Review

Emerging Biomarkers in Pediatric Food Allergy: From Mechanistic Endotyping to Precision Diagnosis and Therapeutic Monitoring

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Emerging Biomarkers in Pediatric Food Allergy: From Mechanistic Endotyping to Precision Diagnosis and Therapeutic Monitoring

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Abstract

Background: Food allergy is a heterogeneous pediatric disease involving IgE-mediated, non-IgE-mediated, and mixed immune mechanisms, with manifestations ranging from mild symptoms to life-threatening anaphylaxis. Current diagnostic tools, including clinical history, skin prick testing, serum specific IgE measurement, and oral food challenge, have limitations in specificity, invasiveness, prognostic value, and ability to guide personalized management. *Methods:* This narrative review summarizes emerging biomarkers in pediatric food allergy and evaluates their diagnostic, prognostic, predictive, and therapeutic potential. A literature search was conducted in PubMed/MEDLINE and Cochrane Central for English-language studies published between December 2015 and March 2026. Eligible studies included original clinical or translational research involving children aged 0–18 years and assessing functional cellular assays, epithelial barrier markers, intestinal permeability, gut microbiota, metabolomics, transcriptomics, proteomics, epigenetics, and immune biomarkers. Findings were synthesized qualitatively according to biomarker category and biological function. *Results:* Functional cellular biomarkers, particularly the basophil activation test, show the greatest translational readiness, with high diagnostic specificity, utility in reaction threshold and severity assessment, and potential value for monitoring oral immunotherapy. Biomarkers of epithelial barrier dysfunction, including zonulin, tight junction proteins, epithelial injury markers, filaggrin variants, and epithelial-derived cytokines, provide mechanistic insight into allergic sensitization and gastrointestinal phenotypes but remain insufficiently validated. Microbiota-derived, metabolomic, transcriptomic, proteomic, epigenetic, and integrated multi-omics approaches offer promising tools for risk prediction, tolerance monitoring, endotype identification, and precision medicine. *Conclusion:* Emerging biomarkers may improve diagnosis, risk stratification, therapeutic monitoring, and personalized care in pediatric food allergy. However, standardized assays, large longitudinal pediatric studies, and external validation are required before routine clinical implementation.

Keywords: pediatric food allergy; biomarkers; basophil activation test; epithelial barrier dysfunction; gut microbiota; precision medicine

1. Introduction

Food allergy is defined as a specific and reproducible immune-mediated adverse reaction to food, with clinical manifestations ranging from mild local symptoms to severe systemic reactions, including anaphylaxis. It represents a major public health concern, particularly because food-induced reactions are among the leading causes of anaphylaxis outside the hospital setting and require accurate diagnosis, risk assessment, and specialized management. Food allergy must be distinguished from food intolerance, which is generally characterized by functional or

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gastrointestinal symptoms and does not involve an immunological mechanism. For many years, the absence of uniform definitions complicated the estimation of global incidence and prevalence [1]. More recently, the 2023 revision of the European Academy of Allergy and Clinical Immunology nomenclature classified food allergies according to their underlying immunopathogenetic mechanisms, distinguishing between immunoglobulin E (IgE)-mediated forms and cell- and tissue-mediated, non-IgE-mediated forms [2].

Over recent decades, the prevalence of food allergy has increased worldwide, although epidemiological estimates remain influenced by differences in study design, diagnostic criteria, geographic area, and whether data are self-reported or confirmed by oral food challenge. The increase appears more pronounced in industrialized countries, including the United States, and affects children more frequently than adults. Analyses based on self-reported data suggest an estimated rise of up to 1.2% per decade; currently, approximately 8% of children are reported to have food allergy, 2.4% have multiple food allergies, and up to 3% report episodes of anaphylaxis [3]. In Europe, Spolidoro et al. reported that cumulative self-reported lifetime prevalence varies by food allergen, reaching 5.7% for cow's milk, 2.4% for egg, 1.6% for wheat, 0.5% for soy, 1.5% for peanut, 0.9% for tree nuts, 1.4% for fish, and 0.4% for crustaceans. However, prevalence estimates are markedly lower when diagnosis is confirmed by oral food challenge, ranging from 0.02% for fish to 0.8% for egg [4].

IgE-mediated food allergy is a prototypical type I hypersensitivity reaction characterized by an immediate immune response to specific food allergens. In this process, T cells contribute to B-cell activation and class switching, leading to the production of allergen-specific IgE, which plays a central role in allergic sensitization and effector responses [5]. Clinically, allergies to cow's milk, egg, soy, and wheat usually arise in early childhood and often resolve before adolescence, whereas allergies to tree nuts, fish, and crustaceans tend to develop later and more frequently persist into adulthood [6]. Non-IgE-mediated food allergies comprise a heterogeneous group of immune reactions independent of IgE, mainly driven by cell-mediated mechanisms, particularly type IVb and V hypersensitivity, and characterized by prominent tissue involvement [7]. Their pathogenesis involves T lymphocytes, eosinophils, and a complex cytokine network [8]. The main clinical entities include food protein-induced enterocolitis syndrome (FPIES), food protein-induced allergic proctocolitis (FPIAP), and food protein-induced enteropathy (FPE) [7]. FPIAP is the most frequently described form, although its true prevalence remains uncertain because of marked variability in epidemiological data [9–11]. FPE is rare and appears to be decreasing over time [9]. FPIES, previously considered uncommon, is now estimated to have a cumulative incidence of approximately 0.3%–0.7% during the first year of life, with differences across studies likely reflecting geographic and methodological factors [12–14]. Mixed forms of food allergy also occur, in which IgE-mediated and non-IgE-mediated mechanisms coexist. These include eosinophilic gastrointestinal diseases, in which the immune response is complex, the contribution of IgE is variable, and clinical manifestations depend on the gastrointestinal segment involved and the degree of eosinophilic infiltration [9].

Accurate diagnosis is essential to guide appropriate dietary, therapeutic, and educational strategies and to prevent severe, potentially life-threatening reactions. At the same time, correct identification of tolerance is crucial to avoid unnecessary dietary restrictions, particularly in light of current preventive approaches supporting the early introduction of potentially allergenic foods [5]. Diagnostic evaluation is based on clinical history and physical examination, supported by evidence of sensitization through skin prick testing (SPT) and/or measurement of serum specific IgE (sIgE) directed against the suspected food allergen [15]. In IgE-mediated food allergy, clinical history remains the cornerstone of the diagnostic work-up but is insufficient on its own to confirm the diagnosis. Confirmation may require a double-blind, placebo-controlled food challenge (DBPCFC), which yields positive results in approximately 30%–40% of suspected cases [16]. Key diagnostic elements include the type of symptoms, their temporal relationship with food ingestion, usually within minutes to 1–2 hours, reproducibility after subsequent exposures, the form and amount of food ingested, and exclusion of alternative conditions that may mimic food allergy. Clinical assessment should also consider cofactors such as febrile infections, asthma exacerbations, physical

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exercise, alcohol intake, and medications, including antacids and nonsteroidal anti-inflammatory drugs, which may increase reaction severity [17].

SPT using commercial allergen extracts is a rapid, simple, and reproducible in vivo method for detecting food-specific IgE bound to cutaneous mast cells [16]. Test reactivity may vary according to age, anatomical site, testing device, allergen extract characteristics, and the use of fresh food [17]. Although SPT has limited positive predictive value, its high negative predictive value, exceeding 90%, makes it particularly useful for excluding food allergy in the appropriate clinical context [16,17]. Measurement of sIgE is also widely used because of its high sensitivity, although specificity is limited. Detectable sIgE alone does not confirm clinical allergy, even though higher levels are generally associated with a greater likelihood of reactivity. Conversely, 10%–25% of patients may experience reactions despite undetectable IgE levels [17]. The oral food challenge (OFC) remains the diagnostic gold standard and consists of controlled administration of the suspected food in gradually increasing doses [16,18]. Absence of symptoms during the procedure defines a negative test and allows reintroduction of the food, whereas objective or persistent symptoms confirm a positive response and the diagnosis of food allergy [16].

Diagnosis of non-IgE-mediated and mixed food allergies is more challenging. Compared with IgE-mediated forms, symptoms usually occur later after ingestion and may follow a chronic or relapsing course, making the association with the causative food less evident. In addition, the lack of reliable laboratory tests limits instrumental support. In clinical practice, diagnosis relies on recognition of a compatible clinical phenotype, symptom improvement during elimination of the suspected food, and recurrence after reintroduction. OFC remains the diagnostic gold standard also in these forms. In selected cases of mixed food allergy, particularly eosinophilic gastrointestinal diseases, endoscopic assessment may help demonstrate eosinophilic infiltration of the gastrointestinal mucosa [19].

Within this diagnostic and clinical framework, the identification of reliable biomarkers capable of predicting the risk of food allergy development, reaction severity, persistence, tolerance acquisition, or response to therapy would represent a major advance. Such biomarkers could support earlier intervention, reduce food allergy–related morbidity and mortality, limit the need for invasive or high-risk diagnostic procedures, and help identify novel therapeutic targets [20]. Biomarkers able to define specific phenotypes and endotypes are also essential to improve diagnostic accuracy and personalize treatment strategies. At present, management remains largely based on patient and caregiver education, allergen avoidance, and prompt treatment of accidental reactions [21]. However, advances in immunology, high-throughput technologies, and bioinformatics have led to the identification of several promising biomarker categories in food allergy [22].

Despite the increasing number of studies in this field, the integration of biomarkers into routine clinical practice remains limited. Current evidence is characterized by substantial heterogeneity in study design, patient populations, laboratory methods, and clinical outcomes. Many candidate biomarkers lack standardized cut-off values, external validation, longitudinal pediatric data, and clear evidence of clinical utility. Moreover, although several reviews have addressed emerging biomarkers in food allergy, few have critically evaluated their diagnostic performance, biological relevance, translational readiness, and potential applicability within precision medicine frameworks, particularly in pediatric populations.

