg white 6.63, peanut 7.5	egg 11, peanut 26
FA	11	1	M	White	99%	peanut,tree nuts	no		yes	yes	60	no	hives (cashew, peanut)	peanut 2.72, cashew 2.75, pistachio 2.83	peanut 21, cashew 20, pistachio 25
FA	12	5	M	White	44%	cow milk, egg, tree	no		yes	no	727	yes	hives, angioedema, rhinorrhea, wheezing	cow milk >100, egg white 3.62, almond 2.4, hazelnut 1.9,	n.a.

						nuts							(cow milk), hives (egg)	cashew 1.73, pistachio 0.52,	
FA	13	4	M	Hispanic	91%	egg, peanut, tree nuts, beans, garlic	no		yes	yes	2,146	no	hives (baked egg, pinto beans, garlic)	egg white 24.6, peanut 54.2, almond 6.4, hazelnut 17.4, cashew 33.2, pistachio 33.6, garlic 13.1	egg 6, peanut 40, almond 10, hazelnut 10, cashew 20, pistachio 22, pinto beans 20, garlic 13,
FA	14	4	M	Hispanic	n.a.	milk, egg	no		no	yes	361	yes	hives, lip swelling, respiratory distress (cow milk formula), vomiting (egg)	cow milk 4.44	milk 17, egg 9
FA	15	6	F	White	91%	peanut, tree nut	no		yes	yes	494	no`	hives (peanut), vomiting (almond)	peanut >100, almond 5.61, cashew 5.14, pistachio 1.53	peanut 20, almond 7, cashew 6
FA	16	10	M	AA	11%	peanut, tree nuts	no		yes	yes	1,767	no	hives, angioedema (peanut)	peanut >100, cashew 13.2, pistachio 19.3	peanut 30, cashew 40, pistachio 30
FA	17	3	M	n.a.	78%	tree nuts	no		yes	yes	87	no	hives (cashew)	cashew 15.5, pistachio 7.3	cashew 18, pistachio 30
FA	18	5	M	White	21%	egg, peanut, tree nuts, sesame, lentils, beef	no		yes	yes	867	yes	hives (egg food challenge), face swelling, difficulty breathing (cashew, lentils, beef)	egg 2.98, peanut 4.36, almond 2.13, hazelnut 6.54, cashew 18.4, pistachio 19.9, walnut 13.7, pecan 3.77, sesame 5.46, lentil 6.1, beef 2.95	egg 12, peanut 21, almond 8, hazelnut 7, cashew 30, pistachio 22, walnut 21, pecan 18, sesame 23, beef 7
FA	19	1	F	White	n.a.	tree nuts	no		no	no	34	no	hives, face swelling (pistachio)	cashew 9.54, pistachio 12	cashew 14, pistachio 7
FA	20	9	M	White	83%	sesame	no		yes	yes	140	no	acute worsening eczema	sesame 1.09	sesame 21
FA	21	9	M	n.a.	36%	tree nuts, banana	no		yes	yes	90	no	hives, throat itching (banana)	pistachio 0.71, walnut 0.41, banana 2.73	walnut 7, pistachio 4
FA	22	2	F	AA	18%	egg, peanut, cashew	no		yes	yes	105	no	hives, facial swelling (egg), hives (peanut food challenge)	egg 7.14, peanut 1.08, cashew 0.92	egg 11, peanut 2, cashew 11
FA	23	3	M	other/mixed	32%	tree nuts	no		yes	no	91	no	hives (almond)	almond 0.59, hazelnut 0.52, cashew 0.44, pistachio 0.77	almond 7, hazelnut 7, cashew 9, pistachio 4
FA	24	10	F	White	65%	peanut	no		yes	no	106	no	hives, lip swelling	peanut 2.01	peanut 17
FA	25	4	F	White	72%	cow milk,goat milk	no		yes	yes	1,420	yes	hives, vomiting, wheezing, hypoxia (goat milk)	cow milk 91.9, goat milk >100	n.a.
FA	26	11	M	White	7%	pineapple,	no		yes	no	1,579	no	hives (pineapple,	pineapple 1.69, kiwi 4.51	pineapple 11

						kiwi							kiwi)		
FA	27	2	F	White	93%	milk, egg, peanut, hazelnut, wheat	no		yes	yes	87	no	acute eczema worsening (cow milk), hives (wheat and egg food challenge),	cow milk 7.74, egg white 5.21, peanut 5.43, hazelnut 3.88, wheat 1.67	cow milk 13, egg 16, peanut 27, wheat neg
FA	28	1	F	Asian	63%	milk, egg, soy, peanut, tree nuts, wheat, oat, peas	no		yes	yes	472	yes	hives, angioedema, vomiting (wheat), hives (sesame),	cow milk 14.2, egg white 35.3, soy 5.47, peanut >100, almond 9.7, hazelnut 7.96, cashew 9.51, pistachio 11.4, walnut 33.8, pecan 6.55, wheat 6.74, wheat 27. 1, sesame 98, oat 10.4, peas 16.3	cow milk 25, egg 25, soy 7, almond , hazelnut 7, cashew 7, walnut 34, pecan 12, wheat 19, oat 13, peas 9
FA	29	2	F	Hispanic	70%	egg, peanut, tree nuts	no		yes	yes	96	no	eczema flare, hives (egg, peanut)	egg white 7.56, peanut 19.6, cashew 15.6, pistachio 13.1	egg 24, peanut 12, cashew 30, pistachio 40
FA	30	6	M	White	25%	milk, peanut, tree nuts	no		yes	no	1,376	no	tongue itching, face redness, abdominal pain (cow milk food challenge), hives, angioedema (mixed nuts)	cow milk 2.16, peanut 1.64, hazelnut 7.03, walnut 44.8, pecan 7.54	cow milk 25, peanut 20, hazelnut 20, walnut 35, pecan 13
FA	31	1	F	White	62%	milk, egg, tree nuts	no		yes	no	51	no	hives, eye swelling, sneezing (cow milk), hives face swelling (egg), hives, eye swelling, sneezing (almond food challenge)	cow mlk 6.24, egg 0.95, almond 0.50, hazelnut 3.56	milk 20, egg 18, almond 6, hazelnut 5
FA	32	5	M	White	96%	peanut	no		no	yes	220	no	facial flushing, hives	peanut 53.9	n.a.
FA	33	2	F	White	37%	egg, lentils, peas	no		yes	yes	110	no	rash, eye swelling (egg), hives, itching (peas, lentils)	egg 4.11, lentil 1.34, peas 1.52	egg 17, pea 4
FA	34	1	M	White	68%	egg	no		no	yes	60	no	sneezing, hives	egg 4.94	egg 13
FA	35	1	F	White	68%	egg, peanut	no		yes	yes	66	yes	lip swelling, face rash (egg), hives, rhinitis, cough, wheezing (peanut food challenge)	egg 1.07, peanut 6.87	egg 11, peanut 5

