GMR
The Original

GUT MICROBIOME DYSBIOSIS IN PEDIATRIC INFLAMMATORY BOWEL DISEASE: FROM FOUNDATIONAL 16S EVIDENCE TO SHOTGUN METAGENOMICS AND A DEVELOPMENTAL INSTABILITY FRAMEWORK

Bashaer E. Alattas[1,2], Reem M. Farsi[1], Turki S. Abujamel[2,3]

[1]Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia.
[2]EcoHealth Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia.
[3]Department of Medical Laboratory Sciences, Faculty of Applied Medical Science, King Abdulaziz University, Jeddah, Saudi Arabia.

ABSTRACT

Pediatric inflammatory bowel disease (IBD) frequently emerges during periods of active immune maturation and microbiome assembly, suggesting that age at disease onset fundamentally shapes host–microbe interactions and disease architecture. Here, we synthesize evidence from foundational 16S rRNA and pediatric shotgun metagenomic studies, integrating taxonomic, functional, and host genetic findings into a developmental instability framework for interpreting dysbiosis in early-onset disease. Across independent pediatric cohorts, particularly in Crohn's disease, a reproducible dysbiosis signature has emerged, characterized by reduced alpha diversity, depletion of major short-chain fatty acid (SCFA)-producing obligate anaerobes, including Faecalibacterium, Roseburia, and Blautia, and expansion of facultative anaerobes, especially members of the Enterobacteriaceae. Building on these compositional observations, shotgun metagenomic studies provide species- and strain-level resolution together with direct functional characterization, revealing pathway-level remodelling that includes reduced butyrate biosynthetic potential, disrupted bile acid and tryptophan metabolism, and altered nitrogen and amino acid metabolism. Longitudinal investigations further demonstrate coordinated ecological restructuring during exclusive enteral nutrition and biologic therapy, while mucosal datasets consistently reveal stronger host–microbe coupling than stool profiles alone. Host genetic variants in NOD2, ATG16L1, IL23R, CARD9, and FUT2 further shape microbial ecosystem development through distinct biological mechanisms whose frequencies and effects vary across ancestries, providing a biological rationale for population-specific pediatric cohorts, including those from Saudi Arabia and the broader Middle East. Collectively, these findings support a developmental instability framework in which inflammation arises within an incompletely stabilized immune–microbiome ecosystem shaped in parallel by host genotype. Future longitudinal, compartment-resolved, age-stratified, and genotype-informed multi-omics studies will be essential to clarify causal relationships and identify therapeutic windows for precision microbiome-based interventions.

KEYWORDS: Pediatric inflammatory bowel disease (IBD), 16S rRNA, Crohn's disease, microbiome .

1. INTRODUCTION

Pediatric-onset inflammatory bowel disease (IBD) is increasingly recognized as a biologically distinct disease entity rather than merely an earlier manifestation of adult IBD (Levine et al., 2011; Ruemmele et al., 2014). The global incidence of pediatric IBD continues to rise, particularly in rapidly industrializing regions, highlighting the contribution of environmental exposures acting during critical windows of developmental susceptibility (Ng et al., 2017; Kuenzig et al., 2022). Beyond these epidemiological trends, pediatric IBD exhibits distinctive clinical, immunological, and molecular characteristics. Compared with adults, affected children more frequently present with extensive colonic involvement in ulcerative colitis (UC) and ileocolonic or pan-enteric Crohn's disease (CD), phenotypes formally captured by the Paris classification (Levine et al., 2011). In addition, age-dependent ileal immune intensification, altered antimicrobial peptide expression, and distinctive mucosal immune responses further distinguish pediatric CD from adult disease (Haberman et al., 2019). Collectively, these observations suggest that host–microbe interactions in pediatric IBD are fundamentally shaped by developmental biology rather than representing a simple manifestation of established microbial dysbiosis.

Pediatric IBD typically develops during a period of active immune maturation and microbiome assembly, when bidirectional communication between the host and the intestinal microbiota remains highly dynamic. Early life represents a critical window for mucosal immune education and the establishment of microbial tolerance, during which microbial exposure shapes regulatory immune networks, epithelial barrier integrity, and long-term immune homeostasis (Belkaid & Hand, 2014; Gensollen et al., 2016). Simultaneously, the gut microbiome undergoes a structured ecological succession, progressively diversifying toward an adult-like community during early childhood (Yatsunenko et al., 2012; Bäckhed et al., 2015). These compositional changes are accompanied by maturation of microbial metabolic functions, including the production of short-chain fatty acids (SCFAs), particularly butyrate, which supports epithelial integrity and regulatory T-cell differentiation (Furusawa et al., 2013). Disruption of these developmental processes, for example through early-life

antibiotic exposure, may induce persistent strain-level alterations that influence immune calibration and microbial resilience long after the initial perturbation (Korpela et al., 2016). Together, these observations indicate that both microbial composition and microbial function are established during a highly dynamic developmental period, rendering childhood uniquely susceptible to ecological perturbations.