For these reasons, the present narrative review aims to provide a comprehensive and critical evaluation of emerging biomarkers in pediatric food allergy, focusing on their diagnostic, prognostic, predictive, and therapeutic potential. Particular attention is given to biomarkers supported by increasing clinical and translational evidence and capable of reflecting distinct pathophysiological mechanisms involved in disease development and progression. The biomarker categories considered in this review include functional cellular biomarkers, such as the basophil activation test (BAT), which may improve diagnostic specificity, risk stratification, and reduce the need for OFCs; biomarkers of epithelial barrier dysfunction and intestinal permeability, which may help characterize gastrointestinal and non-IgE-mediated phenotypes; microbiota-derived and metabolomic

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biomarkers, which are increasingly linked to oral tolerance, immune regulation, and disease persistence; and inflammatory, transcriptomic, proteomic, epigenetic, and other immunological biomarkers that may contribute to endotype identification and future personalized therapeutic strategies. An overview of the main biomarker categories, their biological rationale, potential clinical utility, and current validation status is provided in Table 1.

Table 1. Overview of emerging biomarkers in pediatric food allergy: biological rationale and potential clinical applications.

Biomarker category	Main biomarkers	Biological rationale	Potential clinical utility	Current validation status
Functional cellular biomarkers	BAT, CD63, CD203c, CD-sens, EC50	Measures allergen-induced basophil activation and effector cell responsiveness	Diagnosis, reaction threshold prediction, severity stratification, OIT monitoring	Advanced clinical validation; second-line tool in EAACI guidelines
Intestinal permeability biomarkers	Zonulin, claudins, occludin, ZO-1	Reflect epithelial tight junction dysfunction and increased antigen passage	Disease endotyping, non-IgE phenotype characterization	Investigational
Enterocyte damage biomarkers	I-FABP, DAO	Reflect epithelial injury and mucosal damage	Barrier dysfunction assessment	Investigational
Microbial translocation biomarkers	LBP, soluble CD14, endotoxin-related markers	Indirect markers of increased intestinal permeability/systemic microbial exposure	Experimental mechanistic assessment	Experimental
Fecal inflammatory biomarkers	Calprotectin, sIgA, ECP, EDN	Reflect mucosal inflammation and eosinophilic activity	GI food allergy phenotyping	Limited evidence
Epithelial integrity/genetic biomarkers	*Filaggrin (FLG)* mutations	Barrier dysfunction and transcutaneous sensitization susceptibility	Risk stratification, prediction of persistent/severe disease	Strong mechanistic evidence; not dynamic biomarker
Microbiota/metabolomic biomarkers	SCFAs (especially butyrate), *Clostridiales, Bifidobacterium*	Oral tolerance regulation, immune modulation	Disease prediction, tolerance acquisition	Emerging

Abbreviations: BAT, basophil activation test; CD, cluster of differentiation; CD-sens, baso-phil allergen threshold sensitivity; EC50, half-maximal effective concentration; OIT, oral immunotherapy; EAACI, European Academy of Allergy and Clinical Immunology; ZO-1, zonula occludens-1; IgE, immunoglobulin E; I-FABP, intestinal fatty acid-binding protein; DAO, diamine oxidase; LBP, lipopolysaccharide-binding protein; sCD14, soluble cluster of differentiation 14; sIgA, secretory immunoglobulin A; ECP, eosinophilic cationic protein; EDN, eosinophil-derived neurotoxin; GI, gastrointestinal; FLG, filaggrin; SCFAs, short-chain fatty acids.

By analyzing these biomarker categories, this review aims not only to summarize current evidence but also to identify the main barriers preventing implementation in routine clinical practice, including assay standardization, reproducibility, accessibility, methodological variability, and lack of prospective validation. Ultimately, this review seeks to clarify which biomarkers currently show the strongest translational potential and which remain investigational, thereby supporting the development of precision medicine approaches in pediatric food allergy.

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2. Methods

A systematic literature search was performed in PubMed/MEDLINE and the Cochrane Central databases to identify studies investigating biomarkers in pediatric food allergy. Articles published between December 2015 and March 2026 were considered, and additional landmark references were included when relevant for contextual or background information. The search strategy combined Medical Subject Headings (MeSH) terms and free-text keywords related to food allergy, biomarkers, and pediatric populations, including "food allergy," "biomarkers," "markers," "children," "child," "infant," "toddler," "adolescent," "pediatric," and "paediatric," using appropriate Boolean operators ("AND," "OR"). Additional targeted searches were conducted for specific biomarker categories, including basophil activation test, epithelial barrier biomarkers, intestinal permeability, gut microbiota, transcriptomics, metabolomics, proteomics, epigenetic biomarkers, inflammatory biomarkers, and immune biomarkers. Only peer-reviewed articles published in English were included. Eligible studies were original clinical or translational investigations involving pediatric populations aged 0–18 years that evaluated diagnostic, prognostic, predictive, or mechanistic biomarkers associated with food allergy. Studies were excluded if they included exclusively adult populations, were case reports, conference abstracts, editorials, or expert opinions, lacked sufficient methodological information, focused exclusively on non-food allergic diseases, or were not available in English. After duplicate removal, titles and abstracts were independently screened by two reviewers, and the full texts of potentially eligible studies were assessed for final inclusion; disagreements were resolved through discussion and consensus. From each included study, data were extracted on study design, population characteristics, type of food allergy, biomarker investigated, laboratory methodology, diagnostic and prognostic performance, sensitivity, specificity and area under the curve values when available, and clinical outcomes associated with biomarker expression. The primary outcomes were diagnostic accuracy, association with clinical severity, prediction of oral tolerance acquisition, prediction of reaction thresholds, and utility in monitoring therapeutic response. Secondary outcomes included associations with epithelial barrier dysfunction, correlations with immunological endotypes, and applicability within precision medicine approaches. Because of substantial heterogeneity in study populations, biomarker methodologies, and reported outcomes, a meta-analysis was not performed; findings were therefore synthesized qualitatively and grouped according to biomarker category and biological function.

3. Cellular and Functional Biomarkers

3.1. Basophil Activation Test (BAT)

The BAT is a flow cytometry–based functional assay that measures the upregulation of activation markers, most commonly CD63 and CD203c, on the surface of basophils after stimulation with specific allergens. Since its development, BAT has progressively gained relevance in the diagnosis and monitoring of allergic diseases, emerging as a safe, reproducible, and informative in vitro alternative to in vivo provocation tests. Because it is performed entirely *ex vivo*, BAT is less invasive and safer than oral food challenge (OFC), while also offering potential cost advantages in selected diagnostic pathways [23,24].

In the 2024 update of the European Academy of Allergy and Clinical Immunology (EAACI) guidelines on the diagnosis of IgE-mediated food allergy, BAT was included among recommended diagnostic tools for the first time. It is suggested as a second-line test in patients with suspected food allergy and equivocal results on skin prick testing (SPT) and/or serum specific IgE (sIgE), before proceeding to OFC [25]. This recommendation reflects the growing evidence supporting the diagnostic accuracy of BAT, particularly for peanut and sesame allergy, for which meta-analyses have shown moderate sensitivity, 86% and 89%, respectively, and high specificity, 90% and 93%, respectively, with low heterogeneity across studies [26].

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The biological rationale for BAT is closely linked to the effector mechanisms of IgE-mediated food allergy. Acute allergic reactions and anaphylaxis result from activation and degranulation of mast cells and basophils after allergen-induced cross-linking of IgE bound to the high-affinity IgE receptor FcεRI. Basophils express the tetrameric form of FcεRI and, after allergen stimulation, release vasoactive and inflammatory mediators that contribute directly to acute allergic symptoms. Unlike mast cells, basophils are readily accessible in peripheral blood, making them particularly suitable for functional diagnostic testing [23,24].

BAT evaluates basophil activation after stimulation with allergens or control stimuli. Activated basophils upregulate surface proteins that can be quantified by flow cytometry. CD63, a lysosome-associated four-transmembrane protein localized in secretory granules, translocates to the plasma membrane during degranulation and is the most widely used activation marker. CD203c is also frequently assessed and is commonly expressed as a stimulation index [23,27]. Basophil activation increases with rising allergen concentrations until a plateau is reached. The resulting dose–response curve is influenced by several factors, including the density of epitope–IgE complexes, IgE affinity for the allergen, and intrinsic basophil responsiveness [24,28]. Therefore, BAT optimization requires allergen dose–response curves using multiple, usually 6–8, serial dilutions to identify the most informative diagnostic concentration [29,30]. For each food allergen, specific cut-off values for basophil activation, expressed as the percentage of CD63-positive basophils and/or CD203c upregulation, should be defined to maximize diagnostic accuracy, including area under the curve (AUC), specificity, and sensitivity [29].

Methodological standardization is essential for reliable BAT interpretation. Negative controls usually show 1.5%–2.5% spontaneous basophil activation; therefore, 2.5% CD63-positive basophils is generally accepted as the upper limit for valid negative controls [31,32]. Positive controls should be included in every assay and commonly consist of anti-IgE, anti-FcεRI antibodies, or N-formyl-methionyl-leucyl-phenylalanine (fMLP). Anti-IgE and anti-FcεRI induce IgE-dependent activation through receptor cross-linking, whereas fMLP activates basophils through G-protein–coupled receptors in an IgE-independent manner [33–35]. BAT requires less than 0.1 mL of fresh blood and should ideally be performed within 4 hours of sampling, and no later than 24 hours, because basophil responsiveness declines rapidly over time [36–39].

In food allergy, BAT provides substantially higher specificity than SPT and sIgE, which are sensitive but often limited by poor specificity and may leave patients in a diagnostic “grey zone.” BAT has demonstrated specificity up to 100% for peanut allergy [27,40] and egg allergy [41], and has been useful in distinguishing allergic from tolerant children sensitized to milk or egg, including children with atopic dermatitis [42,43]. Consequently, BAT may reduce the need for diagnostic OFC in patients with equivocal first-line test results.

This advantage is particularly relevant in polysensitized children with suspected tree nut and seed allergy. In a large prospective European multicenter study, 60.7% of children with challenge-confirmed peanut, tree nut, or sesame allergy were allergic to more than one nut or seed, highlighting the difficulty of interpreting multiple positive SPT and sIgE results for foods not regularly consumed [34]. Although sensitization to multiple nuts is common, many patients react clinically to only one or two, with strong cross-reactivity observed between cashew and pistachio and between walnut and pecan [34,35]. In this context, BAT used as a second-line diagnostic tool has been shown to reduce the need for OFC by 5%–15% and the number of positive OFCs by 33%–75%, while achieving diagnostic accuracy between 96% and 100% and no false-negative results [44].

