

A Systematic Review of Metabolic Alterations Underlying IgE-Mediated Food Allergy in Children

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ABSTRACT

Scope: IgE-mediated food allergies (IgE-FA) are characterized by an ever-increasing prevalence, currently reaching up to 10.4% of children in the European Union. Metabolomics has the potential to provide a deeper understanding of the pathogenic mechanisms behind IgE-FA.

Methods and results: In this work, literature was systematically searched using Web of Science, PubMed, Scopus and Embase, from January 2010 until May 2021 including human and animal metabolomic studies on multiple biofluids (urine, blood, feces). In total, 15 studies on IgE-FA were retained and a dataset of 277 potential biomarkers was compiled for in-depth pathway mapping. Decreased IDO-1 activity was hypothesized due to altered plasma levels of tryptophan and its metabolites in IgE-FA children. In feces of children prior to IgE-FA, aberrant metabolization of sphingolipids and histidine was noted. Decreased fecal levels of (branched) SCFAs compelled a shift towards aerobic glycolysis and suggested dysbiosis, associated with an immune system shift towards Th2 responses. During animal anaphylaxis a similar switch towards glycolysis was observed, combined with increased ketogenic pathways. Additionally, altered histidine, purine, pyrimidine and lipid pathways were observed.

Conclusion: To conclude, this work confirms the unprecedented opportunities of metabolomics and supports the in-depth pathophysiological qualification in the quest towards improved diagnostic and prognostic biomarkers for IgE-FA.

1. INTRODUCTION

In 2016, the White House announced its precision medicine initiative enabling the development of individualized care in medicine through research, technology and policies. Precision medicine relies on the determination of the patients' best treatment or prevention method, based on his/her metabolic state.^[1,2] As such, it should provide a metabolic signature of the (patho)physiological state, employing multiple omics disciplines, including genomics, transcriptomics, proteomics and metabolomics.^[1,2] Metabolomics is considered an indispensable tool in biomedical research as it measures all, or a large number of, small molecules present in cells, tissues or biological fluids, constituting the metabolome.^[1,3-6] This metabolome, governed by the interaction of a person's genetics with diet, environmental factors and the gut microbiome, provides the closest representation of the functional phenotype, thus giving insights into metabolic changes in health and disease.^[2,7-9] So far, metabolomic fingerprinting has emerged as a powerful technique to uncover biologically relevant biomarkers and pathway alterations, contributing to a variety of complex diseases, including obesity, diabetes, inflammatory bowel diseases and a variety of cancers.^[3,10-13]

In this regard, the analysis of the metabolome provides unprecedented opportunities in the quest for biomarkers of IgE-mediated allergic diseases. The prevalence of these allergic diseases, including IgE-mediated food allergy (IgE-FA), has been increasing over the last decade, the latter currently affecting up to 10.4 % of children and 4.7 % of adults in the European Union, with numbers still increasing.^[14] Several allergic manifestations, including atopic dermatitis, IgE-FA and respiratory allergies (asthma and allergic rhinitis) belong to the natural history of the atopic march (AM), which generally begins early in life and can persist into adulthood.^[15] AM manifestations are characterized by a type 2 immune mechanism, which has the tendency to produce IgE-antibodies upon exposure to small amounts of commonly tolerated allergens.^[16,17] Each allergic manifestation within the AM represents complex and diverse inflammatory conditions, with markedly heterogeneous clinical phenotypes complicating diagnostics, especially in IgE-FA.^[18,19] However, a correct diagnosis is essential in order to prevent both allergic reactions and unnecessary dietary restrictions, as the latter

could result in nutritional imbalances and growth problems, especially in children.^[20,21] The current diagnostic procedure relies on a thorough assessment of the clinical history of the patient, followed by additional diagnostic tests, including skin prick tests (SPT), immunoassays of serum food-specific IgE levels and, in some cases, a double-blinded placebo-controlled food challenge.^[21,22] In this regard, given the limitations of conventional diagnostic procedures, a deeper understanding into the pathogenic mechanisms behind IgE-FA may reveal superior diagnostic or prognostic biomarkers, as well as novel treatment strategies, or may hint towards diagnostic criteria for non-IgE-mediated food allergies.^[1,2,23,24]

To date, several studies have applied (un)targeted metabolomics in the search for metabolic signatures associated with asthma.^[25–32] However, compared to asthma, very few studies have applied (un)targeted metabolomics to investigate IgE-FA^[19,33–40] and food anaphylaxis^[19,36,37,41–43], even though precision medicine could greatly improve IgE-FA diagnosis, prevention and therapy.^[14,21,44] Therefore, the aim of this systematic review was to provide a comprehensive overview on the use of metabolomics in IgE-FA and anaphylaxis in children and animal models, to aid in the exploration and validation of biomarkers. Subsequent to an extensive literature search, a dataset including all relevant IgE-FA-associated metabolites was created and pathway analysis executed, followed by a more detailed exploration of any up- or downregulated metabolic features and pathways.

2. EXPERIMENTAL SECTION

2.1 Data sources and searches

For this review, we conducted a systematic search of published literature in English in four electronic databases, i.e. PubMed, Web of Science, Scopus and Embase. The literature search included articles published between January 2010 and May 2021. All articles were hand-searched for human and animal studies evaluating metabolite biomarkers associated with IgE-FA. Additional literature was included from references cited in the identified studies. Detailed information regarding

the search strategy is presented in Supplementary Data (Data S1). The study protocol was registered in the PROSPERO registry (ID CRD42020183054).

2.2 Study selection

Initial screening of the retrieved studies was performed by two independent reviewers (EDP and LVG), based on title and abstract, after which duplicates were removed. The full-text copies of potentially relevant studies were obtained and their eligibility for inclusion was independently assessed based on the whole manuscript and inclusion and exclusion criteria (Table 1). While the use of animal models for the study of human disease requires careful interpretation, they are considered invaluable for a better understanding of diseases and for enabling translational discoveries.^[45] Therefore, articles describing both children and animal studies, with metabolites identified in blood (serum or plasma), urine and feces, were retained.

2.3 Data extraction and quality assessment

Once consensus on the included articles was achieved, data were extracted from the full text articles, entered into a table and compared for similarity (Table 2 - 3). Quality assessment relied on the Newcastle-Ottawa Scale (NOS) or the Systematic Review Centre for Laboratory animal Experimentation (SYRCLE) risk of bias tool (Table S1 – S3). More information regarding data extraction and quality assessment is provided in Supplementary Data (Data S2).