Because pediatric IBD frequently manifests during or shortly after these critical phases of immune–microbiome co-maturation, interpretation of dysbiosis requires a developmental perspective. While previous reviews have primarily focused on describing taxonomic alterations associated with pediatric IBD, relatively few have integrated compositional, functional, genetic, and developmental evidence into a unified conceptual framework. In this review, we synthesize evidence from foundational 16S rRNA studies and emerging pediatric shotgun metagenomic investigations while incorporating recent advances in microbial functional profiling, host genetic susceptibility, and multi-omics approaches. Rather than viewing pediatric IBD as a simple deviation from a mature microbial steady state, we propose a developmental instability framework in which disease arises from ecological instability during immune–microbiome co-maturation. Within this framework, microbial composition, metabolic function, and host genetic architecture interact dynamically to shape disease susceptibility, progression, and therapeutic responsiveness.

The principal pediatric IBD microbiome cohorts discussed throughout this review are summarized in Table 1.

Table 1. Representative pediatric IBD microbiome studies included in this review (2014–2026)

Study	Year	Study design	Cohort size	Treatment-naïve	Compartment	Analytical platform	Multi-omics	Key finding
Gevers et al.	2014	Cross-sectional, new-onset	CD: 447; controls: 221	Yes	Stool and rectal biopsies	16S rRNA sequencing	No	Established the foundational pediatric CD dysbiosis signature and demonstrated stronger disease discrimination using mucosal samples than stool.
Haberman et al.	2014	Cross-sectional	Total: 359; group breakdown NR	Yes	Ileal mucosa	16S rRNA sequencing and host transcriptomics	Yes	Linked ileal epithelial antimicrobial dysfunction with coordinated microbiome alterations in pediatric CD.
Quince et al.	2015	Longitudinal interventional study of EEN	NR	NR	Stool	Shotgun metagenomic sequencing	No	Demonstrated extensive taxonomic and functional restructuring of the fecal metagenome during EEN-induced remission.
Shaw et al.	2016	Prospective longitudinal	IBD: 19; controls: 10	NR	Stool	16S rRNA sequencing	No	Associated baseline dysbiosis severity with subsequent treatment response in pediatric IBD.
Dunn et al.	2016	Prospective longitudinal study of EEN outcomes	CD: 10; controls: 5	NR	Stool	16S rRNA sequencing	No	Linked early microbial community restructuring after EEN with sustained clinical remission.
Mottawea et al.	2016	Cross-sectional, new-onset	CD: 65; UC: 21; controls: 42	Yes	Mucosal–luminal interface	16S rRNA sequencing and host proteomics	Yes	Identified an Atopobium parvulum-centred microbial network associated with impaired host H_2S detoxification and demonstrated its colitogenic potential experimentally.
Haberman et al.	2019	Cross-sectional	NR	NR	Ileal mucosa	16S rRNA sequencing and host transcriptomics	Yes	Demonstrated age-of-diagnosis-dependent immune intensification and reduced α-defensin expression despite broadly similar dysbiotic profiles.

Diederen et al.	2020	Longitudinal EEN study	CD: 43; controls: 18	NR	Stool	16S rRNA sequencing and NMR metabolomics	Yes	Identified coordinated microbiome–metabolome changes during EEN, including reductions in trimethylamine and cadaverine.
Jagt et al.	2022	Multicentre case-control study	CD: 40; UC: 38; controls: 105	Yes	Stool	Targeted amino acid profiling by HPLC	Yes	Identified fecal amino acid profiles that differentiated pediatric CD, UC, and healthy controls.
Lv et al.	2024	Longitudinal	CD: 27; controls: 11	NR	Stool and intestinal tissue	Metagenomic sequencing and host miRNA profiling	Yes	Revealed coordinated interactions between microbial composition and intestinal microRNA expression in pediatric CD.
Conrad et al.	2024	Longitudinal	NR	NR	Stool	Shotgun metagenomic sequencing	No	Characterized age-associated variation in the intestinal microbiome across the pediatric-to-adult IBD spectrum.
Kuleckа et al.	2025	Prospective pediatric UC study	UC: 56; controls: NR	Yes	Mucosal biopsies	Quantitative microbial profiling	Yes	Integrated mucosal microbiome, transcriptomic, epigenomic, and host genotype data and identified prognostic host–microbiome signatures.
Vermeer et al.	2026	Cross-sectional	IBD: 103; healthy controls: 356; CGI: 75	NR	Stool	Shotgun metagenomic sequencing	No	Reported distinct metagenomic profiles that differentiated pediatric IBD from healthy and symptomatic controls.