BAT may also support diagnostic decision-making in cow’s milk and egg allergy, the most common food allergies worldwide [45,46]. Approximately 60%–80% of affected patients tolerate these foods in baked form, allowing dietary liberalization and improving quality of life [47]. Baked milk and egg may also be incorporated into oral immunotherapy (OIT) protocols and may accelerate the natural resolution of allergy [47,48]. Because SPT and sIgE poorly predict baked food tolerance, OFC is often required. In this setting, BAT has emerged as a useful tool for selecting patients for challenge. BAT has shown particular utility in predicting baked egg allergy in young children and, when

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combined with sIgE, has been associated with a 30% reduction in OFCs [49]. Increased basophil activation to milk has also been associated with reactivity and severity during baked milk challenges [50].

Beyond diagnosis, BAT provides information on reaction severity and clinical thresholds. Basophil reactivity, usually expressed as the percentage of CD63-positive basophils, correlates with reaction severity, whereas basophil sensitivity parameters, such as CD-sens or EC50, correlate with clinical threshold doses [40,51,52]. These findings have been confirmed in large pediatric cohorts, including participants in the LEAP study. Increased basophil activation has also been associated with severe reactions and epinephrine use in walnut allergy [53], and BAT has emerged as one of the most accurate biomarkers for predicting severity and low reaction thresholds in egg allergy during double-blind placebo-controlled food challenges [54].

BAT may also have a role in therapeutic stratification and monitoring. In patients undergoing OIT or receiving biologics targeting T helper 2 (Th2) inflammation, such as omalizumab, elevated basophil activation has been proposed as a biomarker to identify candidates who may benefit from treatment and to monitor immunological changes over time [55]. Omalizumab induces complex effects on basophils, including reduced FcεRI density and increased intrinsic basophil sensitivity, changes that can be detected by BAT and correlated with clinical outcomes [56,57].

Despite its strong diagnostic performance, BAT remains a second-line test because of limited availability, higher costs compared with SPT and sIgE, and the need for standardized protocols, fresh blood samples, flow cytometry infrastructure, and trained personnel. In addition, approximately 10% of individuals have basophils that do not respond to FcεRI-mediated stimulation, making BAT results uninterpretable in these cases [41]. Furthermore, although evidence is robust for peanut and sesame allergy, BAT has not yet been formally recommended by EAACI for all foods because of insufficient data for meta-analysis.

Overall, BAT represents the most clinically advanced functional biomarker in pediatric food allergy. Its main diagnostic and prognostic features, including activation markers, sample requirements, sensitivity, specificity, ability to reduce OFCs, and utility for severity, threshold, and treatment monitoring, are summarized in Table 2.

Table 2. Diagnostic and prognostic performance of the Basophil Activation Test (BAT) in pediatric food allergy.

Parameter	Findings reported in literature	Clinical implication
Activation markers	CD63, CD203c	Core BAT readouts
Sample requirement	<0.1 mL fresh blood; ideally within 4 h	Pediatric feasibility
Sensitivity	Peanut 86%; Sesame 89%	Good diagnostic sensitivity
Specificity	Peanut 90%; Sesame 93%; up to 100% in selected cohorts	High specificity vs SPT/sIgE
Diagnostic accuracy	96–100% in selected studies	Reduces diagnostic uncertainty
Reduction in OFCs	5–15% fewer OFCs	Less invasive work-up
Reduction in positive OFCs	33–75%	Better patient selection
Severity prediction	CD63 correlates with reaction severity	Risk stratification
Threshold prediction	CD-sens, EC50 correlate with eliciting dose	Threshold estimation
Monitoring utility	OIT and omalizumab response monitoring	Therapeutic follow-up
Main limitations	Need for fresh blood, flow cytometry, trained personnel, non-responder basophils (~10%)	Limited scalability

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Abbreviations: BAT, basophil activation test; CD, cluster of differentiation; CD-sens, basophil allergen threshold sensitivity; EC50, half-maximal effective concentration; OIT, oral immunotherapy; OFC, oral food challenge; sIgE, serum specific immunoglobulin E; SPT, skin prick test.

Wider clinical implementation will require further standardization, external quality assurance, and improved accessibility, but the potential impact of BAT on precision diagnosis and risk stratification in pediatric food allergy is substantial.

4. Inflammatory and Immunological Biomarkers

4.1. Biomarkers of Intestinal Barrier Permeability

The intestinal epithelial barrier plays a central role in maintaining immune homeostasis and oral tolerance to dietary antigens. It consists of a single layer of epithelial cells interconnected by tight junctions, adherens junctions, and gap junctions, which regulate the selective passage of molecules through the paracellular pathway. Tight junctions are the principal regulators of intestinal permeability and are composed of transmembrane proteins, including claudins, occludin, junctional adhesion molecules, and tricellulin. These proteins interact with cytoplasmic scaffold proteins of the zonula occludens family, particularly ZO-1, ZO-2, and ZO-3, which connect the junctional complex to the actin cytoskeleton. Under physiological conditions, this barrier limits the systemic passage of intact food antigens while allowing nutrient absorption and controlled immune sampling. Disruption of epithelial integrity increases intestinal permeability, facilitates allergen translocation into the lamina propria, and promotes immune activation and allergic sensitization [58].

Growing evidence suggests that epithelial barrier dysfunction is a key pathogenic mechanism in food allergy and may define a specific disease endotype characterized by enhanced mucosal permeability, impaired barrier regulation, and altered immune responses. Accordingly, several biomarkers reflecting intestinal barrier integrity, epithelial injury, microbial translocation, and mucosal inflammation have been investigated as potential tools for disease characterization, risk stratification, and monitoring in pediatric food allergy [59]. These biomarkers can be grouped into four main categories, as summarized in Table 3, which provides an overview of their biological significance, main findings in food allergy, and current limitations.

Tight junction–related biomarkers reflect structural or functional alterations of epithelial junctional complexes and include zonulin, claudins, occludin, and zonula occludens proteins, particularly ZO-1. Among these, zonulin is currently the most extensively investigated biomarker in food allergy because of its role in modulating tight junction permeability and regulating paracellular antigen passage [60,61]. Alterations in tight junction–associated proteins may indicate impaired epithelial integrity and increased exposure of the mucosal immune system to food antigens.

Enterocyte damage biomarkers indicate epithelial cell injury and mucosal damage. The most commonly investigated markers include intestinal fatty acid-binding protein (I-FABP) and diamine oxidase (DAO). Increased circulating levels of these molecules may reflect enterocyte injury, impaired mucosal integrity, and increased intestinal permeability, although evidence in pediatric food allergy remains limited and requires further validation [62,63].

Microbial translocation and systemic permeability markers indirectly reflect enhanced passage of luminal microbial products across a compromised intestinal barrier. These include lipopolysaccharide-binding protein (LBP), soluble CD14, and circulating endotoxin-related markers. Such biomarkers have mainly been studied in experimental settings and systemic inflammatory conditions and may provide mechanistic information on the relationship between barrier disruption, microbial exposure, and immune activation [63,64].

Fecal inflammatory and mucosal immunity biomarkers are not specific markers of intestinal permeability but may reflect mucosal inflammation associated with barrier dysfunction. These include fecal calprotectin, secretory IgA, and eosinophil-derived proteins, such as eosinophilic cationic protein (ECP) and eosinophil-derived neurotoxin (EDN). Alterations in these markers have

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been reported in pediatric food allergy, particularly in children with gastrointestinal involvement, non-IgE-mediated phenotypes, and eosinophilic inflammation [59].

Overall, biomarkers of epithelial barrier dysfunction may contribute to the identification of specific food allergy phenotypes, especially in patients with gastrointestinal manifestations and non-IgE-mediated disease. However, most remain investigational because of limited assay standardization, heterogeneity among studies, small pediatric cohorts, and insufficient longitudinal validation. At present, no intestinal permeability biomarker has been fully validated for routine clinical diagnosis of food allergy, although several candidates show promise for future endotype-based and precision medicine approaches [21]. Table 3 summarizes main biomarkers of epithelial barrier dysfunction in pediatric food allergy.

Table 3. Main biomarkers of epithelial barrier dysfunction in pediatric food allergy.

Biomarker class	Biomarkers	Biological significance	Main findings in food allergy	Limitations
Tight junction biomarkers	Zonulin, Claudins, Occludin, ZO-1	Tight junction regulation/permeability	Altered levels associated with increased permeability	Lack of assay standardization
Enterocyte damage biomarkers	I-FABP, DAO	Epithelial injury	Elevated in barrier dysfunction states	Limited pediatric validation
Microbial translocation biomarkers	LBP, soluble CD14, endotoxin markers	Luminal antigen/microbial translocation	Experimental associations only	Mostly preclinical/experimental
Fecal inflammatory biomarkers	Calprotectin, sIgA, ECP, EDN	Mucosal inflammation	Associated with GI involvement/non-IgE phenotypes	Low specificity

Abbreviations: ZO-1, zonula occludens-1; I-FABP, intestinal fatty acid-binding protein; DAO, diamine oxidase; LBP, lipopolysaccharide-binding protein; sCD14, soluble cluster of differentiation 14; sIgA, secretory immunoglobulin A; ECP, eosinophilic cationic protein; EDN, eosinophil-derived neurotoxin; GI, gastrointestinal; IgE, immunoglobulin E.

4.2. Intestinal Barrier Dysfunction as an Early Event in Allergic Sensitization

According to the epithelial barrier hypothesis, impairment of epithelial surfaces represents a central mechanism in the pathogenesis of allergic diseases, including food allergy [59]. Increasing evidence suggests that intestinal barrier dysfunction may occur early in the development of food allergy and may contribute causally to allergic sensitization, rather than representing only a secondary consequence of established inflammation [58]. This concept is supported by clinical observations showing that infants with early-onset atopic dermatitis (AD) are at increased risk of developing food allergy later in childhood. Population-based studies have further demonstrated that eczema and loss-of-function mutations in the *filaggrin* (*FLG*) gene, which impair skin barrier integrity, are major risk factors for allergic sensitization and subsequent food allergy [65,66]. These findings support the existence of an integrated epithelial barrier system in which both cutaneous and intestinal barrier dysfunction may promote allergen exposure, immune activation, and progression toward clinically relevant food allergy.