2.4 Data synthesis and pathway analysis

Subsequent to the literature search, a dataset including all relevant IgE-FA-associated metabolites was constructed - including KEGG ID and chemical class - (Table S4) which served as a basis for

pathway analysis using MetaboAnalyst 5.0.^[46] During pathway analysis, the following parameters were employed: hypergeometric test as over-representation analysis method and relative betweenness centrality to measure the shortest paths going through the node and to account for node importance measure for topological analysis. The pathway libraries *homo sapiens* and *mus musculus* were consulted for, respectively, human and animal studies. The most important pathways (p -value < 0.10) were retained and subjected to a more detailed exploration based on chemical class or origin. For lipids and microbial metabolites, only manual exploration was executed, as none of these were included in the KEGG registry.

3. RESULTS AND DISCUSSION

3.1 Study inclusion and characteristics

The initial search strategy resulted in a total of 3,600 articles. After meticulous inspection, 15 metabolomics studies focusing on IgE-FA were incorporated in our dataset (Table 2, Table S4) and included for consecutive pathway analysis. Within these 15 studies, 12 included data on children while 5 reported on data from animal models, of which 2 studies combined both human and animal data.^[36,47] The process of study selection is summarized in a PRISMA scheme (Figure 1). Even though all studies met the inclusion criteria, one animal study was excluded from this review as scoring of anaphylaxis was not adequately described, thereby negatively impacting its quality score.^[43] Diagnosis of IgE-FA in children relied on SPT, serum IgE measurements and/or food challenges, while IgE-FA in animals was confirmed by food challenge and anaphylaxis scores, combined with serum IgE measurements. One animal study focused on guinea pigs, while 3 studies evaluated IgE-FA in murine models. In total, 11 retrospective and 3 prospective studies were included, with the latter focusing on cord blood ($n = 1$) and feces ($n = 2$). The majority of the studies focused on blood ($n = 8$), followed by feces ($n = 5$) and urine ($n = 3$). The blood metabolome reflects a wide range of biochemical processes and is therefore a highly interesting biofluid for the assessment of metabolic diseases. However, a major drawback is its invasive nature of sample collection, which is especially problematic in (young) children. Therefore, urine and especially feces provide an excellent alternative to plasma collection. Each matrix has a different coverage and the combination thereof could be highly informative for disease-related biomarker detection and pathway elucidation.^[48] For this reason, multiple matrices were included in the pathway analysis, however, they were analyzed separately in MetaboAnalyst 5.0, with a minimum of 3 studies per matrix. As such, a dataset was constructed including 277 metabolites for in-depth pathway mapping. The latter included metabolites from studies that obtained basal blood and fecal samples of children with IgE-FA. No pathway analysis was performed for urine, although, the results thereof were compared to observations in blood and feces. Prospective studies were not included in any pathway analysis, nevertheless,

metabolites identified in these studies were discussed in relation to results from retrospective studies. Animal samples were collected prior to ^[36,41] and/or during anaphylactic reactions,^[41,42] and were regarded as a separate entity for pathway analysis. Within the group of children with IgE-FA, a positive history of anaphylaxis as well as the manifestation of multiple IgE-FAs were considered as a retrospective parameter for IgE-FA severity. However, no predictive value was assigned to these potential markers.

3.2 Pathway analysis

Pathway analysis included all compounds for which a KEGG ID could be assigned, resulting in 86 metabolites, of which 20 were detected in blood and 5 in feces of children with IgE-FA, while 61 were retrieved in blood of animals (Table S5 – S7). In IgE-FA children's plasma, differential expressions could be noted in amino acid (AA) metabolism, i.e. most importantly the tryptophan (p -value = $1.23e^{-03}$) metabolism (Table S8). In samples of anaphylactic animals, the main pathways entailed AA metabolism, with most importantly histidine metabolism (p -value = $2.44e^{-04}$), next to purine (p -value = $7.52e^{-04}$) and pyrimidine (p -value = $6.29e^{-02}$) metabolism and TCA cycle (p -value = $4.05e^{-02}$) (Figure 2, Table S9). Additional exploration based on chemical class revealed alterations in microbial metabolite production in feces of children with IgE-FA (Table S10), whereas in animals undergoing anaphylaxis, the lipid metabolism was involved.

3.3 Biomarkers related to carbohydrate and energy metabolism

Decreased levels of several simple sugars, a.o. glucose-6-phosphate and sucrose, were prospectively noted in cord blood, and in feces of 3 – 6 month old children with future IgE-FA by the age of 3 years (Figure 2, carbohydrate metabolism (purple)).^[34] It could be hypothesized that, under conditions of dysbiosis early in life, the latter being characterized by a lower presence of butyrate

producing anaerobic bacteria and thus also lower levels of the short chain fatty acid (SCFA) butyrate, colonocytes tend to shift to aerobic glycolysis to convert glucose into lactate and pyruvate, thereby depleting the luminal concentration of these simple sugars.^[35,40,49–51] The latter was confirmed by altered lactate concentrations in blood of patients with IgE-FA and a history of anaphylaxis (Figure 3).^[19] Additionally, a shift towards aerobic glycolysis can be hypothesized in immune cells as they are known to alter their metabolism to cope with the increasing demand resulting from the need to synthesize biological precursors associated with immune cell activation. Within the rapidly dividing and proliferating immune cells, glycolytic metabolism can be regarded as the most dominant and essential pathway to ensure adequate energy supply.^[52]

In an animal study of anaphylaxis, Hu et al (2012)^[42] described reduced serum glucose levels in guinea pigs (Figure 2, carbohydrate metabolism (purple)). Additionally, decreased TCA intermediates (succinic anhydride, fumarate) (Figure 2, TCA cycle (black)), and an increase of the ketone body 3-hydroxybutyrate were observed.^[41,42] The authors suggested a higher energy expenditure during anaphylaxis, for which a sharp increase in glucose consumption occurred, causing lower glucose levels.^[42] As a consequence of the higher energy needs and limited glucose supply, the organism is obliged to switch to the degradation of triglycerides as alternative energy source, with the resulting free fatty acids subsequently giving rise to the formation of acetyl-CoA via β -oxidation in the mitochondrial membrane.^[43] Under normal conditions, acetyl-CoA undergoes oxidation in the TCA cycle to yield cofactors for ATP-production. However, decreased levels of citrate^[41] and other TCA intermediates^[42] pointed towards a disruption of the mitochondrial TCA cycle, which may lead to mitochondrial toxicity (Figure 2, TCA cycle (black)).^[43] The latter could induce the excess acetyl-CoA to be dissipated via ketogenesis, evoking an increase in ketone body formation (Figure 3).^[42] As such, increased cellular energy requirement and a disrupted TCA cycle might be hypothesized, especially since mitochondrial function and elevated serum IgE levels are known to be correlated.^[53–55] Moreover, a disrupted TCA cycle early in life could be hypothesized due to decreased levels of the