NR = not reported
CGI = Children with Gastrointestinal Symptoms/Indications

2.Foundational 16S rRNA Evidence in Pediatric IBD

The initial characterization of the pediatric IBD microbiome was largely driven by 16S rRNA gene sequencing studies conducted in treatment-naïve Crohn's disease (CD) cohorts. These investigations established a reproducible dysbiosis signature that has remained remarkably consistent across geographically distinct populations. The landmark multicentre study by Gevers et al. provided the first comprehensive description of this microbial profile in newly diagnosed pediatric CD, demonstrating reduced alpha diversity, expansion of Enterobacteriaceae, Pasteurellaceae, Veillonellaceae, and Fusobacteriaceae, together with depletion of obligate anaerobic members of the order Clostridiales (Gevers et al., 2014). Importantly, mucosal biopsies outperformed stool samples in discriminating disease status, emphasizing spatial heterogeneity and indicating that host–microbe interactions are more pronounced at the mucosal interface. This study established the conceptual foundation for subsequent pediatric microbiome research by demonstrating that microbial alterations are reproducible, compartment-dependent, and detectable at disease onset.

Subsequent investigations validated these findings across independent pediatric cohorts. De Meij et al. and Kowalska-Duplaga et al. independently reported reduced microbial diversity, depletion of obligate anaerobic commensals, and expansion of facultative bacteria in newly diagnosed pediatric IBD despite differences in geography, cohort characteristics, and study design (de Meij et al., 2018; Kowalska-Duplaga et al., 2019; El Mouzan et al., 2025a). Together, these studies established that pediatric CD is characterized by a conserved microbial signature rather than cohort-specific observations.

The biological relevance of these compositional alterations was further strengthened by integration with host transcriptomic profiling. Haberman et al. demonstrated coordinated ileal transcriptomic and microbiome changes that linked epithelial antimicrobial dysfunction with microbial dysbiosis in pediatric CD (Haberman et al., 2014). In a subsequent study, the same group showed that older children exhibited greater Th1-associated immune activation and reduced α-defensin expression despite broadly similar microbial profiles, suggesting that comparable dysbiotic communities may interact differently with age-dependent immune environments (Haberman et al., 2019). These findings support the concept that pediatric IBD should be interpreted within the context of immune–microbiome co-maturation rather than microbial composition alone.

Across these studies, several bacterial taxa consistently emerged as hallmarks of pediatric dysbiosis. Treatment-naïve pediatric CD is characterized by depletion of major SCFA-producing obligate anaerobes, particularly Faecalibacterium, Roseburia, and Blautia, together with expansion of facultative anaerobes, most notably members of the Enterobacteriaceae family (Gevers et al., 2014; Mottawea et al., 2016). Additional genera, including Akkermansia, Bacteroides, Collinsella, Veillonella, and Fusobacterium, have also been implicated, although their abundance patterns appear more variable and are influenced by disease location, inflammatory activity, treatment status, and sampling compartment (Zheng et al., 2023). Rather than representing isolated taxonomic changes, these recurrent alterations indicate coordinated ecological restructuring of the pediatric gut microbiome.

Despite establishing a robust compositional framework, 16S rRNA sequencing remains inherently limited by its genus-level taxonomic resolution and inability to directly quantify microbial functional capacity or resolve strain-level heterogeneity (Quince et al., 2017). Consequently, while these studies identified the microorganisms consistently associated with pediatric IBD, they provided only limited insight into the metabolic pathways and biological mechanisms through which dysbiosis contributes to disease pathogenesis. Addressing these questions required the transition to shotgun metagenomics, which enables direct characterization of microbial genes, metabolic pathways, and functional potential.

3.Emergence of Shotgun Metagenomics: From Compositional Dysbiosis to Functional Remodelling

While foundational 16S rRNA studies established a reproducible compositional dysbiosis signature in pediatric Crohn's disease (CD), they were inherently limited by insufficient taxonomic resolution and the inability to directly characterize microbial functional capacity (Quince et al., 2017). The introduction of shotgun metagenomic sequencing represented a major methodological advance by enabling species- and strain-level profiling together with comprehensive characterization of microbial genes and metabolic pathways. Rather than simply identifying which microorganisms are present, shotgun metagenomics provides insight into how microbial communities function and how these functions are altered during disease progression and therapeutic intervention.