Experimental data further indicate that the transition from skin sensitization to intestinal allergic inflammation is a critical step in the development of IgE-mediated food allergy. In models of epicutaneous sensitization with thymic stromal lymphopoietin (TSLP) and ovalbumin, blockade of TSLP alone does not completely prevent food allergy development, suggesting that additional intestinal immune pathways contribute to disease amplification [67]. The gastrointestinal mucosa is the largest immune interface exposed to dietary antigens. Under physiological conditions, $CD103^{+}$

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tolerogenic dendritic cells promote oral tolerance by inducing antigen-specific regulatory T cells. However, disruption of epithelial integrity, increased antigen passage, and altered epithelial cytokine signaling may impair tolerogenic pathways and favor type 2 immune responses [68].

Among epithelial-derived cytokines, interleukin-25 (IL-25) has emerged as a potential mediator linking epithelial dysfunction to intestinal allergic inflammation. IL-25, constitutively produced by tuft cells, promotes type 2 immunity through activation of group 2 innate lymphoid cells (ILC2s) and enhanced production of interleukin-5 (IL-5) and interleukin-13 (IL-13). Experimental studies have shown that increased IL-25 signaling enhances susceptibility to food allergy, whereas deletion of its receptor confers protection in animal models [68]. Despite its strong mechanistic relevance, IL-25 remains an experimental biomarker without standardized clinical application in food allergy. Similarly, other epithelial alarmins involved in barrier dysfunction, including TSLP and interleukin-33 (IL-33), are promising candidates for endotype characterization but currently lack validated cut-off values, standardized assays, and longitudinal pediatric data supporting routine clinical use [21,22].

Overall, current evidence supports the hypothesis that epithelial barrier dysfunction is an early pathogenic event in allergic sensitization and may help identify food allergy endotypes characterized by impaired mucosal tolerance, enhanced antigen penetration, and type 2 inflammation. Nevertheless, most barrier-associated biomarkers remain investigational and require analytical standardization, prospective validation, and longitudinal pediatric studies before they can be implemented in routine clinical practice.

4.3. Zonulin as a Biomarker of Intestinal Permeability

Among biomarkers of intestinal barrier dysfunction, zonulin is the most extensively investigated in food allergy and other immune-mediated gastrointestinal disorders. Unlike many permeability-associated biomarkers that remain mainly exploratory, zonulin deserves separate consideration because of the larger body of available clinical evidence, its direct relationship with tight junction regulation, and its potential relevance for identifying barrier dysfunction–associated food allergy endotypes. Zonulin is a physiological modulator of intercellular tight junctions and plays a key role in regulating intestinal permeability. Increased zonulin signaling promotes tight junction disassembly, resulting in enhanced paracellular trafficking of luminal antigens and microbial products across the intestinal epithelium [60,61].

Several studies have reported elevated serum zonulin levels in children with food allergy. In a study evaluating selected intestinal permeability markers in pediatric patients with food allergy, serum zonulin concentrations were significantly higher than in healthy controls and were particularly increased in children with gastrointestinal manifestations and non-IgE-mediated food allergy [59]. Additional evidence supporting the association between zonulin and allergic disease comes from pediatric studies on AD, a condition strongly linked to food sensitization and food allergy. Sheen et al. reported that serum zonulin levels were significantly associated with both the presence and severity of atopic dermatitis in children, independently of total IgE and eosinophil counts, supporting the hypothesis that epithelial barrier dysfunction contributes to allergic inflammation beyond classical IgE-mediated mechanisms [69]. Together, these findings suggest that increased intestinal permeability may coexist with impaired epithelial barrier integrity and chronic allergic inflammation in food allergy and related atopic disorders.

Despite these promising associations, the clinical utility of zonulin remains controversial. A major limitation concerns the analytical validity of currently available commercial enzyme-linked immunosorbent assay (ELISA) kits. Several studies suggest that many commercial assays may not specifically measure pre-haptoglobin-2, the molecule originally identified as zonulin, but instead detect structurally related proteins or other circulating molecules, thereby limiting assay specificity and reproducibility [70,71]. Additional limitations include small sample sizes, heterogeneous study populations, lack of standardized cut-off values, and absence of longitudinal pediatric studies validating zonulin as a predictive or monitoring biomarker in clinical practice.

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At present, elevated zonulin levels should therefore be interpreted as a potentially informative marker of epithelial barrier dysfunction rather than as a validated diagnostic biomarker for food allergy [21,22]. Nevertheless, zonulin remains the most studied intestinal permeability biomarker in this field and may help identify patients characterized by epithelial barrier impairment, particularly among those with gastrointestinal manifestations and non-IgE-mediated phenotypes. Future studies using standardized analytical methods, clearly defined clinical phenotypes, and prospective longitudinal designs are needed to determine whether zonulin can be integrated into precision medicine approaches for pediatric food allergy.

4.4. Biomarkers Associated with Epithelial Barrier Integrity

Increasing evidence indicates that epithelial barrier dysfunction plays a central role in the pathogenesis of allergic diseases, including food allergy. Beyond biomarkers of intestinal permeability that reflect dynamic epithelial injury or inflammation, markers associated with epithelial barrier integrity have emerged as potential indicators of susceptibility to allergic sensitization and disease progression [72,73]. These include structural epithelial proteins, tight junction–associated molecules, epithelial-derived cytokines, and genetic variants involved in barrier formation and maintenance.

In contrast to circulating inflammatory biomarkers, epithelial integrity markers may identify endotypes characterized by primary barrier impairment and increased allergen penetration through epithelial surfaces [72]. Among these markers, *FLG* is the most extensively studied because of its strong genetic association with atopic dermatitis, transcutaneous sensitization, and food allergy development. *FLG* therefore represents a key biomarker linking inherited or early-life epithelial barrier dysfunction to allergic disease progression and is discussed separately below.

4.5. Filaggrin: Linking Skin Barrier Dysfunction to Food Allergy

Filaggrin is a key structural protein involved in epidermal differentiation and maintenance of skin barrier integrity. It contributes to keratin aggregation, epidermal hydration, and prevention of transepidermal water loss. Loss-of-function mutations in the FLG gene impair skin barrier function, facilitating allergen penetration through the epidermis and promoting transcutaneous sensitization [74]. The association between FLG mutations and AD is well established, and accumulating evidence indicates that impaired skin barrier integrity may represent an early step in the allergic march leading to food allergy and asthma [73,75].

Several studies have demonstrated that children carrying *FLG* loss-of-function variants are at increased risk of developing peanut allergy and other IgE-mediated food allergies, particularly in the presence of early-onset AD [76]. In addition to increasing susceptibility, *FLG* mutations have been associated with greater disease severity and persistence. Astolfi et al. reported that *FLG* loss-of-function mutations were significantly associated with severe food allergy in children with atopic dermatitis, supporting the hypothesis that genetically impaired epithelial barriers may contribute not only to sensitization but also to more severe clinical phenotypes [67].

Despite their pathogenic and prognostic relevance, *FLG* mutations should be considered susceptibility biomarkers rather than dynamic disease biomarkers. Unlike circulating inflammatory mediators or markers of epithelial permeability, *FLG* variants are genetically determined and remain stable over time. Therefore, they are more useful for identifying children at increased risk of allergic sensitization, persistent disease, or severe phenotypes than for monitoring disease activity or therapeutic response in clinical practice [21].

Overall, *FLG* mutations provide important evidence supporting epithelial barrier dysfunction as a central mechanism in food allergy pathogenesis. Their current clinical relevance lies mainly in risk stratification and in the identification of barrier dysfunction–associated allergic endotypes, particularly in pediatric patients with early-onset atopic dermatitis.

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5. Gut Microbiota as a Potential Biomarker in Pediatric Food Allergies

The gut microbiota has emerged as a promising source of candidate biomarkers in pediatric food allergy because of its central role in oral tolerance, epithelial barrier integrity, and immune regulation. Early-life microbial dysbiosis has been repeatedly associated with increased susceptibility to food allergy, supporting the hypothesis that microbiota-related signatures may contribute to disease prediction, endotype definition, and monitoring of tolerance acquisition [77–79]. However, despite growing evidence, microbiota-derived biomarkers remain largely exploratory and are not yet standardized for clinical use.

Microbiota-derived biomarkers in food allergy can be broadly classified into four overlapping categories: compositional biomarkers, including bacterial taxa and microbial diversity patterns; metabolomic biomarkers, such as short-chain fatty acids (SCFAs), bile acids, and tryptophan-derived metabolites; functional microbial pathways involved in immune regulation and epithelial integrity; and integrated multi-omics signatures combining metagenomics, metabolomics, transcriptomics, and immunophenotyping data. Rather than representing a single biomarker, the gut microbiota should therefore be considered a multidimensional biomarker platform with potential relevance for disease stratification and precision medicine.

Several studies have identified reduced microbial diversity and depletion of specific commensal taxa in children with food allergy. In particular, lower abundances of *Clostridiales*, *Bifidobacterium*, and butyrate-producing bacteria have been associated with impaired oral tolerance and increased allergic sensitization [80,81]. These alterations may reduce regulatory T-cell (Treg) induction, weaken epithelial barrier function, and favor T helper 2 (Th2)-skewed immune responses. Nevertheless, most available evidence remains associative rather than causal. Moreover, compositional microbial signatures show substantial inter-study variability because of differences in age, diet, geographic setting, breastfeeding, antibiotic exposure, sequencing methodology, and bioinformatic pipelines. These factors limit inter-cohort reproducibility and currently reduce the reliability of microbiota composition as a standalone diagnostic biomarker.

Among microbiota-derived candidates, SCFAs, particularly butyrate, are among the most extensively investigated functional biomarkers. SCFAs contribute to epithelial barrier maintenance, mucus production, peripheral Treg differentiation, and immune tolerance [79,81,82]. Reduced fecal butyrate concentrations have been associated with food allergy and persistent cow's milk allergy, suggesting a possible prognostic role in tolerance acquisition [82]. In longitudinal pediatric cohorts, lower fecal butyrate levels during infancy have been linked to an increased risk of persistent allergic phenotypes, with some studies reporting moderate discriminatory performance for tolerance prediction. However, SCFA concentrations are strongly influenced by diet, age, sample collection and storage, and analytical methodology. Validated cut-off values and externally validated sensitivity and specificity thresholds are still lacking, preventing routine clinical application.