FAA	36	8	M	White	52%	soy	yes	intermittent	yes	no	303	no	hives	soy 0.85	soy 10
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FAA	37	2	M	Hispanic	87%	egg, peanut, tree nuts	yes	intermittent	no	yes	422	yes	hives, eye swelling (egg), hives, vomiting, raspy voice, wheezing (pistachio)	egg 4.34, peanut 2.78, almond 2.08, hazelnut 1.53, cashew 11.3, pistachio 15.1, walnut 36.2, pecan 10.4	egg 10, peanut 9, almond 11, hazelnut 13, cashew 22, pistachio 21, walnut 11, pecan 13
FAA	38	7	M	AA	15%	peanut, tree nuts	yes	mild persistent	yes	no	584	no	cough, wheezing (peanut)	peanut 1.87, almond 5.2, hazelnut 27.7, cashew 8.96, pistachio 14.1	n.a.
FAA	39	8	M	White	98%	milk egg, peanut, tree nuts, beef	yes	moderate persistent	yes	yes	500	yes	hives, throat itching, shortness of breath (milk, egg, peanut)	milk 6.7, egg 0.73, peanut 1.67, cashew 0.80, pistachio 1.6	egg 9
FAA	40	5	F	White	15%	milk, egg, peanut	yes	intermittent	yes	yes	886	yes	cough, wheezing (cow milk), hives (peanut)	cow milk 40.5, egg 2.3, peanut 13.5	milk 22, egg 15, peanut 30
FAA	41	9	F	White	90%	peanut, tree nuts	yes	mild persistent	yes	yes	2238	no	hives, eczema flare (peanut)	peanut >100, almond 5.54, hazelnut 2.34	peanut 45, almond 7, hazelnut 7
FAA	42	2	M	Other/Indian	3%	egg	yes	mild persistent	yes	yes	416	no	hives	egg white 16.6	egg 9
FAA	43	1	M	White	48%	egg	yes	intermittent	no	yes	51	no	hives	egg white 3.78	egg 18
FAA	44	3	M	Hispanic	66%	lentils, chickpeas	yes	intermittent	yes	yes	142	no	hives, lip swelling, eye swelling (lentils, chickpea)	lentil 48.1, chickpea 52.1	n.a.
FAA	45	2	M	White	15%	egg, peanut, tree nuts	yes	intermittent	yes	yes	167	no	hives (egg, peanut)	egg 7.73, peanut 1.94, walnut 20.3, pecan 5.29	egg 13, peanut 21, walnut 25, pecan 4
FAA	46	7	F	White	94%	peanut, tree nuts, sesame, banana	yes	mild persistent	yes	yes	2,553	yes	hives, lip swelling, difficulty breathing (peanut), hives (banana)	peanut >100, almond 6.43, hazelnut 18.2, cashew 85.8, pistachio >100, walnut 49.9, pecan 20.8, sesame 8.29,	peanut 21, cashew 20, pistachio 5, walnut 30, sesame 4, banana 3

														banana 8.69	
FAA	47	3	F	White	96%	peanut	yes	mild persistent	yes	no	88	no	hives, lip swelling	peanut 32.9	peanut 19
FAA	48	7	M	Hispanic	15%	milk, egg, peanut, tree nuts, wheat, barley, oat	yes	moderate persistent	yes	no	1881	yes	hives, vomiting, wheezing (cow milk, egg)	cow milk 39.1, egg 4.61, peanut 4.58, almond 13.8, hazelnut 38.2, wheat >100, barley >100, oat 65.8	cow milk 39, egg 30, peanut 13, almond 11, hazelnut 15, wheat 30, barley 28, oat 26
FAA	49	6	M	White	89%	peanut	yes	intermittent	yes	no	244	no	hives	peanut 11.1	peanut 20
FAA	50	8	M	White	69%	cow milk, egg, peanut, tree nuts, beef	yes	mild persistent	yes	yes	1192	no	hives (cow milk, egg)	cow milk 31.3, egg 24.8, peanut >100, cashew 7.7, pistachio 5.5, walnut 4.65, beef 4.8	cow milk 21, egg 21, peanut 31, cashew 22, pistachio 19, walnut 6, beef 15
FAA	51	7	F	n.a.	49%	egg, peanut, tree nuts	yes	intermittent	yes	yes	206	no	hives (egg, peanut)	egg 23.1, peanut 26.5, cashew 7.48, pistachio 13.3	egg 25, peanut 20, cashew 15, pistachio 20
FAA	52	6	F	AA	31%	peanut	yes	intermittent	yes	no	1,041	no	throat hurting, vomiting	peanut >100	peanut 15
FAA	53	8	M	Hispanic	63%	peanut, tree nuts, fish	yes	moderate persistent	yes	yes	104	no	hives, face itching (fish), hives (peanut)	n.a.	peanut 13, cashew 25, pistachio 20, salmon 20, codfish 15, trout 16, flounder 9
FAA	54	7	F	Hispanic	95%	tree nuts	yes	intermittent	yes	yes	490	no	face rash, lip swelling (walnut)	walnut 73, pecan 30.3, cashew 10.9, pistachio 18.9	walnut 32, pecan 15, cashew 14, pistachio 16
FAA	55	4	F	Hispanic	23%	egg, milk, peanut	yes	moderate persistent	yes	yes	1,210	yes	rash, vomiting (cow milk), itching, vomiting, lethargy (egg)	cow milk >100, egg white >100, peanut 1.4	cow milk 20. egg 20, peanut 13
FAA	56	6	M	AA	93%	fish	yes	moderate persistent	yes	no	989	yes	hives, difficulty breathing (tilapia)	tilapia 38.3, codfish 33.5, flounder 31.9, salmon 7.69	n.a.
FAA	57	3	F	AA	45%	milk, egg, peanut, tree	yes	moderate persistent	yes	yes	2528	no	hives, vomiting	cow milk >100, egg white >100, soy	cow milk 35, egg 21, soy 13, peanut 26, almond 15, cashew 33, pistachio