TCA intermediates isocitrate and α -ketoglutarate in cord blood,^[56] with the latter being a key molecule in the determination of the overall rate of the TCA cycle.^[43]

3.4 Biomarkers related to lipid metabolism

3.4.1 Sphingolipids

Marked alterations could be observed in feces of children who will develop IgE-FA, as well as in blood of patients already diagnosed with IgE-FA, more specifically in metabolites associated with *de novo* sphingolipid syntheses, including sphinganine, sphingomyelins and ceramides.^[19,34] Sphingolipids are major components of cellular membranes and participate in multiple cellular functions. They can be synthesized *de novo* as components of cellular membranes, derived from dietary sources or produced by *Bacteroides* species in the gut.^[19,34] When prospectively comparing food sensitized children to patients with clinical IgE-FA at 3 years old, higher levels of stool sphingolipids were detected at the age of 3 – 6 months, which could suggest a protective effect of *B. fragilis*.^[34] On the other hand, the levels of ceramides and sphingomyelins, both negative regulators of mast cell activation, were decreased in serum of patients with IgE-FA, especially in children with a positive history of anaphylaxis. The latter might suggest an increased mast cell responsiveness towards a hyperresponsive state, predisposing to severe anaphylactic reactions.^[19] The decline of both ceramides and sphingomyelins has been attributed to a reduced production of ceramides as well as to a drop in functionality of the ceramide-converting enzymes sphingomyelin synthase and sphingomyelinase.^[19] However, both enzymes are critical in the control of Th17 cell differentiation, while Th17 cells in their turn are important in intestinal inflammation, and perturbations in their response may contribute to the pathogenesis of inflammatory diseases.^[57] Therefore, altered enzyme functionalities may hint towards an immune-regulatory role for this pathway, resulting in a systemic, non-allergen specific perturbation in Th17 response.^[19,58]

3.4.2 Lipid allergy mediators

Evidence of allergic inflammation was observed in serum of children with a history of anaphylaxis, due to unspecified alterations in the pro-inflammatory 12-hydroxy eicosatetraenoic acid (12-HETE), which is a platelet-derived 12-lipoxygenase metabolite of arachidonic acid.^[59] Additionally, it was demonstrated that the concentration of urinary tetranor PGDM, a major prostaglandin D₂ (PGD₂) metabolite, increased along the severity of immediate allergic reactions following oral food challenge. Moreover, urinary levels of this metabolite were significantly higher in patients with IgE-FA compared to those in healthy volunteers and patients with other types of allergic diseases.^[47,60]

In serum of anaphylactic mice, decreased levels of choline could be observed.^[41] Next to its neuro-active properties, choline is known as essential component of phosphatidylcholine lipids, including platelet-activating-factor (PAF), a potent anaphylactic mediator.^[41] Therefore, decreased choline levels suggest increased PAF synthesis. Additionally, altered concentrations of several serum phosphatidylcholines and lysophosphatidylcholines were observed prior to, in the early and the late phases of anaphylaxis.^[36,41] Such components are chemically similar to PAF, and could therefore function as direct mediators of anaphylaxis, or as chemical precursors to PAF and/or other yet to be discovered phosphatidylcholine-based mediators (Figure 2, glycerophospholipid metabolism (blue)).^[36,41] Phosphatidylcholines and lysophosphatidylcholines are also the main constituents of cellular membranes. Their altered levels may thus indicate a release from degranulated mast cells and basophils, after which they are scavenged into leukotrienes and PAF. The latter presumably causes an accumulation of PAF itself, and 1-arachidonoyl-GPC, a plasmalogen and precursor of PAF, thereby triggering a signaling event towards anaphylaxis.^[41,43] Similar to observations in IgE-FA children, urinary tetranor-PGDM levels reflected the severity of allergic symptoms in mice, as they increased with disease progression. PGD₂ is mainly produced by mast cells, which infiltrate in the intestinal mucosa in the case of IgE-FA, thereby causing increased urinary concentrations of its metabolite. In contrast, in murine models of non-allergic intestinal inflammation and other allergic models, only a slight increase in urinary PGDM was observed.^[47]

3.4.3 Fatty acids and derivatives

Fatty acids are of particular interest due to their regulation of the immune system and their anti-inflammatory roles in asthma and atopy.^[37,61,62] These include linoleic acid derived omega-3 polyunsaturated fatty acids, docosapentanoate (22:5n3) and docosahexaenoate (22:6n3), which were found at lower concentrations in feces of children who will develop IgE-FA.^[34] Additionally, children with multiple IgE-FAs or a history of anaphylaxis displayed reduced levels of branched fatty acids and acylcarnitines in their serum.^[19]

Similarly, blood of animals undergoing anaphylaxis mainly exhibited decreased levels of propionylcarnitine,^[41] revealing disturbed energy metabolism. Under normal conditions, carnitines play a key role as carrier of fatty acids in the β -oxidation.^[41,53] Their decreased concentration could point towards an increased β -oxidation of fatty acids to form acetyl-CoA.

3.5 Biomarkers related to amino acid catabolism

In the case of IgE-FA in children, a disturbed tryptophan metabolism was observed in blood,^[39,63] while histidine metabolism appeared to be most affected in feces of children prior to the onset of IgE-FA.^[34]

Anaphylaxis in animals was defined by variations regarding histidine metabolism and several other AAs, including creatinine as well as diminished levels of proline, alanine, leucine, creatine a.o.^[41,43]