Only a limited number of studies have evaluated microbiota-derived biomarkers using robust quantitative performance metrics. Available evidence suggests that integrated microbiota–metabolomic models generally outperform single microbial taxa, showing moderate-to-good discriminatory capacity in differentiating allergic from non-allergic children, with area under the curve values varying according to the cohort and omics platform used [83]. Similarly, some longitudinal studies suggest that early-life microbial and metabolomic signatures may predict food allergy persistence or tolerance acquisition, supporting their potential value as dynamic prognostic biomarkers [79,84]. However, sensitivity, specificity, positive predictive value, and hazard ratio estimates are inconsistently reported, and few candidate biomarkers have been externally validated in independent pediatric cohorts. Consequently, current evidence remains insufficient to define clinically applicable diagnostic thresholds or standardized predictive models.

Beyond SCFAs, increasing attention has focused on other metabolomic signatures, including secondary bile acids and tryptophan-derived metabolites. Altered bile acid metabolism has been associated with food allergy development and may reflect disrupted microbial metabolic activity and altered mucosal immune signaling [79]. Similarly, perturbations in tryptophan metabolism may

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affect aryl hydrocarbon receptor (AhR)-mediated immune regulation and epithelial homeostasis, supporting their potential role as biomarkers of immune dysregulation in food allergy [82]. Preliminary metabolomic studies have shown promising discriminatory accuracy in differentiating allergic from non-allergic children, although reproducibility across independent pediatric cohorts remains limited. These findings therefore require confirmation in larger, longitudinal, multicenter studies before clinical implementation.

Recent multi-omics approaches integrating metagenomics, metabolomics, transcriptomics, and immunophenotyping have further expanded the biomarker landscape. De Paepe et al. identified integrated microbiota–metabolome signatures capable of discriminating children with food allergy from healthy controls, with improved predictive performance compared with individual microbial taxa alone [83]. Additional studies have reported associations between IgA-coated bacteria and allergic phenotypes, suggesting that host–microbiota immune interactions may provide novel stratification biomarkers [77]. Integrated multi-omics models may ultimately improve disease classification and prediction compared with single biomarkers, although few candidate signatures have undergone external validation in large pediatric longitudinal cohorts.

Importantly, microbiota-related biomarkers may have different clinical applications. Dysbiosis patterns and metabolomic alterations could serve as susceptibility biomarkers for early food allergy risk prediction, whereas SCFAs and functional microbial pathways may provide prognostic information on disease persistence or tolerance acquisition. Multi-omics signatures and immune–microbiota interaction profiles may instead support disease endotyping and patient stratification within precision medicine frameworks.

Despite these promising findings, several limitations currently prevent translation into routine practice. Most studies are characterized by small sample sizes, cross-sectional designs, heterogeneous populations, and lack of external validation. Major methodological variability also exists in stool collection procedures, sequencing technologies, 16S rRNA sequencing versus shotgun metagenomics, metabolomic platforms, and bioinformatic analyses, resulting in inconsistent findings and limited reproducibility. In addition, diet, age, antibiotic exposure, breastfeeding, environmental factors, and geography strongly influence microbiota composition and metabolite profiles, further complicating interpretation and limiting generalizability.

Overall, the gut microbiota represents a highly promising but still exploratory biomarker platform in pediatric food allergy. Future research should prioritize longitudinal multicenter cohorts, standardized sample collection and analytical pipelines, quantitative validation metrics, and integrated multi-omics approaches to define the clinical utility, reproducibility, diagnostic performance, and predictive accuracy of microbiota-derived biomarkers. Such advances may support precision medicine strategies aimed at improving early diagnosis, prognostic stratification, tolerance monitoring, and personalized therapeutic interventions in children with food allergy.

6. Biomarkers in Emerging Therapies and Precision Medicine

6.1. Epigenetics and Gene Regulation

Epigenetic regulation, defined as heritable and reversible changes in gene expression that do not alter the underlying DNA sequence, has emerged as an important dimension of food allergy pathogenesis. Epigenetic mechanisms may help explain how genetic susceptibility, environmental exposures, microbiota-derived signals, dietary factors, and immunological maturation interact during critical windows of immune development. Among these mechanisms, DNA methylation and microRNA-mediated gene regulation are the most extensively investigated in food allergy and are increasingly considered potential tools for disease prediction, endotype characterization, and monitoring of response to immunomodulatory interventions.

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6.1.1. DNA Methylation Signatures in Food Allergy

DNA methylation at cytosine–phosphate–guanine (CpG) dinucleotides is one of the best characterized epigenetic modifications in allergic disease. When occurring within gene promoter regions, CpG methylation generally suppresses gene transcription, although its functional effect depends on genomic location, cell type, and immune context. The T helper 2 (Th2)-skewed immune response that characterizes IgE-mediated food allergy is tightly regulated at the epigenetic level. Candidate-gene studies have shown altered methylation of key cytokine loci, including IL4, IL5, IL10, IFNG, and IL12B, in food-allergic individuals compared with healthy controls. In a systematic review including 16 studies, Safar et al. found that epigenomic associations with food allergy were mainly located in genes involved in Th1/Th2 balance, regulatory T-cell (Treg) function, Toll-like receptor signaling, and intestinal barrier integrity. Negative correlations between promoter methylation of IL-4, IL-5, IL-10, and interferon-γ (IFN-γ) and the corresponding circulating cytokine concentrations have also been reported, supporting the expected suppressive effect of promoter hypermethylation on gene expression [85].

A particularly relevant epigenetic target is *FOXP3*, the master transcription factor required for Treg identity and function. Demethylation of *FOXP3* CpG sites, especially within the Treg-specific demethylated region (TSDR), is associated with stable Treg differentiation and the acquisition of immunological tolerance [86]. In a pivotal study by Syed et al., patients with peanut allergy who achieved sustained unresponsiveness after oral immunotherapy (OIT) showed significantly lower *FOXP3* methylation in antigen-induced Tregs than patients who regained clinical sensitivity after peanut avoidance. Importantly, resensitization was accompanied by remethylation of the same CpG sites, indicating that *FOXP3* methylation may dynamically reflect both acquisition and loss of clinical tolerance [87]. Complementary preclinical evidence suggests that combining OIT with the botanical extract B-FAHF-2 may provide greater and more durable protection than OIT alone, with enhanced efficacy associated with favorable epigenetic changes, including IL-4 promoter remethylation and IFN-γ and *FOXP3* promoter demethylation. Final challenge symptom scores were inversely correlated with IL-4 methylation levels, further supporting the potential role of epigenetic marks as pharmacodynamic biomarkers in OIT research [88]. Together, these findings position *FOXP3* methylation as a candidate pharmacoepigenomic biomarker for monitoring OIT-induced tolerance.

Epigenetic alterations may also appear early in life. In a study of infants younger than six months, CpG methylation profiles of IL-4, IL-5, IL-10, IFN-γ, and *FOXP3* differed significantly between allergic infants, defined as those with food allergy and/or atopic dermatitis, and healthy controls, with lower *FOXP3* methylation observed among allergic infants [86]. This finding requires cautious interpretation. Unlike TSDR-focused studies, in which *FOXP3* hypomethylation reflects stable Treg differentiation and tolerance, the study by Gorzkiewicz et al. used whole-blood high-resolution melting polymerase chain reaction (PCR) targeting a CpG amplicon outside the TSDR. Because methylation changes outside the TSDR may have different functional implications, and because whole blood contains mixed cell populations dominated by non-Treg lineages, these results are not directly comparable with analyses performed in sorted T-cell subsets. Nevertheless, they suggest that disease-associated epigenetic marks may emerge during the first months of life and could contribute to early risk stratification.

At the genome-wide level, epigenome-wide association studies (EWASs) have identified 11 differentially methylated *loci* replicated in at least two independent food allergy studies, including genes involved in T-cell development, antigen presentation, and chromatin remodeling, such as *RPS6KA2*, *HDAC4*, *CAMTA1*, and *DOCK1* [85]. More recently, machine learning integration of whole-genome DNA methylation array data identified *LDHC* and *SLC35G2* as candidate epigenetic biomarkers for food allergy classification, with validation across two independent cohorts [89]. These findings illustrate the growing convergence of epigenomics, computational modeling, and precision medicine.

Overall, DNA methylation studies provide strong biological plausibility for the development of diagnostic, prognostic, and treatment-monitoring biomarkers in food allergy. However, clinical

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translation remains limited by substantial methodological heterogeneity, including differences in tissue source, such as whole blood, peripheral blood mononuclear cells, and sorted T-cell populations; methylation platforms, including bisulfite pyrosequencing and Illumina EPIC arrays; analytical pipelines; and clinical food allergy phenotyping. Most candidate-gene and EWAS studies have included relatively small and heterogeneous cohorts, often fewer than 100 participants, and only a limited number of methylation loci have shown partial replication across independent populations. Reproducible changes have most consistently involved *FOXP3*, IL-4, IL-5, and other Th2-regulatory pathways, but cross-study concordance remains modest. Thus, DNA methylation signatures remain promising but exploratory biomarkers requiring further validation.

6.1.2. MicroRNAs Associated with the Allergic Response

MicroRNAs (miRNAs) are short, endogenous, single-stranded non-coding RNA molecules, approximately 18–22 nucleotides in length, that regulate gene expression post-transcriptionally by binding to complementary sequences in the 3′ untranslated region of target messenger RNAs, leading to translational repression or mRNA degradation. Because miRNAs regulate a large proportion of protein-coding genes and remain relatively stable in biological fluids, they are attractive candidates as minimally invasive biomarkers and important modulators of immune cell function.

In allergic disease, miR-21, miR-146a, and miR-155 are among the most extensively studied miRNAs and are consistently dysregulated across several atopic conditions. miR-21 promotes Th2 polarization and dendritic cell differentiation from monocytes, and its upregulation has been documented in murine models of cow's milk allergy and ovalbumin sensitization [90,91]. miR-146a appears to exert predominantly immunomodulatory effects by limiting follicular T helper cell expansion, upregulating *FOXP3*, and enhancing Treg activity through suppression of the HIPK3/STAT3 axis. This mechanism has been demonstrated in an allergic conjunctivitis model, in which miR-146a targeting of HIPK3 reduced phosphorylated STAT3 and increased *FOXP3* expression in transforming growth factor-β (TGF-β)-induced thymocytes, suggesting a potential role as a molecular brake on type 2 inflammation [92]. miR-155 has a more complex and context-dependent function. It promotes dendritic cell maturation, B-cell-to-plasma-cell conversion, IgE production, and follicular T helper cell differentiation, but may also restrain Th2 cytokine secretion at the T-cell level. This dual activity complicates its interpretation as either a purely pro-allergic or tolerogenic signal.