						nuts, soy, wheat, coconut, flax							(cow milk), hives (egg)	31.7, peanut >100, almond 78.8, hazelnut 91.7, cashew >100, pistachio >100	30, wheat 7
FAA	58	6	F	Hipanic	n.a.	peanut	yes	mild persistent	yes	no	n.a.	no	hives	n.a.	peanut 10
FAA	59	10	M	Hispanic	81%	peanut, tree nuts	yes	moderate persistent	yes	no	5245	no	hives (peanut)	peanut >100, cashew 44.2	peanut 35, cashew 11
FAA	60	8	F	Hispanic	86%	peanut, tree nuts, lentils, shellfish	yes	intermittent	yes	no	485	yes	hives, eye swelling, cough, rhinorrhea (peanut), itchy throat, abdominal pain (lentil)	peanut >100, cashew 2.57, pistachio 6.43, lentil 8.99, shrimp 1.84, lobster 0.62	peanut 15, cashew 11, shrimp 16, lobster 11
FAA	61	4	F	Hispanic	64%	peanut, tree nuts	yes	mild persistent	yes	yes	n.a.	no	hives, face swelling (peanut)	n.a.	peanut 35, walnut 20, pecan 11
FAA	62	7	F	White	46%	tree nuts	yes	mild persistent	no	no	383	no	face swelling, vomiting (pistachio)	cashew >100, pistachio >100, walnut >100, pecan 28.7	cashew 45, pistachio 25, walnut 13, pecan 9
FAA	63	1	M	White	52%	milk, egg, soy, peanut, tree nuts, sesame	yes	mild persistent	yes	no	179	no	hives, sneezing, itching (baked egg food challenge), hives (cow milk)	cow milk 8.58, egg white 17.6, peanut 17.7, cashew 6.4, pistachio 10.1	cow milk 21, egg 16, soy 7, peanut 16, cashew 27, pistachio 27
FAA	64	8	M	White	8%	milk, egg, peanut, tree nuts	yes	mild persistent	yes	yes	1307	yes	sneezing, vomiting (cow milk), vomiting (egg)	cow milk 81.7, egg white 37.7, peanut 40.3	cow milk 18, egg 20, peanut 15
FAA	65	7	F	White	90%	peanut, tree nuts	yes	intermittent	yes	no	351	no	abdominal pain, nausea, cough, wheezing (walnut)	peanut 8.02, cashew 6.56, pistachio 14.4, walnut 49.7. pecan 16.5	peanut 23, cashew 20, pistachio 13, walnut 11, pecan 9

FAA	66	3	M	AA	58%	egg, peanut, tree nuts, sesame	yes	mild persistent	no	no	1220	no	vomiting (egg), hives, vomiting (sesame)	egg 98, peanut 35.7, cashew 34.7, pistachio 44.9, walnut 13.5, pecan 3.16, sesame >100	egg 11, peanut 22, cashew 15, pistachio 13, sesame 16
FAA	67	9	M	Asian	95%	peanut, tree nuts, shellfish	yes	intermittent	yes	yes	2524	no	hives (nuts, shellfish)	peanut >100, almond 85.9, hazelnut >100, cashew >100, pistachio >100, walnut >100, pecan >100, shrimp 30.3, lobster 26.9, crab 22	peanut 25, almond 7, hazelnut 15, cashew 25, pistachio 25, walnut 25, pecan 7, shrimp 9, lobster 15, crab 14
FAA	68	5	M	White	77%	milk, egg, tree nuts	yes	mild persistent	no	yes	311	yes	hives, vomiting (cow milk)	cow milk 5.52, egg white 2.87, cashew 3.89, pistachio 3.95, walnut 4.95, pecan 2.02	cow milk 50, egg 40, cashew 25, pistachio 25, walnut 35, pecan 15
FAA	69	4	M	White	34%	egg, peanut, tree nuts, soy, peas	yes	intermittent	yes	yes	488	no	hives (egg, soy, peas)	egg white 7.54, soy 13.8, peanut 6.35, almond 4.67, hazelnut 5.07, cashew 2.5, pistachio 5.47, walnut 2.33, pecan 0.78, peas 6.26	egg 16, soy 11, peannut 9, pea 7, almond 22, hazelnut 11, cashew 5, pistachio 5, walnut 7,
FAA	70	1	M	Asian	53%	egg	yes	mild persistent	yes	no	389	yes	hives	egg white 5.9	egg 14

Asthma	71	7	M	n.a.	91%	no		yes	intermittent	no	no	n.a.
Asthma	72	5	F	White	39%	no		yes	mild persistent	yes	no	n.a.
Asthma	73	10	M	White	95%	no		yes	moderate persistent	yes	no	n.a.
Asthma	74	5	M	Hispanic	99%	no		yes	mild persistent	yes	yes	n.a.
Asthma	75	6	M	Hispanic	22%	no		yes	intermittent	no	yes	n.a.
Asthma	76	8	F	Hispanic	99%	no		yes	moderate persistent	yes	no	n.a.

Asthma	77	6	F	Hispanic	18%	no		yes	intermittent	no	no	n.a.
Asthma	78	9	F	Hispanic	56%	no		yes	mild persistent	yes	no	n.a.
Asthma	79	8	F	White	18%	no		yes	mild persistent	no	no	n.a.
Asthma	80	11	F	AA	19%	no		yes	intermittent	yes	no	n.a.
Asthma	81	8	M	Hispanic	99%	no		yes	mild persistent	yes	no	n.a.
Asthma	82	8	M	Hispanic	99%	no		yes	moderate persistent	no	no	n.a.
Asthma	83	5	M	AA	n.a.	no		yes	mild persistent	yes	yes	n.a.
Asthma	84	9	F	Hispanic	17%	no		yes	mild persistent	no	no	n.a.
Asthma	85	8	M	AA	68%	no		yes	moderate persistent	yes	yes	n.a.
Asthma	86	7	F	Hispanic	18%	no		yes	intermittent	no	no	n.a.
Asthma	87	7	F	AA	14%	no		yes	intermittent	no	no	n.a.
Asthma	88	9	M	Hispanic	78%	no		yes	moderate persistent	yes	no	n.a.
Asthma	89	9	M	White	n.a.	no		yes	mild persistent	yes	no	n.a.
Asthma	90	11	M	AA	n.a.	no		yes	moderate persistent	yes	yes	n.a.
Asthma	91	8	F	n.a.	99%	no		yes	mild persistent	no	no	n.a.
Asthma	92	6	M	Hispanic	5%	no		yes	mild persistent	yes	no	n.a.
Asthma	93	7	F	AA	48%	no		yes	intermittent	yes	no	n.a.
Asthma	94	6	M	AA	n.a.	no		no	intermittent	no	no	n.a.
Asthma	95	10	F	AA	n.a.	no		yes	intermittent	yes	no	n.a.
Asthma	96	8	M	AA	61%	no		yes	intermittent	no	no	n.a.
Asthma	97	10	F	AA	18%	no		yes	intermittent	no	no	n.a.
Asthma	98	5	M	AA	63%	no		yes	intermittent	no	no	n.a.
Asthma	99	8	M	AA	86%	no		yes	mild persistent	yes	no	n.a.