3.5.1 Tryptophan catabolism

IgE-FA children had significantly higher levels of serum tryptophan, while the levels of its catabolites in the kynurenine and serotonin (5-hydroxytryptamine; 5-HT) pathway decreased according to disease burden, including multiple IgE-FAs or AM manifestations. Additionally, the serum metabolome of IgE-FA children with a positive history of anaphylaxis was characterized by increased levels of tryptophan catabolites in the indolic pathway,^[19,63] which were, however, reported to be reduced in children with low communication scores and IgE-FA (Figure 2, tryptophan metabolism (light green)).^[39] Tryptophan is an essential aromatic AA and is metabolized through 3 main pathways (Figure 4): (a) direct transformation by the gut microbiota into proteins or indolic metabolites, including ligands of the aryl hydrocarbon receptor (AhR); (b) kynurenine pathway in both immune and epithelial cells through the action of the rate-limiting indoleamine 2,3-dioxygenase-1 (IDO-1); and (c) transformation to serotonin in enterochromaffin cells through tryptophan hydroxylase-1 (TH1).^[19,64] Changes in the 5-HT pathway have been associated with psychological and psychiatric conditions, such as depression and autism, which have previously been associated with FAs.^[33,39] Both the kynurenine as well as indolic compound pathways are important in immune responses at mucosal barriers and contribute to intestinal homeostasis, due to direct downregulation of T cell activity or induction of various Treg cells.^[19,65] Metabolites within the indole pathway, a.o. indole propionate and 5-hydroxyindoleacetate were respectively increased and decreased in patients

with a history of anaphylaxis or AM manifestations. As this pathway is mediated by direct transformation by the gut microbiota, aberrant levels of these metabolites point towards dysbiosis, especially since colonization with indole producing bacteria is known to attenuate intestinal inflammation and a reduced microbial indole production has been associated with inflammatory bowel disease.^[66] Finally, tryptophan degradation via the kynurenine pathway, and more specifically the IDO-1 enzyme, is one of the central regulatory pathways in allergy development, with IDO-1 associated with Th1 inhibition via tryptophan depletion, but additionally controlling Th2 inflammation through the action of Treg cells.^[58,60,62] As such, elevated tryptophan levels suggest inactivation of the IDO-1 enzyme, which is associated with a downregulation of the kynurenine pathway. The latter would interfere with the development of Treg cells and oral tolerance, by disrupting the Th1/Th2 balance. This hypothesis is partially confirmed by Buyuktiryaki et al (2016) who observed a reduced kynurenine/tryptophan ratio in allergic patients, indicating low IDO-1 activity.^[63] Under normal conditions, IDO-1 activity is increased by bacterial components via toll-like receptors and therefore supports the popular “hygiene hypothesis”, i.e. that frequent contact with microbial components in (early) childhood favors differentiation of T cells towards the Th1 direction and thus reduces the risk of Th2-associated diseases.^[65]

A murine model of cow's milk allergy showed increased levels of serotonin and 5-HT positive cells, next to lowered levels of 5-hydroxyindoleacetic acid (5-HIAA) in the intestine.^[33] A rise in intestinal serotonin levels could result from synthesis and release by mast cells, an increase in the proportion of serotonin-secreting enterochromaffin cells in the gut, or decreased serotonin transporter activity,^[33,39,67] with the latter being supported by lowered blood serotonin levels in children with multiple IgE-FA.^[19]

3.5.2 Histidine catabolism

Alterations in histidine metabolism were observed in feces of children prior to the development of IgE-FA, displaying decreased levels of the AA histidine, which could point towards a higher intestinal absorption (Figure 2, histidine metabolism (dark green)).^[34]

Animal studies evidenced a strong increase in biogenic amines, namely histamine and 1-methylhistamine during anaphylaxis, almost immediately following oral challenge.^[41] Those compounds are well-known markers of mast cell degranulation and play an important role in eliciting anaphylaxis. Increased synthesis of histamine could account directly for the decreased histidine levels in feces of children prone to develop IgE-FA.^[34,43,69]

3.6 Biomarkers related to the nucleotide metabolism

The purine pathway was associated with peanut allergy in the study by Kong et al (2015),^[36] as levels of uric acid were increased in serum of IgE-FA children.^[36] Additionally, increased levels of the pyrimidine uridine and the purine adenosine were observed in cord blood of children who will develop IgE-FA by the age of 3 years old.^[56]

Alterations within the nucleotide metabolism were most striking in serum of sensitised mice prior to anaphylaxis, with increased uric acid concentrations, and decreased levels of adenosine, adenine, inosine, hypoxanthine and xanthine.^[36,41] Uric acid is an important endogenous alarmin, indicative for tissue damage or cell necrosis and is therefore excessively present in the extracellular compartment in case of IgE-FA and especially during anaphylaxis. It is thought to play a critical role in allergic sensitization and may hint towards a subclinical state of underlying inflammation, altered dietary habits or actual changes in metabolism.^[36] As adenosine, adenine, inosine, hypoxanthine and xanthine are precursors of uric acid, their drop suggests an increased uric acid synthesis. In addition, a reduction in uric acid metabolization can be hypothesised during sensitisation, due to a decrease of its catabolite allantoin.^[36,43,70,71] Pyrimidine metabolism may also be implicated in eliciting anaphylaxis in animal models, with decreased concentrations of cytidine, and unspecified alterations in uridine and uracil as a consequence of sensitisation. The latter is known as potent inducer of histamine release,

with both cytidine and uridine as its precursors. Declined cytidine levels prior to anaphylaxis suggest increased production of uracil, which will subsequently effectuate histamine release in anaphylaxis.^[72]

3.7 Biomarkers related to microbiome composition

The gut microbiome has wide-ranging local and systemic effects. Indeed, it has been implicated in local disorders, such as inflammatory bowel diseases and an array of systemic conditions, including, but not limited to, disorders of the nervous, cardiovascular and immune system.^[48,61] Microbiome-associated metabolomics has been employed to investigate and characterize a range of clinically relevant features, as it allows a more functional approach to microbial ecology.^[35] The latter could provide crucial information on the pathogenesis of several diseases, as microbial stimulation, both from commensals and pathogens, may be important for the maturation of the immune system, thereby driving the postnatal development of a normal Th1-/Th2-balance.^[49]

3.7.1 Short chain fatty acids

SCFAs, such as acetate, propionate and butyrate as well as branched SCFAs (BSCFAs), such as isobutyrate and valerate, are formed during fermentation of undigested dietary fibers and proteins by anaerobic microbes in the human colon and provide an important carbon source for the host. SCFAs promote the growth of beneficial intraluminal bacteria and homeostasis through the maintenance of an intact GI barrier.^[35,40,73] Additionally, they have been shown to exert anti-inflammatory effects in animal models, as they support the expansion of Treg cells and upregulate the production of IL-10 by increasing the expression of transcription factor FOXP3 via inhibition of histone deacetylation (Figure 3).^[40,73,74] Several authors observed lower levels of acetate, propionate, butyrate, valerate, isobutyrate and isovalerate in feces of allergic children,^[35,73] or children who will develop IgE-FA (> 1 year old),^[40] while Thompson-Chagoyan et al (2011) showed higher concentrations of fecal butyrate and