A systematic review by Rana et al., including both in vivo models and clinical data, identified miR-146a, miR-155, and miR-30a-5p as the miRNAs most consistently dysregulated across food allergy studies [90]. However, the direction of change and the specific molecular targets varied across allergen types, including peanut, cow's milk, and egg allergy. These findings indicate that no universal food allergy miRNA signature has yet been identified and that allergen-specific and phenotype-specific profiling may be necessary. Other candidates, such as miR-143-3p, have been investigated in peanut allergy and non-celiac wheat sensitivity, while early-life miRNA patterns, including elevated miR-21 in neonatal blood, have been associated with antenatal IgE production and later allergic disease, suggesting potential predictive value during prenatal or early postnatal immune development.

The therapeutic relevance of miRNAs has also been explored in dietary and immunomodulatory interventions. In infants with cow's milk allergy receiving extensively hydrolyzed casein formula supplemented with *Lactobacillus rhamnosus* GG, differential modulation of miR-155, miR-146a, miR-128, and miR-193a was observed, together with changes in *FOXP3* methylation [93,94]. This coordinated epigenetic response suggests that miRNA regulation and DNA methylation may be mechanistically linked in the immunomodulatory effects of early nutritional interventions. In allergen-specific immunotherapy, miRNA profile changes associated with tolerance induction have also been reported, including upregulation of miR-146a together with increased IL-10 and TGF-β production, a pattern consistent with Treg expansion and broadly aligned with methylation changes observed during OIT [91,95].

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Despite these promising findings, the clinical applicability of miRNAs as biomarkers in food allergy remains incompletely defined. Most studies do not report formal diagnostic performance metrics, such as sensitivity, specificity, or area under the receiver operating characteristic curve, limiting assessment of their discriminatory value. Additional challenges include pre-analytical variability related to sample type, including serum, plasma, and whole blood; hemolysis; sample processing and storage; and the absence of universally accepted normalization strategies. Furthermore, although circulating miRNAs are attractive because of their non-invasive accessibility, their relationship with tissue-specific immune events in the gut, skin, or lymphoid tissues remains uncertain. Distinguishing exosomal from cell-free circulating miRNA fractions adds further complexity, as these compartments may differ in biological origin and biomarker relevance.

6.1.3. Evidence Status: Predominantly Exploratory

Despite strong biological plausibility and increasing mechanistic insight, the evidence supporting DNA methylation signatures and miRNA panels as clinically actionable biomarkers in food allergy remains predominantly exploratory. No epigenetic or miRNA-based biomarker has yet achieved the analytical validation, reproducibility, or regulatory endorsement required for routine use in diagnosis, prognosis, risk stratification, or therapeutic monitoring.

Several limitations explain this gap between discovery and clinical application. First, most studies include small and clinically heterogeneous cohorts, often with variable diagnostic criteria for food allergy. Second, analytical platforms differ substantially across studies, including pyrosequencing, methylation arrays, bisulfite sequencing, quantitative reverse transcription PCR, small RNA sequencing, and digital PCR, limiting cross-platform comparability. Third, many studies rely on surrogate tissues such as whole blood, which may not accurately reflect immune events occurring in the intestinal mucosa, skin, or allergen-specific T-cell subsets. Fourth, longitudinal pediatric data remain limited, despite the fact that epigenetic marks evolve dynamically with age, microbiome development, diet, and environmental exposures. Finally, most candidate loci and miRNA signatures have not been independently replicated.

Nevertheless, the field is advancing. The sensitivity of *FOXP3* methylation to OIT-induced tolerance and its reversibility during resensitization provide proof-of-concept that epigenetic biomarkers may be useful for monitoring therapeutic response [87,88]. Machine learning approaches applied to methylation array data are beginning to identify reproducible epigenetic signatures beyond single-locus analyses [89]. In addition, the convergence of miRNA and DNA methylation changes in dietary intervention studies suggests that multi-layered epigenomic panels may ultimately be more informative than single biomarkers [95,96]. Future studies should include adequately powered, longitudinal, multicenter pediatric cohorts, standardized omics platforms, rigorous cell-type annotation, and validated clinical phenotyping to translate epigenomic findings into precision medicine tools.

6.2. Multi-Omics Profiling

Multi-omics profiling integrates complementary high-throughput technologies to examine biological systems across multiple regulatory layers, including the genome, epigenome, transcriptome, proteome, metabolome, and microbiome. In pediatric food allergy, these approaches are increasingly used to characterize coordinated alterations in gene expression, protein abundance, metabolic pathways, and microbiome-derived signals that underlie allergic sensitization, clinical reactivity, tolerance acquisition, and response to therapy [97]. Rather than focusing on isolated biomarkers, multi-omics analyses reconstruct interconnected molecular networks that link immune dysregulation, epithelial barrier dysfunction, metabolic adaptation, and host–microbiome interactions to clinically observable phenotypes.

Transcriptomic studies conducted during oral food challenges have identified gene expression patterns that correlate with clinical reaction thresholds, including pathways related to Fcγ receptor–mediated phagocytosis and Toll-like receptor signaling, supporting the concept that coordinated

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gene expression programs contribute to inter-individual variability in allergic responsiveness [98]. Metabolomic profiling of plasma and stool samples collected during infancy and childhood has revealed alterations in lipid species, bile acids, steroid-related metabolites, and sphingolipid pathways in children with food allergy or allergic sensitization. These signatures likely reflect both host metabolic programming and microbiota-driven biotransformation, highlighting the importance of immune–metabolic crosstalk in shaping allergic risk [99,100]. Age-stratified analyses further suggest that metabolic pathways differ across developmental stages, with bile acid and polyamine metabolism more prominent in early life and fatty acid and acylcarnitine perturbations more evident later in childhood, emphasizing the dynamic nature of pediatric food allergy phenotypes [85].

Proteomic approaches, although less extensively studied than transcriptomics and metabolomics, provide complementary information by directly measuring protein expression and post-translational regulation. In pediatric immune-mediated conditions, deep proteome profiling has successfully identified immune-relevant protein signatures and candidate biomarkers, supporting the feasibility of applying similar strategies to food allergy to capture functional immune changes not evident at the transcript or metabolite level [101]. Overall, integrating transcriptomic, proteomic, metabolomic, and microbiome data may generate multidimensional biomarker panels that better reflect the biological complexity of pediatric food allergy than any single data type. Such approaches may enable earlier diagnosis, mechanism-based risk stratification, identification of molecular endotypes, and precision-oriented management strategies [97].

6.3. Proteomic Profiling

Proteomic profiling is increasingly being explored in pediatric food allergy as a strategy to identify protein expression patterns that reflect immune perturbation, allergic sensitization, and tolerance development. Because proteins represent functional mediators of immune and epithelial responses, proteomics may provide clinically relevant information that is not fully captured by transcriptomic or metabolomic analyses.

Early plasma proteomic studies in infancy have identified inflammation-related and immune-associated proteins associated with later development of allergic phenotypes, suggesting that systemic protein-level changes may reflect early deviations in immune maturation toward atopy [97]. More targeted high-resolution mass spectrometry analyses have detected distinct serum protein–peptide signatures in atopic children sensitized to plant storage proteins. In these cohorts, proteins involved in cell-cycle regulation and transcriptional repression helped discriminate allergic from non-allergic atopic individuals, suggesting potential utility for early identification of sensitization patterns and risk stratification [102].

Proteomic methodologies have also been applied to specific IgE-mediated food allergies, including cow's milk allergy, through immunoproteomic mapping of allergen-reactive proteins and antibody-binding profiles. These studies have identified immunodominant milk protein targets and patterns of antibody recognition that distinguish allergic from tolerant pediatric patients, providing mechanistic insight into antigen-specific immune responses and informing the development of molecularly guided diagnostic tools [103]. In parallel, proteomics has been used to characterize the molecular composition of food allergens, including allergenic proteins, isoforms, and IgE-binding epitopes. This high-resolution molecular information supports component-resolved diagnostic strategies and may improve risk assessment by capturing structural and sequence heterogeneity across allergen sources that influences clinical reactivity and cross-sensitization [104].

More recently, metaproteomic analyses of the gut microbial ecosystem have begun to clarify how microbial protein expression relates to mucosal immune regulation and the balance between oral tolerance and allergic sensitization. Microbiota-derived enzymes and structural proteins linked to immune-modulatory metabolic pathways may serve as indirect indicators of host–microbe crosstalk relevant to pediatric food allergy [105]. These findings support the concept that proteomic and metaproteomic signatures could contribute to endotype characterization, particularly when integrated with microbiome and metabolomic data.

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Despite these advances, most proteomic biomarkers in food allergy remain at an early translational stage. Several candidate protein signatures have been associated with susceptibility, clinical phenotype, persistence, and tolerance acquisition, but external validation is limited. Inflammation-associated proteins involved in epithelial barrier dysfunction, innate immune activation, complement pathways, acute-phase responses, and Th2-skewed inflammation have been reported in children with IgE-mediated food allergy compared with non-allergic controls. Similarly, serum and cellular proteomic profiles may help distinguish persistent from transient cow's milk allergy phenotypes. However, these findings remain preliminary and have not yet shown sufficient reproducibility for routine clinical use.

The quantitative strength of current proteomic evidence is also limited. Many studies include small cohorts, use heterogeneous analytical platforms, and lack independent replication. Only a minority report formal performance metrics, such as fold-change magnitude, receiver operating characteristic (ROC) analyses, sensitivity, specificity, or predictive accuracy. Additional challenges include age-dependent variability in circulating protein profiles, difficulty detecting low-abundance immune mediators in small pediatric samples, and variability in sample processing, mass spectrometry workflows, and bioinformatic pipelines. Standardized protocols, adequately powered multicenter cohorts, and integration with transcriptomic and metabolomic layers are needed to validate proteomic biomarkers and support their clinical implementation for early diagnosis, endotype stratification, tolerance prediction, and personalized management [97].

6.4. Metabolomic Profiling

Metabolomic profiling captures dynamic changes in small-molecule metabolites generated by immune activation, nutritional exposures, host metabolic programming, and microbial biotransformation. Because metabolites represent downstream products of cellular and microbial activity, metabolomics provides a functional snapshot of biological processes relevant to pediatric food allergy, including epithelial barrier integrity, immune effector function, and microbiota–host metabolic signaling [106].