Asthma	100	8	F	AA	29%	no		yes	mild persistent	yes	yes	n.a.
Asthma	101	7	M	Hispanic	78%	no		yes	intermittent	yes	no	n.a.
Asthma	102	4	M	Hispanic	65%	no		yes	mild persistent	yes	yes	n.a.
Asthma	103	7	M	White	n.a.	no		yes	moderate persistent	yes	yes	n.a.
Asthma	104	7	F	AA	30%	no		yes	intermittent	no	no	n.a.
Asthma	105	5	M	White	85%	no		yes	mild persistent	yes	no	n.a.

Control	106	8	M	Hispanic	61%	no		no		no	no	14
Control	107	3	M	Hispanic	77%	no		no		no	no	16
Control	108	3	F	Hispanic	97%	no		no		no	no	49
Control	109	18	M	White	32%	no		no		no	no	n.a.
Control	110	1	M	White	68%	no		no		no	no	5
Control	111	8	F	White	80%	no		no		no	no	n.a.
Control	112	3	M	Hispanic	89%	no		no		no	no	5
Control	113	10	F	White	62%	no		no		no	no	n.a.
Control	114	18	F	AA	88%	no		no		no	no	1
Control	115	3	M	White	90%	no		no		no	no	n.a.
Control	116	17	M	Hispanic	98%	no		no		no	no	50
Control	117	2	M	White	61%	no		no		no	no	2
Control	118	1	F	AA	n.a.	no		no		no	no	5
Control	119	1	F	White	90%	no		no		no	no	15
Control	120	13	F	n.a.	73%	no		no		no	no	64
Control	121	2	F	AA	50%	no		no		no	no	1
Control	122	1	M	AA	n.a.	no		no		no	no	72
Control	123	12	F	Hispanic	53%	no		no		no	no	5
Control	124	1	F	Hispanic	60%	no		no		no	no	2

Control	125	1	M	White	n.a.	no		no		no	no	18
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Supplementary Table 2. List of metabolites significantly different ($p \leq 0.005$) in pairwise comparisons of children with FA and non-atopic controls. Metabolite name, pathway, unadjusted and Atopic Dermatitis (AD) adjusted p-values are listed.

	Metabolite	Pathway	Unadjusted p-value	AD-adjusted p-value
1	1-(1-enyl-palmitoyl)-2-palmitoyl-GPC (P-16:0/16:0)	Plasmalogen	0.002	0.001
2	1-(1-enyl-oleoyl)-GPE (P-18:1)	Lysoplasmalogen	0.002	0.001
3	1-(1-enyl-stearoyl)-GPE (P-18:0)	Lysoplasmalogen	0.0004	0.0002
4	1-(1-enyl-palmitoyl)-GPC (P-16:0)	Lysophospholipid	0.001	0.002
5	1-(1-enyl-palmitoyl)-GPE (P-16:0)	Lysophospholipid	0.005	0.002
6	1-arachidonoyl-GPC (20:4n6)	Lysophospholipid	0.00003	0.00006
7	1-arachidonoyl-GPE (20:4n6)	Lysophospholipid	0.001	0.0002
8	1-linolenoyl-GPC (18:3)	Lysophospholipid	0.005	0.011
9	1-linoleoyl-GPC (18:2)	Lysophospholipid	0.001	0.001
10	1-oleoyl-GPC (18:1)	Lysophospholipid	0.003	0.003
11	1-palmitoleoyl-GPC (16:1)	Lysophospholipid	0.002	0.002
12	1-palmitoyl-GPC (16:0)	Lysophospholipid	0.003	0.002
13	glycerophosphoinositol	Phospholipid	0.00002	0.0002
14	1-linoleoyl-2-arachidonoyl-GPC (18:2/20:4n6)	Phosphatidylcholine	0.004	0.013
15	1-myristoyl-2-arachidonoyl-GPC (14:0/20:4)	Phosphatidylcholine	0.004	0.010
16	3-hydroxyhexanoate	Fatty Acid, Monohydroxy	0.004	0.007
17	heptenedioate (C7:1-DC)	Fatty Acid, Dicarboxylate	0.004	0.030
18	octadecanedioate (C18-DC)	Fatty Acid, Dicarboxylate	0.002	0.016
19	stearoylcarnitine (C18)	Fatty Acid Metabolism (Acyl Carnitine)	0.003	0.008
20	oleoyl-arachidonoyl-glycerol (18:1/20:4)	Diacylglycerol	0.001	0.001
21	palmitoyl-arachidonoyl-glycerol	Diacylglycerol	0.004	0.029

	(16:0/20:4)			
22	stearoyl-arachidonoyl-glycerol (18:0/20:4)	Diacylglycerol	0.002	0.001
23	ceramide (d18:1/20:0, d16:1/22:0, d20:1/18:0)	Ceramides	0.002	0.023
24	glycosyl-N-stearoyl-sphingosine (d18:1/18:0)	Ceramides	0.003	0.001
25	lactosyl-N-nervonoyl-sphingosine (d18:1/24:1)	Ceramides	0.001	0.001
26	lactosyl-N-palmitoyl-sphingosine (d18:1/16:0)	Ceramides	0.001	0.001
27	behenoyl dihydrosphingomyelin (d18:0/22:0)	Sphingolipids	0.004	0.003
28	lignoceroyl sphingomyelin (d18:1/24:0)	Sphingolipids	0.003	0.005
29	myristoyl dihydrosphingomyelin (d18:0/14:0)	Sphingolipids	0.001	0.002
30	palmitoyl dihydrosphingomyelin (d18:0/16:0)	Sphingolipids	0.005	0.001
31	palmitoyl sphingomyelin (d18:1/16:0)	Sphingolipids	0.004	0.001
32	sphingomyelin (d17:1/14:0, d16:1/15:0)	Sphingolipids	0.002	0.021
33	sphingomyelin (d17:1/16:0, d18:1/15:0, d16:1/17:0)	Sphingolipids	0.003	0.017
34	sphingomyelin (d18:0/20:0, d16:0/22:0)	Sphingolipids	0.0002	0.003
35	sphingomyelin (d18:1/14:0, d16:1/16:0)	Sphingolipids	0.001	0.004
36	sphingomyelin (d18:1/22:1, d18:2/22:0, d16:1/24:1)	Sphingolipids	0.001	0.016
37	sphingomyelin (d18:1/22:2, d18:2/22:1, d16:1/24:2)	Sphingolipids	0.0005	0.003
38	sphingomyelin (d18:2/14:0, d18:1/14:1)	Sphingolipids	0.002	0.0002
39	sphingomyelin (d18:2/16:0, d18:1/16:1)	Sphingolipids	0.00001	0.00002
40	sphingomyelin (d18:2/18:1)	Sphingolipids	0.001	0.016
41	sphingomyelin (d18:2/21:0, d16:2/23:0)	Sphingolipids	0.004	0.017
42	sphingomyelin (d18:2/23:0, d18:1/23:1, d17:1/24:1)	Sphingolipids	0.001	0.007
43	sphingomyelin (d18:2/23:1)	Sphingolipids	0.001	0.007
44	tricosanoyl sphingomyelin (d18:1/23:0)	Sphingolipids	0.002	0.007