BSCFA in children with IgE-FA (< 1 year old).^[38] The latter could result from an immaturity of the microbiome, as was suggested by Wopereis et al (2017), who hypothesized that the establishment of butyrate-producing bacteria, around 26 weeks of age, might prove important for establishing and maintaining homeostasis with the immune system during this critical state of development.^[75] Nevertheless, altered (B)SCFA levels point towards the presence of dysbiosis in IgE-FA.^[38]

3.7.2 Bile and related metabolites

In both retrospective and prospective fecal samples of children with IgE-FA, alterations of (secondary) bile metabolites could be observed.^[19,34] It was suggested that alterations in secondary bile acids (BAs) were caused by differential processing of primary BAs through the action of a dysregulated bacterial metabolism, or dysbiosis.^[19] Additionally, Crestani et al. (2020) observed increased levels of ursodeoxycholic acid and its conjugates in serum of children with IgE-FA,^[19] similar to prospective observations in cord blood made by Ross et al. (2020),^[56] while Lee-Sarwar et al. (2018) noticed declined levels of its sulfated form ursodeoxycholate sulfate in feces of children developing IgE-FA.^[34] Sulfation of secondary BAs decreases toxicity and intestinal absorption, suggesting lower sulfation in children with developing IgE-FA, leading to increased intestinal absorption of the respective BA and increased toxicity.^[76] Next, heme catabolites, including I-urobilinogen, D-urobilin and bilirubin, were decreased in feces of children developing an IgE-FA.^[34] The latter results from a combination of altered microbial and host metabolic activity, an increase in host production and/or a change in the microbial enzymatic activity on their substrates.^[61]

4. CONCLUDING REMARKS

In recent years, metabolomics has surfaced as an expedient technology in the field of biomarker discovery and pathophysiological pathway elucidation, as it provides a snapshot of the functional phenotype of an individual. Indeed, metabolomics is a highly promising technique to provide a deeper

understanding into the pathogenic mechanisms behind IgE-FA, which is imperative to bolster the discovery of improved diagnostic and prognostic biomarkers as well as novel treatment strategies. In the last decade, however, the majority of allergy-related metabolomics studies have been focusing on asthma.

In essence, this systematic review emphasizes the importance of metabolomics in the quest for potential biomarkers for IgE-FA in children and its mechanistic elucidation. To do so, metabolomic studies on feces, urine and blood of children with (future) IgE-FA and on food anaphylaxis in animal models were selected, after which a food allergy dataset was constructed and used for in-depth pathway analysis. The latter revealed alterations in carbohydrate and energy metabolism, as well as lipid, AA, nucleotide and microbial metabolism. With respect to the energy metabolism in IgE-FA children, a pronounced shift towards aerobic glycolysis was evinced by aberrant concentrations of simple sugars in feces, arising as a direct metabolic consequence of dysbiosis. The switch towards glycolytic pathways was even more emphasized in animal models of anaphylaxis, which was combined with a shift towards ketogenesis. Decreased levels of serum glucose during IgE-FA-mediated anaphylaxis suggested a higher energy expenditure, evoking a switch to triglycerides as alternative energy source. Moreover, it has been demonstrated that reduced TCA functionality, resulting in IgE-mediated mitochondrial toxicity, promotes acetyl-CoA to shift towards ketogenic pathways. Inflammatory conditions in the gut were coincided by different levels of pro-inflammatory lipids, a.o. 12-HETE and urinary PGDM. Additional lipid changes in blood, including the synthesis of sphingolipids, were suggested as indicator of severity, i.e. history of anaphylaxis, pointing towards a hyperresponsive mast cell state due to alterations in the enzymes sphingomyelin synthase and sphingomyelinase, which are critical in the control of Treg cell/Th17 cell differentiation. Differences in lipid allergy mediators, such as choline and its metabolites, present in cellular membranes, suggested increased PAF synthesis during anaphylaxis. Interestingly, increased levels of tryptophan and decreased levels of its catabolites were observed, implicating reduced IDO-1 activity, thereby creating a Th2-skewed immune environment and thus increasing the risk of Th2-associated diseases.

Furthermore, decreased levels of fecal histidine were suggestive of increased biogenic histamine production prior to anaphylaxis, which was confirmed by a strong increase in biogenic amines (histamine and 1-methylhistamine) in blood during anaphylaxis. A dysregulated purine pathway was considered critical for allergic sensitization, and even more so during anaphylaxis with increased levels of uric acid, which is an endogenous alarmin, hinting towards an (a)symptomatic state of underlying inflammation, even prior to anaphylactic responses. During anaphylactic reactions, a.o. an increased production of uracil was observed, pointing towards an involvement of pyrimidine metabolism. Finally, differences in microbial metabolites, i.e. SCFAs and bile acids pointed towards a combination of altered microbial and host metabolic activity. The depletion of butyrate might support a pro-inflammatory environment, as this anti-inflammatory compound normally supports the expansion of Treg cells and upregulates the production of IL-10.

To conclude, our results suggested several pathway alterations in case of IgE-FA in children and, thereby offered a solid basis for future research. The latter should include the integration of all relevant pathways combined, next to the pathophysiological importance of each separate pathway. Also, more comprehensive metabolomics studies, evaluating a vast number patients and exploring a broad range of biological matrices should enable the translation of clinical phenotypes into metabolic signatures, in addition to the confirmation of suggested biomarkers with higher statistical relevance. Both *in vivo* and *in vitro* studies, combined with target intervention studies, should provide a more in-depth evaluation of the suggested pathophysiological basis of IgE-FA.

5. SHORTCOMINGS

The main shortcoming of this work relates to the limited number of papers that attained our quality standards for inclusion in the systematic review. However, until now, the number of research studies on this topic remains scarce. Therefore, multiple diagnostic procedures were included in this review, i.e. SPT, serum IgE measurements and food challenges. Additionally, this research combined

the results of both human and animal studies, since humans and animal models can differ in their metabolic pathways and concentration of metabolites, it is of utmost importance to interpret these data with caution. Moreover, this paper did not include other types of chronic intestinal inflammations, such as inflammatory bowel disease. Therefore, the suggested biomarkers or metabolic pathways could be specific for IgE-FA, however, they could also reflect a wide range of gastrointestinal inflammations.

6. CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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8. AUTHOR CONTRIBUTIONS

Conceptualization: E.D.P., L.V.; methodology: E.D.P., M.D.S., L.V.; literature search: E.D.P., L.V.G., L.V.; writing: E.D.P., L.V.G., M.D.S., E.C., L.V.