Untargeted plasma metabolomic studies in IgE-mediated pediatric food allergy have consistently identified perturbations in lipid-associated pathways, including reduced sphingolipids, sphingomyelins, and ceramide species. These lipid classes contribute to membrane architecture and lipid raft organization, both of which are important for antigen presentation and receptor-mediated signaling in immune and epithelial cells. Altered sphingolipid metabolism may therefore reflect impaired epithelial barrier stability and enhanced immune responsiveness during early sensitization [97]. Pathway-based analyses also suggest abnormalities in mitochondrial energy metabolism, including fatty acid β-oxidation and acylcarnitine turnover, indicating that immune cell metabolic reprogramming accompanies allergic inflammation. Such bioenergetic shifts may influence effector T-cell differentiation, cytokine production, and persistence of inflammatory circuits [106].

Age-stratified metabolomic studies indicate that food allergy-associated metabolic perturbations vary across developmental stages. In early childhood, alterations in bile acid composition and polyamine metabolism appear prominent, both of which may influence epithelial turnover and regulatory T-cell differentiation. In older children, dysregulation of long-chain fatty acids and acylcarnitines is more frequently observed, consistent with metabolic adaptation during chronic immune activation. These findings highlight the importance of interpreting metabolomic biomarkers within age-specific biological contexts [84].

Longitudinal birth-cohort analyses have identified early-life endocrine–metabolic patterns associated with later food allergy risk, including inverse associations between androgenic or pregnenolone-derived steroid metabolites and allergic outcomes. Specific phosphoinositol-containing lipids and complex phospholipid species measured during early childhood have also shown predictive value for allergic trajectories, suggesting that membrane lipid remodeling and phosphoinositide signaling may influence immune activation thresholds relevant to food allergy development [100]. Integrated gut metabolome–microbiome profiling in infants with IgE-mediated

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cow's milk allergy has shown coordinated depletion of bile acids, short-chain fatty acids, and tryptophan-derived indole metabolites before overt allergic inflammation. These findings implicate impaired microbial metabolic support of regulatory immune pathways in the breakdown of oral tolerance [83].

Collectively, metabolomic data support the concept that pediatric food allergy involves coordinated systemic and intestinal immune–metabolic remodeling driven by both host-intrinsic immune activation and environmentally shaped microbial metabolic outputs. However, clinical translation is limited by marked inter-individual variability, dietary confounding, rapid developmental changes in pediatric metabolism, and heterogeneity across IgE-mediated, non-IgE-mediated, and mixed food allergy phenotypes [106]. Additional methodological challenges include variation in biospecimen type and collection timing, analytical platforms, batch effects, and metabolite annotation pipelines. Most candidate metabolomic biomarkers currently lack standardized pre-analytical protocols, validated diagnostic or prognostic thresholds, and prospective multicenter validation. As a result, metabolomic signatures cannot yet be used as standalone clinical biomarkers. Future studies should prioritize harmonized analytical pipelines, longitudinal sampling, external validation, and integration with proteomic and transcriptomic data to define clinically actionable biomarker panels for diagnosis, prognosis, endotype stratification, and precision-oriented management [97].

6.5. Transcriptomic Profiling

Transcriptomic approaches enable detailed characterization of gene expression programs and immune cell activation states involved in allergic sensitization, acute clinical reactivity, and responses to immunomodulatory interventions. By integrating bulk RNA sequencing with cell-resolved profiling strategies, transcriptomics can reveal coordinated immune programs that are not apparent from conventional immunophenotyping alone, offering mechanistic insight into the molecular architecture of pediatric food allergy [107].

Dynamic analyses of whole-blood transcriptomes during double-blind, placebo-controlled oral food challenges in children with peanut allergy have shown that allergen exposure induces rapid and reproducible transcriptional changes that are absent after placebo exposure. These acute allergen-induced signatures include co-regulated gene networks enriched for acute-phase responses, pro-inflammatory mediators, and innate immune activation. Notable peanut-induced transcripts include *LTB4R, PADI4, IL1R2, PPP1R3D, KLHL2,* and *ECHDC3*, which have been proposed as key drivers of transcriptional networks during clinical reactions [108]. Network-based analyses of the same challenge datasets showed that gene modules associated with individual reaction thresholds are enriched for Fcγ receptor–mediated phagocytosis and Toll-like receptor signaling pathways. Their interaction with circulating neutrophil abundance suggests coordinated regulation across innate and adaptive immune compartments and supports a role for transcriptional network architecture in determining variability in clinical reactivity [98].

Beyond acute challenge settings, whole-blood transcriptomic profiling in infants at elevated risk of peanut allergy has identified preclinical alterations in innate immune signaling, including dysregulated type I interferon responses, enrichment of neutrophil-associated gene modules, and reduced regulatory T-cell-associated signatures before overt disease onset [109]. These findings suggest that transcriptional biomarkers may help identify at-risk children during early immune development. Longitudinal transcriptomic analyses in pediatric OIT trials for egg allergy have shown progressive remodeling of gene expression during treatment. Successful desensitization is associated with downregulation of pro-inflammatory and Th17-related pathways and relative enhancement of innate immune response signatures, suggesting that immunotherapy redirects inflammatory transcriptional circuits toward less pathogenic states [110]. Systematic syntheses of transcriptomic data across OIT studies further indicate that durable immunomodulation is characterized by suppression of Th2-skewed transcriptional programs and reinforcement of regulatory networks,

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including features consistent with T-cell anergy and modulation of type I interferon-associated pathways [107].

Independent studies using weighted gene co-expression network analysis in pediatric nut allergy have identified hub transcripts, such as *IFIH1* and *DRAM1*, within modules associated with type I interferon signaling, neutrophil activation, and reduced regulatory T-cell networks, reinforcing the concept that innate immune bias and impaired regulation contribute to food allergy pathogenesis in early life [109]. Epigenetically informed transcriptomic profiling of naïve $CD4^+$ T-cell activation in infants with challenge-confirmed egg allergy has also revealed dysregulation of cell-cycle-associated transcriptional programs, including E2F- and MYC-regulated gene sets, together with remodeling of immune receptor loci such as *IL1R* and *IL18RAP*. These abnormalities correlate with impaired lymphoproliferative responses and may reflect defective oral tolerance induction during critical windows of immune development [111].

From a translational perspective, transcriptomic biomarkers in pediatric food allergy can be grouped into four main categories. Diagnostic biomarkers include acute allergen-induced signatures detectable during oral food challenges, characterized by rapid induction of inflammatory and innate immune genes such as *LTB4R*, *PADI4*, and *IL1R2*. Prognostic biomarkers include early-life transcriptional patterns associated with later disease development or reaction severity, such as type I interferon modules, neutrophil-associated gene networks, and attenuated regulatory T-cell signatures. OIT-response biomarkers include dynamic changes associated with successful desensitization, including suppression of Th2/Th17 inflammatory pathways and reinforcement of regulatory or anergy-associated programs. Finally, endotype-associated transcriptomic signatures may reflect more stable molecular architectures, such as interferon-driven, neutrophil-enriched, or regulatory-deficient subphenotypes, with potential value for precision stratification [108,110].

Despite their promise, transcriptomic biomarkers are not yet ready for routine clinical use. Key challenges include tissue specificity of gene expression, temporal variability between acute reaction and steady-state sampling, differences in RNA sequencing and sample-processing protocols, and heterogeneity in analytical pipelines. Larger multicenter validation studies, standardized transcriptomic workflows, and integration with proteomic, metabolomic, epigenetic, and microbiome data are needed to identify robust transcriptional biomarkers capable of informing early diagnosis, endotype definition, treatment selection, and therapeutic monitoring in pediatric food allergy [107].

6.6. Future Directions for Multi-Omics Integration

Converging evidence from epigenomic, transcriptomic, proteomic, metabolomic, and microbiome studies indicates that pediatric food allergy arises from coordinated alterations in immune signaling, epithelial barrier function, metabolic programming, gene regulation, and host–microbiome interactions. These perturbations are dynamic, developmentally regulated, and influenced by environmental exposures, collectively defining biologically distinct disease endotypes. The integration of multi-layered omics data offers a promising route toward biomarker panels that move beyond single-analyte diagnostics and better capture the complexity of pediatric food allergy.

Realizing this translational potential will require rigorous clinical phenotyping, standardized sample collection and analytical pipelines, longitudinal cohort designs, external validation, and broader representation of diverse pediatric populations. Computational approaches, including machine learning and network-based modeling, may help identify reproducible biomarker combinations, but these models must be interpretable, clinically feasible, and validated across independent cohorts. With these advances, multi-omics approaches may help transform pediatric food allergy from a condition defined primarily by clinical history and challenge outcomes into a molecularly stratified disease, enabling earlier diagnosis, more accurate risk prediction, improved monitoring of tolerance acquisition, and personalized therapeutic decision-making.

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7. Clinical Translation and Future Perspectives

The expanding field of biomarker research in pediatric food allergy reflects the urgent need for diagnostic, prognostic, and monitoring tools that are more precise, less invasive, and more informative than conventional approaches alone. Current clinical evaluation still relies primarily on medical history, skin prick testing, serum specific IgE, and OFC. Although these tools remain essential, they have important limitations: sensitization tests may lack specificity, OFCs are time-consuming and resource-intensive and carry a risk of allergic reactions, and currently available methods provide limited information on reaction severity, eliciting dose, disease persistence, tolerance acquisition, or therapeutic response. Biomarkers could help address these unmet needs by improving diagnostic accuracy, reducing unnecessary food challenges, identifying children at higher risk of severe reactions, monitoring the development of tolerance, and guiding personalized therapeutic strategies.

Among the biomarkers reviewed, functional cellular assays, particularly the BAT, currently show the greatest translational readiness. BAT has demonstrated higher specificity than skin prick testing and serum specific IgE in several IgE-mediated food allergies, especially peanut and sesame allergy, and has been incorporated as a second-line diagnostic tool in recent EAACI guidance for selected patients with equivocal first-line test results. Its ability to reflect effector cell responsiveness also makes BAT clinically attractive beyond diagnosis, because parameters such as basophil reactivity and sensitivity may provide information on reaction severity, threshold dose, and response to immunomodulatory interventions, including oral immunotherapy and biologic treatment. Nevertheless, broader implementation remains limited by the need for fresh blood, flow cytometry infrastructure, trained personnel, harmonized protocols, standardized allergen preparations, and validated food-specific cut-off values.