45	taurodeoxycholate	Secondary Bile Acid	0.0004	0.002
46	lysine	Lysine Metabolism	0.0001	0.001
47	N2-acetyllysine	Lysine Metabolism	0.0005	0.0001
48	threonine	Glycine, Serine and Threonine Metabolism	0.002	0.011
49	valine	Leucine, Isoleucine and Valine Metabolism	0.003	0.019
50	isoleucine	Leucine, Isoleucine and Valine Metabolism	0.005	0.021
51	glutamate	Glutamate Metabolism	0.004	0.001
52	cholesterol	Sterol	0.0002	0.0002
53	gamma-tocopherol/beta-tocopherol	Tocopherol Metabolism	0.003	0.0008

Supplementary Table 3. List of metabolites significantly different ($p \leq 0.005$) in pairwise comparisons of children with FA and asthma. Metabolite name, pathway, unadjusted and Atopic Dermatitis (AD) adjusted p-values are listed.

	Metabolite	Pathway	Unadjusted p-value	AD-adjusted p-value
1	1-(1-enyl-palmitoyl)-GPC (P-16:0)	Lysoplasmalogen	0.00003	0.00002
2	1-arachidonoyl-GPC (20:4n6)	Lysophospholipid	0.00004	0.00007
3	1-arachidonoyl-GPI (20:4)	Lysophospholipid	0.003	0.0003
4	1-linoleoyl-GPC (18:2)	Lysophospholipid	0.0001	0.0008
5	1-linoleoyl-GPG (18:2)	Lysophospholipid	0.001	0.001
6	1-linoleoyl-GPI (18:2)	Lysophospholipid	0.00004	0.00003
7	1-palmitoleoyl-GPC (16:1)	Lysophospholipid	0.002	0.002
8	1-palmitoyl-GPC (16:0)	Lysophospholipid	0.0002	0.00008
9	1-palmitoyl-GPI (16:0)	Lysophospholipid	0.00005	0.00001
10	1-stearoyl-GPC (18:0)	Lysophospholipid	0.00002	0.00002
11	1-stearoyl-GPG (18:0)	Lysophospholipid	0.002	0.0002
12	1-palmitoyl-2-linoleoyl-GPI (16:0/18:2)	Phosphatidylinositol (PI)	0.00002	0.00002
13	glycerophosphoinositol	Phospholipid Metabolism	0.00002	0.00002
14	glycerol	Glycerolipid Metabolism	0.002	0.001
15	1-arachidonoylglycerol (20:4)	Monoacylglycerol	0.0003	0.00005
16	1-dihomo-linolenylglycerol (20:3)	Monoacylglycerol	0.000009	0.000004
17	1-linolenoylglycerol (18:3)	Monoacylglycerol	0.0005	0.002
18	1-linoleoylglycerol (18:2)	Monoacylglycerol	0.00006	0.00003
19	1-oleoylglycerol (18:1)	Monoacylglycerol	0.005	0.004
20	1-palmitoleoylglycerol (16:1)	Monoacylglycerol	0.002	0.007

21	2-linoleoylglycerol (18:2)	Monoacylglycerol	0.001	0.001
22	diacylglycerol (16:1/18:2 [2], 16:0/18:3 [1])	Diacylglycerol	0.0002	0.001
23	palmitoleoyl-linoleoyl-glycerol (16:1/18:2)	Diacylglycerol	0.003	0.001
24	palmitoyl-linoleoyl-glycerol (16:0/18:2)	Diacylglycerol	0.0004	0.001
25	linoleoyl-arachidonoyl-glycerol (18:2/20:4)	Diacylglycerol	0.003	0.002
26	2-hydroxypalmitate	Fatty Acid, Monohydroxy	0.003	0.001
27	2-hydroxystearate	Fatty Acid, Monohydroxy	0.0004	0.0003
28	arachidonate (20:4n6)	Polyunsaturated Fatty Acid (n3 and n6)	0.001	0.0001
29	dihomo-linolenate (20:3n3 or n6)	Polyunsaturated Fatty Acid (n3 and n6)	0.001	0.001
30	docosapentaenoate (n6 DPA; 22:5n6)	Polyunsaturated Fatty Acid (n3 and n6)	0.002	0.001
31	dihomo-linolenoyl-choline	Fatty Acid Metabolism (Acyl Choline)	0.004	0.002
32	arachidonoylcholine	Fatty Acid Metabolism (Acyl Choline)	0.001	0.003
33	linoleoylcholine	Fatty Acid Metabolism (Acyl Choline)	0.001	0.0007
34	nervonoylcarnitine (C24:1)	Fatty Acid Metabolism (Acyl Carnitine)	0.002	0.003
35	oleoylcholine	Fatty Acid Metabolism (Acyl Choline)	0.006	0.005
36	palmitoylcarnitine (C16)	Fatty Acid Metabolism (Acyl Carnitine)	0.007	0.005
37	palmitoylcholine	Fatty Acid Metabolism (Acyl Choline)	0.002	0.0002
38	stearoylcholine	Fatty Acid Metabolism (Acyl Choline)	0.001	0.0001
39	malonylcarnitine	Fatty Acid Synthesis	0.001	0.010
40	stearate (18:0)	Long Chain Fatty Acid	0.004	0.005
41	palmitate (16:0)	Long Chain Fatty Acid	0.004	0.003
42	1-methyl-4-imidazoleacetate	Histidine Metabolism	0.001	0.004
43	1-ribosyl-imidazoleacetate	Histidine Metabolism	0.0004	0.020
44	4-imidazoleacetate	Histidine Metabolism	0.000003	0.00003