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10. FIGURE LEGENDS

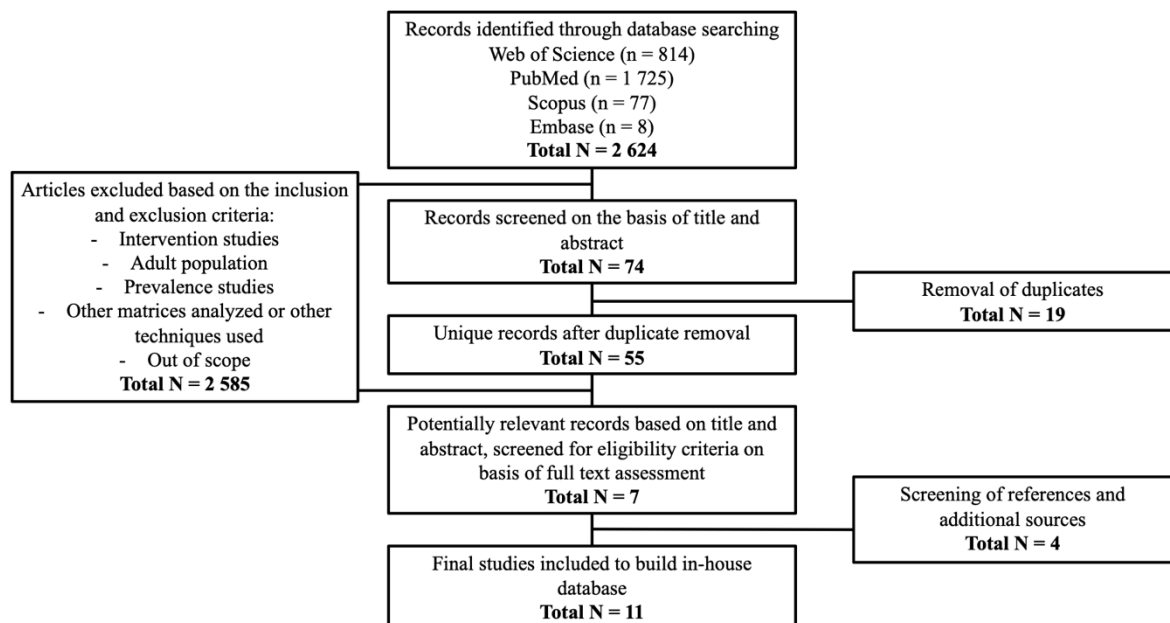


Figure 1. PRISMA scheme of the included studies.

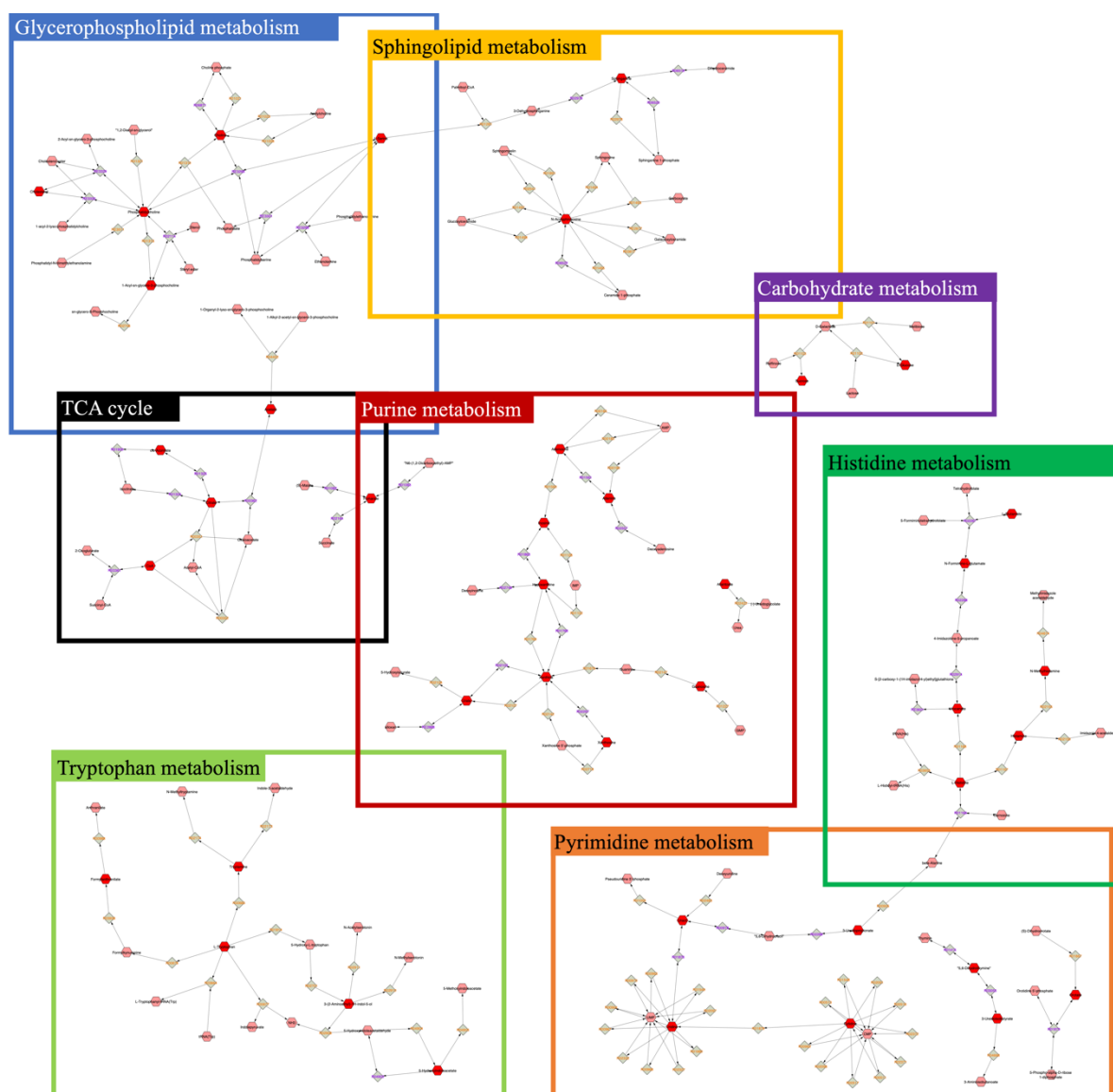


Figure 2. Network analysis summarizing the shortest route that may explain the interactions among metabolites identified in this systematic review. There is a clear interplay of several pathways, especially for the AAs, which are dispersed over multiple squares. Red circles = imported data; Pink circles = linking metabolites; squares = metabolic reactions.