In contrast, biomarkers related to epithelial barrier dysfunction and intestinal permeability remain at an earlier stage of clinical development. Markers such as zonulin, intestinal fatty acid-binding protein, diamine oxidase, tight junction proteins, and fecal inflammatory mediators provide biologically plausible information on mucosal integrity, epithelial injury, and gastrointestinal inflammation. These biomarkers may be particularly relevant for non-IgE-mediated and mixed gastrointestinal food allergy phenotypes, in which conventional IgE-based testing is often uninformative. However, their translation into routine care is currently limited by assay variability, uncertain specificity, lack of validated thresholds, small pediatric cohorts, and insufficient longitudinal evidence. Zonulin is the most extensively studied permeability marker, but concerns about the analytical specificity of commercial assays currently restrict its interpretation to a research or exploratory endotyping context rather than a validated diagnostic role.

Genetic and epithelial integrity biomarkers, particularly *filaggrin* loss-of-function variants, provide important mechanistic insight into the link between barrier dysfunction, atopic dermatitis, transcutaneous sensitization, and food allergy development. Their greatest potential lies in early-life risk stratification, especially among children with early-onset atopic dermatitis or multiple atopic features. However, because genetic variants are stable and do not reflect dynamic disease activity, they are unlikely to be useful for monitoring tolerance acquisition or therapeutic response. Their future clinical value may therefore depend on integration with other dynamic biomarkers, such as immune, microbiota-derived, or metabolomic signatures.

Microbiota-derived and metabolomic biomarkers are among the most promising candidates for precision medicine because they capture host–environment interactions, immune maturation, epithelial barrier function, and oral tolerance mechanisms. Reduced microbial diversity, depletion of butyrate-producing taxa, altered short-chain fatty acid profiles, and perturbations in bile acid or tryptophan metabolism have all been associated with food allergy development, persistence, or impaired tolerance acquisition. However, these markers are highly influenced by age, diet, breastfeeding, antibiotic exposure, geography, sample handling, sequencing platforms, and bioinformatic pipelines. As a result, reproducibility across cohorts remains limited. At present,

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microbiota and metabolomic signatures should be considered exploratory tools with substantial potential for future risk prediction and endotyping, rather than clinically actionable tests.

Multi-omics approaches, including transcriptomic, proteomic, metabolomic, epigenetic, and microbiome-integrated models, offer the most comprehensive strategy for capturing the biological complexity of pediatric food allergy. These approaches may identify molecular endotypes, predict disease trajectories, distinguish transient from persistent allergy, and monitor response to interventions such as oral immunotherapy or biologics. Epigenetic biomarkers, including *FOXP3* methylation and selected microRNA profiles, are particularly relevant because they may reflect immune regulation and tolerance induction. Transcriptomic signatures during oral food challenges and immunotherapy may provide objective molecular correlates of clinical reactivity and treatment response. Proteomic and metabolomic profiles may add functional information on inflammatory pathways, epithelial integrity, and immune–metabolic remodeling. However, most multi-omics findings remain investigational, with limited external validation, small cohorts, heterogeneous analytical methods, and uncertain clinical feasibility.

A major barrier across all biomarker categories is the lack of standardization. Studies differ widely in patient selection, age range, food allergens evaluated, diagnostic confirmation methods, disease phenotype definitions, sample type, timing of collection, laboratory methodology, and statistical analysis. This heterogeneity makes comparison across studies difficult and limits the development of clinically meaningful cut-off values. Future studies should use harmonized definitions of IgE-mediated, non-IgE-mediated, and mixed food allergy; standardized oral food challenge protocols when ethically and clinically appropriate; uniform reporting of sensitivity, specificity, positive and negative predictive values, likelihood ratios, and area under the curve; and prospective validation in independent pediatric cohorts.

Another critical issue is clinical utility. A biomarker should not only distinguish allergic from tolerant children but should also improve decision-making compared with existing tools. For clinical implementation, candidate biomarkers must demonstrate that they can reduce unnecessary OFCs, predict severe reactions or low eliciting thresholds, identify children likely to outgrow allergy, select candidates for OIT or biologic therapy, monitor treatment response, or support safe dietary liberalization. Biomarkers that provide mechanistic insight but do not change management may remain valuable for research and endotyping, but their role in routine practice will be limited.

Future biomarker development should therefore move from discovery-based studies toward clinically oriented validation pathways. Priority should be given to large, multicenter, longitudinal pediatric cohorts with standardized phenotyping, age-specific analyses, adequate representation of different food allergens and disease mechanisms, and external validation across diverse populations. Biomarkers should be evaluated both individually and in combination with established clinical variables, including history, SPT, sIgE, component-resolved diagnostics, comorbid atopic disease, and reaction history. Integrated models combining clinical data with functional, barrier-related, microbiota-derived, and omics-based biomarkers may ultimately outperform single-marker approaches and provide more accurate tools for precision medicine.

Implementation in routine care will also require practical considerations. Assays must be reproducible, affordable, accessible, and compatible with pediatric sampling constraints. Turnaround time, required sample volume, need for specialized equipment, technical expertise, and cost-effectiveness will strongly influence adoption. BAT, for example, has strong clinical potential but requires fresh blood and flow cytometry, whereas genetic markers are stable and easy to measure but provide limited dynamic information. Microbiota and omics-based biomarkers may offer deeper biological insight but will require simplified, validated, and clinically interpretable platforms before widespread use.

In the future, biomarker-guided care could support a more personalized approach to pediatric food allergy. Children with uncertain diagnosis could undergo BAT or other functional testing to reduce unnecessary OFCs. Patients with barrier dysfunction or microbiota-derived risk profiles could be identified early and targeted for preventive or tolerance-promoting strategies. Children

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undergoing OIT could be monitored using functional, epigenetic, transcriptomic, or metabolomic markers to assess desensitization, sustained unresponsiveness, and risk of relapse. Ultimately, biomarker integration may allow clinicians to move beyond a binary allergic-versus-tolerant classification toward a multidimensional model incorporating mechanism, severity, threshold, persistence, and treatment responsiveness.

Overall, current evidence indicates that BAT is the most clinically advanced biomarker in pediatric food allergy, whereas epithelial barrier markers, microbiota-derived signatures, metabolomic profiles, epigenetic markers, and broader multi-omics approaches remain largely investigational but highly promising. A comparative summary of the translational potential, current limitations, and degree of clinical readiness of emerging biomarkers is provided in Table 4.

Table 4. Translational readiness of emerging biomarkers in pediatric food allergy.

Biomarker	Main utility	Strengths	Limitations	Readiness for clinical practice
BAT	Diagnosis, severity, threshold, monitoring	High specificity, OFC reduction, functional assay	Cost, standardization, fresh blood	High (second-line)
Zonulin	Barrier dysfunction endotyping	Most studied permeability marker	Poor assay specificity, no cut-offs	Low–moderate
FLG mutations	Risk prediction	Strong genetic association with food allergy/AD	Static susceptibility marker only	Moderate
SCFAs/butyrate	Tolerance prediction	Mechanistic relevance, microbiota link	High inter-study variability	Low
Gut microbiota signatures	Endotyping, tolerance prediction	Multi-omics potential	Poor reproducibility, no standardization	Low
IL-25 / TSLP / IL-33	Mechanistic endotyping	Strong biologic rationale	Experimental only	Experimental

Abbreviations: AD, atopic dermatitis; BAT, basophil activation test; *FLG, filaggrin*; IL, interleukin; OFC, oral food challenge; SCFAs, short-chain fatty acids; TSLP, thymic stromal lymphopoietin.

8. Conclusions

Food allergy remains a highly heterogeneous pediatric disease resulting from complex interactions among immune dysregulation, epithelial barrier impairment, environmental exposures, genetic susceptibility, and microbial determinants. This systematic review highlights the rapid expansion of biomarker research in pediatric food allergy and underscores the potential of emerging biomarkers to improve diagnosis, risk stratification, tolerance prediction, therapeutic monitoring, and precision medicine approaches.

Among currently available tools, the BAT appears to be the most clinically advanced biomarker. Its high diagnostic specificity, ability to distinguish sensitization from clinically relevant allergy, and potential role in predicting reaction severity and eliciting thresholds make it particularly useful in selected patients with equivocal first-line test results. BAT may also reduce the need for OFCs and support monitoring of immunological changes during OIT or biologic treatment. However, broader implementation requires further standardization, improved accessibility, external quality assurance, and validated food-specific cut-off values.

By contrast, biomarkers related to intestinal permeability and epithelial barrier integrity, including zonulin, tight junction proteins, epithelial injury markers, *FLG* variants, and epithelial-derived cytokines, provide important mechanistic insights into food allergy pathogenesis and endotype characterization. These biomarkers may be especially relevant for gastrointestinal, non-IgE-

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mediated, and mixed phenotypes, where conventional IgE-based tests have limited utility. Nevertheless, most remain investigational because of assay variability, lack of validated thresholds, limited longitudinal pediatric data, and insufficient prospective validation.

Microbiota-derived, metabolomic, transcriptomic, proteomic, and epigenetic biomarkers offer substantial promise for identifying early-life risk signatures, predicting disease persistence or tolerance acquisition, and monitoring response to emerging therapies. Multi-omics approaches are particularly attractive because they can capture the biological complexity of pediatric food allergy more effectively than single-analyte strategies. However, these candidates remain largely exploratory due to small cohorts, heterogeneous study designs, limited reproducibility, methodological variability, and absence of clinically validated diagnostic or prognostic models.

Overall, current evidence supports a gradual transition from isolated biomarkers toward integrated, multidimensional biomarker panels combining clinical data with functional, barrier-related, microbiota-derived, and omics-based signatures. Future research should prioritize large multicenter longitudinal studies, harmonized phenotyping, standardized analytical pipelines, age-specific validation, and external replication across diverse pediatric populations. The integration of validated biomarker-based endotyping into pediatric allergology may ultimately improve early diagnosis, prognostic stratification, therapeutic selection, monitoring of tolerance acquisition, and personalized management of children with food allergy.

Progress in this field will depend on standardization, longitudinal validation, integration with clinical decision-making, and demonstration of real-world clinical benefit. With these advances, biomarkers have the potential to transform pediatric food allergy management by enabling earlier diagnosis, improved risk stratification, safer monitoring, and more individualized therapeutic strategies.

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