45	N-acetylcarnosine	Histidine Metabolism	0.000003	0.00007
46	N-acetylhistidine	Histidine Metabolism	0.00007	0.004
47	3-(4-hydroxyphenyl)lactate	Tyrosine Metabolism	0.001	0.002
48	3-methoxytyramine sulfate	Tyrosine Metabolism	0.0003	0.020
49	4-methoxyphenol sulfate	Tyrosine Metabolism	0.0003	0.029
50	phenol glucuronide	Tyrosine metabolism	0.0002	0.008
51	phenol sulfate	Tyrosine metabolism	0.003	0.020
52	5-hydroxyindoleacetate	Tryptophan Metabolism	0.001	0.035
53	N-palmitoyl-sphingadienine (d18:2/16:0)	Sphingolipids	0.002	0.001
54	sphingomyelin (d18:1/18:1, d18:2/18:0)	Sphingolipids	0.002	0.002
55	sphingomyelin (d18:1/20:1, d18:2/20:0)	Sphingolipids	0.001	0.001
56	sphingomyelin (d18:2/14:0, d18:1/14:1)	Sphingolipids	0.003	0.009
57	sphingomyelin (d18:2/16:0, d18:1/16:1)	Sphingolipids	0.002	0.0001
58	sphingomyelin (d18:2/24:2)	Sphingolipids	0.002	0.002
59	glycohyocholate	Secondary Bile Acid	0.002	0.020
60	glycoursodeoxycholate	Secondary Bile Acid	0.004	0.005
61	taurocholate	Primary Bile Acid	0.003	0.029
62	taurohyocholate	Secondary Bile Acid	0.001	0.015
63	tauroursodeoxycholate	Secondary Bile Acid	0.001	0.022
64	aspartate	Alanine and Aspartate Metabolism	0.0001	0.00006
65	beta-citrylglutamate	Glutamate Metabolism	0.006	0.006
66	N-acetyl-aspartyl-glutamate (NAAG)	Glutamate Metabolism	0.0001	0.005
67	gamma-glutamylalanine	Gamma-glutamyl Amino Acid	0.001	0.003

68	N2-acetyllysine	Lysine Metabolism	0.001	0.0004
69	2-methylserine	Glycine, Serine and Threonine Metabolism	0.001	0.002
70	S-methylmethionine	Methionine, Cysteine, SAM and Taurine Metabolism	0.001	0.025
71	glycylvaline	Dipeptide	0.001	0.0002
72	leucylalanine	Dipeptide	0.000009	0.00005
73	N-acetyl-isoputrescine	Polyamine Metabolism	0.002	0.010
74	N-acetylneuraminate	Aminosugar Metabolism	0.005	0.036
75	N-delta-acetylornithine	Urea cycle; Arginine and Proline Metabolism	0.0001	0.009
76	gamma-tocopherol/ beta-tocopherol	Tocopherol Metabolism	0.000003	0.00006
77	pyridoxate	Vitamin B6 Metabolism	0.002	0.010
78	creatinine	Creatine Metabolism	0.000006	0.00003
79	cysteine-glutathione disulfide	Glutathione Metabolism	0.001	0.001
80	dihydroorotate	Pyrimidine Metabolism, Orotate containing	0.00003	0.00006
81	fumarate	TCA Cycle	0.002	0.0004

Supplementary Table 4. List of metabolites significantly different ($p \leq 0.005$) in pairwise comparisons of children with asthma and non-atopic controls. Metabolite name, pathway, unadjusted and Atopic Dermatitis (AD) adjusted p-values are listed.

	Metabolite	Pathway	Unadjusted p-value	AD-adjusted p-value
1	1-(1-enyl-palmitoyl)-2-oleoyl-GPC (P-16:0/18:1)	Plasmalogen	0.001	0.0003
2	1-(1-enyl-oleoyl)-GPE (P-18:1)	Lysoplasmalogen	0.001	0.0004
3	1-(1-enyl-stearoyl)-GPE (P-18:0)	Lysoplasmalogen	0.005	0.003
4	1-arachidonoyl-GPE (20:4n6)	Lysophospholipid	0.002	0.011
5	1,2-dipalmitoyl-GPC (16:0/16:0)	Phosphatidylcholine (PC)	0.001	0.001
6	1-myristoyl-2-arachidonoyl-GPC (14:0/20:4)	Phosphatidylcholine (PC)	0.001	0.004
7	1-myristoyl-2-palmitoyl-GPC (14:0/16:0)	Phosphatidylcholine (PC)	0.002	0.026
8	1-palmitoyl-2-stearoyl-GPC (16:0/18:0)	Phosphatidylcholine (PC)	0.004	0.007
9	1-stearoyl-2-arachidonoyl-GPI (18:0/20:4)	Phosphatidylinositol (PI)	0.003	0.002
10	3-hydroxyhexanoate	Fatty Acid, Monohydroxy	0.0002	0.0002
11	caproate (6:0)	Medium Chain Fatty Acid	0.001	0.001
12	heptenedioate (C7:1-DC)	Fatty Acid, Dicarboxylate	0.0004	0.006
13	cholesterol	Sterol	0.005	0.004
14	oleoyl-arachidonoyl-glycerol (18:1/20:4)	Diacylglycerol	0.005	0.009
15	behenoyl dihydrosphingomyelin (d18:0/22:0)	Sphingolipids	0.001	0.0001
16	myristoyl dihydrosphingomyelin (d18:0/14:0)	Sphingolipids	0.0005	0.00006
17	palmitoyl dihydrosphingomyelin (d18:0/16:0)	Sphingolipids	0.0005	0.00002
18	sphingomyelin (d17:1/14:0, d16:1/15:0)	Sphingolipids	0.001	0.002
19	sphingomyelin (d18:0/20:0, d16:0/22:0)	Sphingolipids	0.001	0.003
20	sphingomyelin (d18:1/14:0, d16:1/16:0)	Sphingolipids	0.0005	0.0004