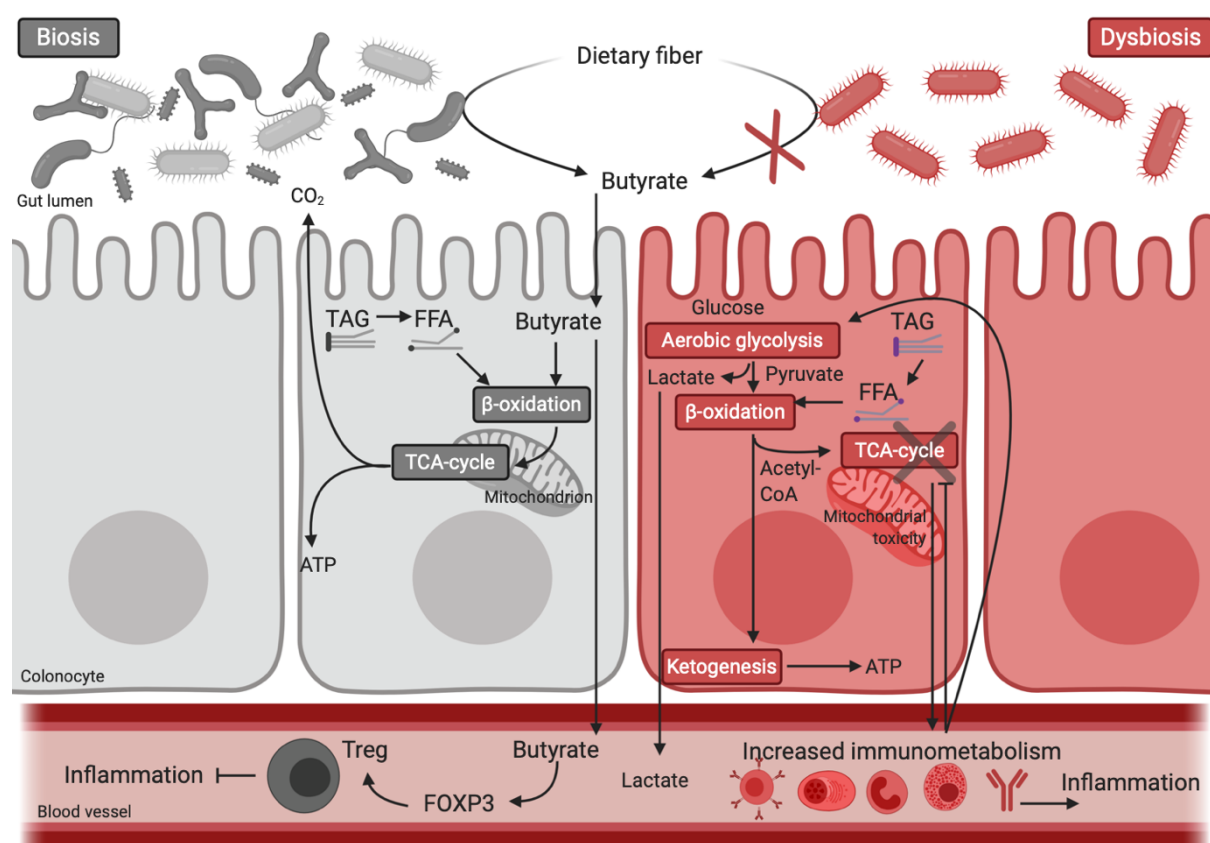


Figure 3. Overview of the altered energy metabolism, created with BioRender.com. Under normal conditions, butyrate is considered the major energy source of the cell, and has anti-inflammatory effects as it supports the expansion of Treg cells. In conditions of dysbiosis, less butyrate is available and the cell is obliged to switch to aerobic glycolysis for energy supply. The obtained pyruvate undergoes β -oxidation to yield acetyl-CoA, which normally is transported to the mitochondria for the TCA-cycle. However, due to IgE mediated mitochondrial toxicity, the acetyl-CoA is processed through ketogenesis to obtain ATP (TAG: triacylglycerol; FFA: free fatty acid).