21	ceramide (d16:1/24:1, d18:1/22:1)	Ceramides	0.004	0.008
22	lactosyl-N-nervonoyl-sphingosine (d18:1/24:1)	Ceramides	0.002	0.001
23	taurochenodeoxycholate	Primary Bile Acid	0.001	0.005
24	taurocholate	Primary Bile Acid	0.0004	0.001
25	glycocholate	Primary Bile Acid	0.001	0.002
26	lysine	Lysine Metabolism	0.00001	0.0003
27	2-aminoadipate	Lysine Metabolism	0.001	0.002
28	N2-acetyllysine	Lysine Metabolism	0.001	0.002
29	leucine	Leucine, Isoleucine and Valine Metabolism	0.002	0.011
30	N-acetylleucine	Leucine, Isoleucine and Valine Metabolism	0.005	0.025
31	isoleucine	Leucine, Isoleucine and Valine Metabolism	0.002	0.013
32	valine	Leucine, Isoleucine and Valine Metabolism	0.001	0.004
33	N-acetylvaline	Leucine, Isoleucine and Valine Metabolism	0.0002	0.001
34	3-methylglutaconate	Leucine, Isoleucine and Valine Metabolism	0.00008	0.00004
35	gamma-glutamylalanine	Gamma-glutamyl Amino Acid	0.004	0.001
36	gamma-glutamyl-alpha-lysine	Gamma-glutamyl Amino Acid	0.002	0.011
37	gamma-glutamylthreonine	Gamma-glutamyl Amino Acid	0.003	0.016
38	glutamate	Glutamate Metabolism	0.0004	0.001
39	glutamine	Glutamate Metabolism	0.004	0.003
40	N-acetylthreonine	Glycine, Serine and Threonine Metabolism	0.0003	0.00008

41	sarcosine	Glycine, Serine and Threonine Metabolism	0.004	0.010
42	threonine	Glycine, Serine and Threonine Metabolism	0.001	0.007
43	methionine	Methionine, Cysteine, SAM and Taurine Metabolism	0.002	0.017
44	N-formylmethionine	Methionine, Cysteine, SAM and Taurine Metabolism	0.003	0.004
45	N-acetylmethionine	Methionine, Cysteine, SAM and Taurine Metabolism	0.002	0.0003
46	N-acetylhistidine	Histidine Metabolism	0.004	0.001
47	4-hydroxyphenylpyruvate	Tyrosine Metabolism	0.002	0.005
48	orotate	Pyrimidine Metabolism, Orotate containing	0.001	0.003
49	3-aminoisobutyrate	Pyrimidine Metabolism, Thymine containing	0.001	0.0004
50	dimethylarginine	Urea cycle; Arginine and Proline Metabolism	0.005	0.005
51	N-acetylneuraminate	Aminosugar Metabolism	0.0002	0.0002

Supplementary Table 5. List of metabolites statistically different ($p \leq 0.05$) in pairwise comparisons between children with FA allergy to one and multiple food items. Metabolite name, pathway, and p-values are listed.

	Metabolite	Pathway	p-value
1	1-(1-enyl-palmitoyl)-2-oleoyl-GPC (P-16:0/18:1)	Plasmalogen	0.047
2	1-(1-enyl-palmitoyl)-2-palmitoleoyl-GPC (P-16:0/16:1)	Plasmalogen	0.020
3	1-(1-enyl-palmitoyl)-2-palmitoyl-GPC (P-16:0/16:0)	Plasmalogen	0.035
4	1-methyl-4-imidazoleacetate	Histidine metabolism	0.025
5	1-myristoyl-2-palmitoyl-GPC (14:0/16:0)	Phosphatidylcholine	0.029
6	glycerol	Glycerolipid Metabolism	0.042
7	1-myristoylglycerol (14:0)	Monoacylglycerol	0.024
8	1-pentadecanoylglycerol (15:0)	Monoacylglycerol	0.007
9	oleoyl-arachidonoyl-glycerol (18:1/20:4)	Diacylglycerol	0.029
10	linoleoyl-arachidonoyl-glycerol (18:2/20:4)	Diacylglycerol	0.007
11	arachidoylcarnitine (C20)	Fatty Acid Metabolism (Acyl Carnitine)	0.049
12	cis-4-decenoylcarnitine (C10:1)	Fatty Acid Metabolism (Acyl Carnitine)	0.043
13	arachidonoylcarnitine (C20:4)	Fatty Acid Metabolism (Acyl Carnitine)	0.034
14	dihomo-linoleoylcarnitine (C20:2)	Fatty Acid Metabolism (Acyl Carnitine)	0.045
15	linolenoylcarnitine (C18:3)	Fatty Acid Metabolism (Acyl Carnitine)	0.012
16	linoleoylcarnitine (C18:2)	Fatty Acid Metabolism (Acyl Carnitine)	0.007
17	13-methylmyristate (i15:0)	Fatty acid, branched	0.027
18	N-trimethyl 5-aminovalerate	Lysine Metabolism	0.012
19	2-aminoadipate	Lysine metabolism	0.019
20	3-hydroxy-2-ethylpropionate	Leucine, Isoleucine and Valine metabolism	0.013
21	isobutyrylcarnitine (C4)	Leucine, Isoleucine and Valine Metabolism	0.019

22	tiglylcarnitine (C5:1-DC)	Leucine, Isoleucine and Valine Metabolism	0.009
23	4-hydroxyphenylpyruvate	Tyrosine metabolism	0.031
24	kynurenine	Tryptophan Metabolism	0.001
25	kynurenate	Tryptophan Metabolism	0.027
26	serotonin	Tryptophan Metabolism	0.026
27	N-formylanthranilic acid	Tryptophan Metabolism	0.008
28	formiminoglutamate	Histidine Metabolism	0.015
29	quinolate	Nicotinate and Nicotinamide Metabolism	0.019
30	N1-Methyl-4-pyridone-3-carboxamide	Nicotinate and Nicotinamide Metabolism	0.032
31	Phenylpyruvate	Phenylalanine Metabolism	0.004
32	S-methylcysteine sulfoxide	Methionine, Cysteine, SAM and Taurine Metabolism	0.029
33	N1-methyladenosine	Purine Metabolism, Adenine containing	0.036
34	orotate	Pyrimidine Metabolism, Orotate containing	0.028
35	N-acetylproline	Urea cycle; Arginine and Proline Metabolism	0.045
36	N-acetylneuraminate	Aminosugar Metabolism	0.038
37	sphingomyelin (d18:2/16:0, d18:1/16:1)	Sphingolipid Metabolism	0.003
38	lactosyl-N-palmitoyl-sphingosine (d18:1/16:0)	Ceramides	0.022
39	glycochenodeoxycholate	Primary Bile Acid Metabolism	0.017
40	succinylcarnitine (C4-DC)	TCA Cycle	0.004
41	cortisone	Corticosteroids	0.022

Supplementary Table 6. List of metabolites significantly different ($p \leq 0.05$) in pairwise comparisons between food allergic children with and without history of anaphylaxis. Metabolite name, pathway, and p-values are listed.

	Metabolite	Pathway	p-value
1	12-HETE	Eicosanoid	0.007
2	1-linoleoyl-GPA (18:2)	Lysophospholipid	0.050
3	1-linolenoylglycerol (18:3)	Monoacylglycerol	0.048
4	acetylcarnitine (C2)	Fatty Acid Metabolism (Acyl Carnitine)	0.047
5	ximenoylcarnitine (C26:1)	Fatty Acid Metabolism (Acyl Carnitine)	0.023
6	hexanoylglycine	Fatty Acid Metabolism (Acyl Glycine)	0.013
7	10-undecenoate (11:1n1)	Medium chain FA	0.038
8	Indolepropionate	Tryptophan Metabolism	0.031
9	5-bromotryptophan	Tryptophan metabolism	0.041
10	methionine sulfone	Methionine, Cysteine, SAM and Taurine Metabolism	0.029
11	N-acetylproline	Urea cycle; Arginine and Proline Metabolism	0.019
12	N-carbamoylalanine	Alanine and Aspartate Metabolism	0.005
13	lactate	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	0.012
14	N-palmitoyl-heptadecasphingosine (d17:1/16:0)	Sphingolipid Metabolism	0.029
15	sphingomyelin (d17:1/16:0, d18:1/15:0, d16:1/17:0)	Sphingolipid Metabolism	0.043
16	sphingomyelin (d18:1/24:1, d18:2/24:0)	Sphingolipid Metabolism	0.033
17	pyridoxal	Vitamin B6 Metabolism	0.008
18	pantothenate	Pantothenate and CoA Metabolism	0.018
19	(N(1) + N(8))-acetylspermidine	Polyamine metabolism	0.044

Supplementary Table 7. List of metabolites significantly different ($p \leq 0.05$) in pairwise comparison between food allergic children without atopic dermatitis (n=29) and non-atopic controls (n=20). Metabolite name, pathway and p-values are listed.

	Metabolite	Pathway	p-value
1	1-(1-enyl-palmitoyl)-2-palmitoyl-GPC (P-16:0/16:0)	Plasmalogen	0.002
2	1-(1-enyl-oleoyl)-GPE (P-18:1)	Lysoplasmalogen	0.001
3	1-(1-enyl-stearoyl)-GPE (P-18:0)	Lysoplasmalogen	0.002
4	1-(1-enyl-palmitoyl)-GPC (P-16:0)	Lysophospholipid	0.003
5	1-(1-enyl-palmitoyl)-GPE (P-16:0)	Lysophospholipid	0.005
6	1-arachidonoyl-GPC (20:4n6)	Lysophospholipid	0.0001
7	1-arachidonoyl-GPE (20:4n6)	Lysophospholipid	0.001
8	1-linolenoyl-GPC (18:3)	Lysophospholipid	0.038
9	1-linoleoyl-GPC (18:2)	Lysophospholipid	0.011
10	1-oleoyl-GPC (18:1)	Lysophospholipid	0.004
11	1-palmitoleoyl-GPC (16:1)	Lysophospholipid	0.002
12	1-palmitoyl-GPC (16:0)	Lysophospholipid	0.008
13	glycerophosphoinositol	Phospholipid	0.012
14	1-linoleoyl-2-arachidonoyl-GPC (18:2/20:4n6)	Phosphatidylcholine	0.021
15	1-myristoyl-2-arachidonoyl-GPC (14:0/20:4)	Phosphatidylcholine	0.005
16	3-hydroxyhexanoate	Fatty Acid, Monohydroxy	0.023
17	stearoylcarnitine (C18)	Fatty Acid Metabolism (Acyl Carnitine)	0.031
18	oleoyl-arachidonoyl-glycerol (18:1/20:4)	Diacylglycerol	0.003
19	stearoyl-arachidonoyl-glycerol (18:0/20:4)	Diacylglycerol	0.001

20	ceramide (d18:1/20:0, d16:1/22:0, d20:1/18:0)	Ceramides	0.004
21	glycosyl-N-stearoyl-sphingosine (d18:1/18:0)	Ceramides	0.001
22	lactosyl-N-nervonoyl-sphingosine (d18:1/24:1)	Ceramides	0.005
23	lactosyl-N-palmitoyl-sphingosine (d18:1/16:0)	Ceramides	0.001
24	behenoyl dihydrosphingomyelin (d18:0/22:0)	Sphingolipids	0.007
25	lignoceroyl sphingomyelin (d18:1/24:0)	Sphingolipids	0.005
26	myristoyl dihydrosphingomyelin (d18:0/14:0)	Sphingolipids	0.001
27	palmitoyl dihydrosphingomyelin (d18:0/16:0)	Sphingolipids	0.0001
28	palmitoyl sphingomyelin (d18:1/16:0)	Sphingolipids	0.001
29	sphingomyelin (d17:1/14:0, d16:1/15:0)	Sphingolipids	0.019
30	sphingomyelin (d17:1/16:0, d18:1/15:0, d16:1/17:0)	Sphingolipids	0.010
31	sphingomyelin (d18:0/20:0, d16:0/22:0)	Sphingolipids	0.005
32	sphingomyelin (d18:1/14:0, d16:1/16:0)	Sphingolipids	0.003
33	sphingomyelin (d18:1/22:1, d18:2/22:0, d16:1/24:1)	Sphingolipids	0.026
34	sphingomyelin (d18:1/22:2, d18:2/22:1, d16:1/24:2)	Sphingolipids	0.003
35	sphingomyelin (d18:2/14:0, d18:1/14:1)	Sphingolipids	0.004
36	sphingomyelin (d18:2/16:0, d18:1/16:1)	Sphingolipids	0.00004
37	sphingomyelin (d18:2/18:1)	Sphingolipids	0.034
38	sphingomyelin (d18:2/21:0, d16:2/23:0)	Sphingolipids	0.024
39	sphingomyelin (d18:2/23:0, d18:1/23:1, d17:1/24:1)	Sphingolipids	0.007
40	sphingomyelin (d18:2/23:1)	Sphingolipids	0.005
41	tricosanoyl sphingomyelin (d18:1/23:0)	Sphingolipids	0.012
42	taurodeoxycholate	Secondary Bile Acid	0.007
43	lysine	Lysine Metabolism	0.001

44	N2-acetyllysine	Lysine Metabolism	0.015
45	threonine	Glycine, Serine and Threonine Metabolism	0.015
46	valine	Leucine, Isoleucine and Valine Metabolism	0.003
47	isoleucine	Leucine, Isoleucine and Valine Metabolism	0.035
48	glutamate	Glutamate Metabolism	0.001
49	cholesterol	Sterol	0.0003
50	gamma-tocopherol/beta-tocopherol	Tocopherol Metabolism	0.003