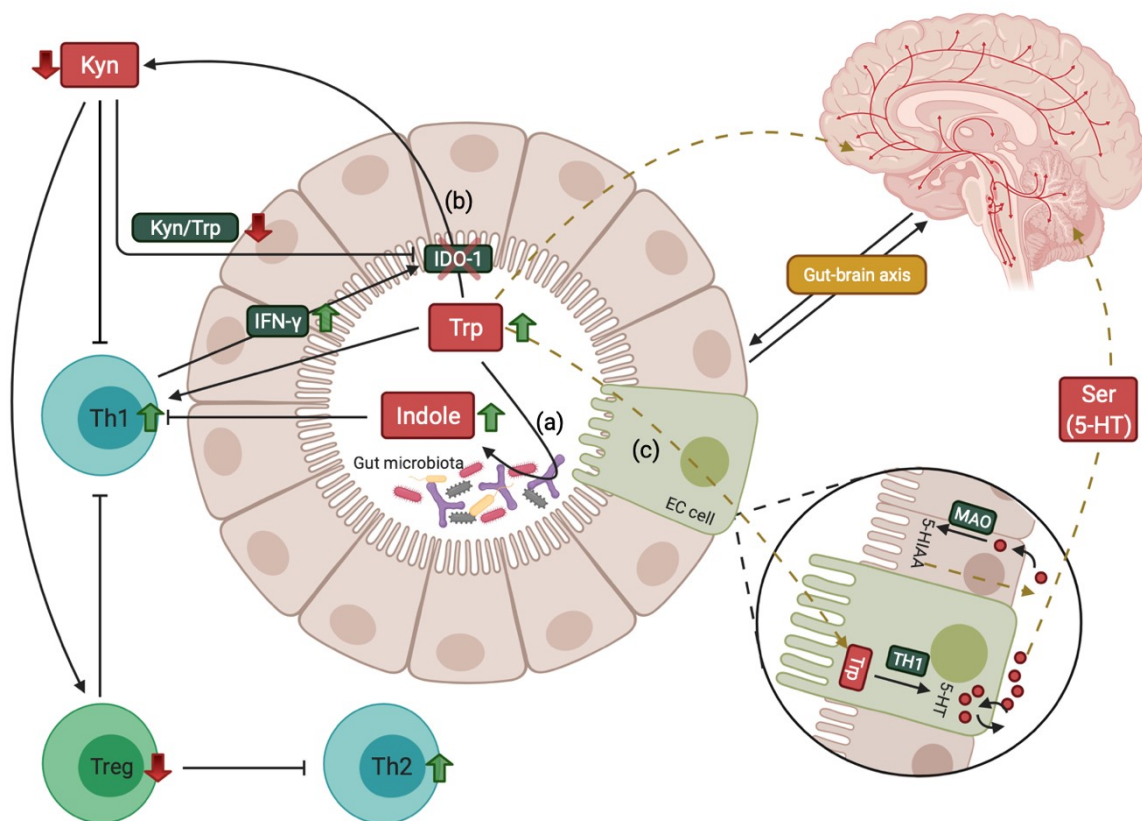


Figure 4. Overview of the tryptophan catabolism, created with BioRender.com. Tryptophan is an essential is metabolized through 3 main pathways: (a) direct transformation by the gut microbiota into proteins or indolic metabolites; (b) kynurenine pathway through the action of indoleamine 2,3-dioxygenase-1 (IDO-1); and (c) transformation into serotonin in enterochromaffin cells through tryptophan hydroxylase-1 (TH1). Both the kynurenine and indole pathways are important in immune responses at mucosal barriers and contribute to intestinal homeostasis, due to direct downregulation of T cell activity or induction of various Treg cells. Green arrow = increased in children with IgE-FA; Red arrow = decreased in children with IgE-FA.

11. TABLES

Table 1. Inclusion/exclusion criteria for screening studies

Inclusion criteria	Study attributes/characteristics of groups studied
Study design	Cohort, case-control, case-series or animal trial
Study population	Children or animals with IgE-mediated food allergy
Biofluid	Urine, feces, blood (serum or plasma)
Diagnosis	Clinician-based (food challenge, SPT [†] , serum IgE-measurement,...)
Exclusion criteria	Study attributes/characteristics of groups studied
Language	Language other than English
Format	Conference abstract

[†]SPT: Skin Prick Test

Table 2. Human food allergy dataset

Author	Country	Study design	Method	Participants	Age	Biofluid	Diagnosis
Buyuktiryaki et al. 2016 ^[63]	Turkey	Case-Control	HPLC-UV	212 (100 IgE-FA ^a ; 112 healthy)	1.6 – 7.5 years	Serum	Food challenge, SPT ^b , serum IgE, clinical symptoms
Crestani et al. 2019 ^[19]	USA	Case-Control	UPLC-MS	55 (35 IgE-FA; 20 healthy)	< 18 years	Serum	SPT, serum IgE, clinical symptoms
Goldberg et al. 2020 ^[73]	Israel	Case-Control	GC-MS	115 (84 IgE-FA; 31 healthy)	48 – 114 months	Feces	Food challenge, SPT, serum IgE, clinical symptoms
Inagaki et al. 2018 ^[60]	Japan	Case-Control	LC-MS/MS	157 (112 IgE-FA; 45 healthy)	3 – 14 years	Urine	Food challenge, clinical symptoms
Kong et al. 2015 ^[36]	Canada	Case-Control	LC-MS	23 (17 IgE-FA; 6 healthy)	< 18 years	Serum	SPT, serum IgE, clinical symptoms
Lee-Sarwar et al. 2018 ^[34]	USA	Cohort	LC-MS/MS	81 (12 IgE-FA; 32 FS ^c ; 37 healthy)	3 – 6 months	Feces	SPT, clinical symptoms, questionnaire
Maeda et al. 2017 ^[47]	Japan	Case-Control	LC-MS/MS	98 (9 IgE-FA; 5 AD ^d ; 8 AR ^e ; 37 A ^f ; 37 healthy)	NA ^g	Urine	Food challenge, serum IgE, clinical symptoms
Roduit et al. 2018 ^[40]	Switzerland	Cohort	HPLC-MS	301 (33 IgE-FA; 268 healthy)	1 – 6 years	Feces	Serum IgE

Ross et al. 2020 ^[56]	Sweden	Cohort	GC-MS	44 (14 IgE-FA; 30 healthy)	0 – 8 years	Cord blood	Food challenge, serum IgE, clinical symptoms
Sandin et al. 2008 ^[35]	Sweden	Cohort	GC-MS	53 (10 IgE-FA; 43 healthy)	4 years	Feces	SPT, questionnaire
Thompson-Chagoyan et al. 2011 ^[38]	Spain	Case-Control	GC-MS	92 (46 IgE-FA; 46 healthy)	2 – 12 months	Feces	Food challenge, SPT, serum IgE
Yadama et al. 2020 ^[39]	USA	Cohort	LC-MS	289 (37 IgE-FA; 252 healthy)	3 years	Plasma	Serum IgE, clinical symptoms, questionnaire

^aIgE-FA: IgE-mediated food allergy; ^bSPT: Skin prick test; ^cFS: Food sensitised; ^dAD: Atopic dermatitis; ^eAR: Allergic rhinitis; ^fA: Asthma; ^gNA: Not available

Table 3. Animal food allergy dataset

Author	Country	Study design	Method	Participants	Age	Biofluid	Diagnosis
Chalcraft et al. 2014 ^[41]	USA	Animal study (mouse)	LC-MS	84	6 – 8 weeks	Serum	Food challenge, serum IgE, anaphylaxis score
Hu et al. 2012 ^[42]	China	Animal study (guinea pig)	GC-MS	36	300 – 350 g	Serum	Food challenge, serum IgE, clinical symptoms, anaphylaxis score
Khoo et al. 2018 ^[43]	Malaysia	Animal study (rat)	H-NMR	36	9 weeks	Plasma, urine	Food challenge
Kong et al. 2015 ^[36]	Canada	Animal study (mouse)	LC-MS	28	6 – 8 weeks	Serum	Food challenge, serum IgE, anaphylaxis score
Maeda et al. 2017 ^[47]	Japan	Animal study (mouse)	LC-MS/MS	> 5 per group	6 – 8 weeks	Urine	Food challenge, anaphylaxis score

1 **Supporting information**

2 Table S1. Identification of proteins in exercised 2D gel by mass spectrometry.

Spot	Protein	Accession number	Unique peptides	pI	MW
1	Glutenin, high molecular weight subunit	P08489	3	6.18	89.1
2	Glutenin, low molecular weight subunit	P10385	6	8.68	41
3	Glutenin, low molecular weight subunit	P10385	6	8.68	41
4	GTP-binding protein	P31584	3	6.21	22.5
4	Actin-82 (Fragment)	P93584	1	5.82	37.2
5	Alpha/beta-gliadin	P02863	12	7.75	32.9
6	Alpha/beta-gliadin	P04722	9	7.23	33.6

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