One of the earliest applications of shotgun metagenomics in pediatric IBD examined the effects of exclusive enteral nutrition (EEN), the first-line induction therapy for pediatric CD. Quince et al. demonstrated that EEN induced coordinated restructuring of both microbial composition and functional gene content, indicating that clinical remission is accompanied by ecological reorganization rather than a simple restoration of the pre-disease microbiota (Quince et al., 2015). Subsequent integration of metagenomics with metabolomic profiling further strengthened this concept. Diederen et al. identified coordinated microbiome–metabolome changes during EEN-induced remission, including alterations in amino acid-related metabolites and reductions in compounds such as trimethylamine and cadaverine, suggesting that microbial metabolic activity may better reflect disease status and therapeutic response than taxonomic composition alone (Diederen et al., 2020).

These studies shifted the interpretation of pediatric dysbiosis from a purely compositional phenomenon toward one of functional remodelling. Gene-centric metagenomic analyses enable direct reconstruction of microbial genes and metabolic pathways, providing mechanistic insight beyond that achievable with 16S rRNA sequencing. Consequently, attention has increasingly focused on metabolic pathways disrupted in pediatric IBD, including short-chain fatty acid (SCFA) biosynthesis, bile acid transformation, nitrogen and amino acid metabolism, and microbial tryptophan catabolism.

Among the functional alterations identified to date, impairment of SCFA production has emerged as one of the most consistent features of pediatric IBD. SCFAs, particularly butyrate, are produced through bacterial fermentation of dietary fiber and serve as the primary energy source for colonocytes while promoting epithelial barrier integrity and regulatory T-cell differentiation (Furusawa et al., 2013; Smith et al., 2013). Shotgun metagenomic analyses have demonstrated reduced representation of butyrate biosynthetic genes in pediatric CD, paralleling the depletion of major butyrate-producing genera, including Faecalibacterium, Roseburia, and Blautia (Quince et al., 2015; Mottawea et al., 2016). These findings provide direct functional evidence that depletion of beneficial anaerobes translates into diminished metabolic capacity, thereby establishing a mechanistic link between microbial dysbiosis and impaired mucosal immune homeostasis (Sokol et al., 2008).

Functional alterations extend beyond SCFA metabolism to include profound disturbances in bile acid transformation. Under physiological conditions, intestinal bacteria convert primary bile acids into secondary bile acids through deconjugation and 7α-dehydroxylation, generating metabolites that regulate epithelial renewal, barrier integrity, and immune signalling through receptors such as FXR and TGR5. Pediatric IBD is associated with depletion of bacterial taxa responsible for these transformations, resulting in reduced production of secondary bile acids and disruption of host signalling pathways involved in intestinal homeostasis (Peterson et al., 2025). Notably, many microorganisms responsible for bile acid metabolism overlap with those involved in SCFA production, suggesting that depletion of a relatively small group of obligate anaerobes simultaneously compromises multiple protective metabolic functions.

Another important aspect of functional remodelling involves alterations in nitrogen and amino acid metabolism. Metagenomic and metabolomic studies have demonstrated changes in microbial pathways related to amino acid utilization together with altered concentrations of fecal amino acids in newly diagnosed pediatric IBD (Jagt et al., 2022). In parallel, expansion of hydrogen sulfide-producing pathobionts, including Atopobium parvulum and other inflammation-associated taxa, has been linked to impaired mitochondrial function and epithelial injury at the mucosal surface (Mottawea et al., 2016). These findings indicate that functional remodelling extends beyond energy metabolism to include pathways directly involved in epithelial injury and immune activation.

Tryptophan metabolism represents another important component of this functional landscape. Commensal bacteria convert dietary tryptophan into indole derivatives that activate the aryl hydrocarbon receptor (AhR), thereby promoting IL-22 production, epithelial repair, and maintenance of mucosal barrier function (Zelante et al., 2013). Reduced abundance

of tryptophan-metabolizing bacteria may therefore limit AhR signalling and contribute to chronic intestinal inflammation (Moutusy & Ohsako, 2024). This pathway also provides an important biological connection between microbial function and host genetics, as variants in genes such as CARD9 influence microbial production of AhR ligands (Lamas et al., 2016), highlighting the close interplay between microbial metabolism and host susceptibility.

Beyond individual metabolic pathways, shotgun metagenomics has increasingly been integrated with complementary multi-omics approaches to provide a more comprehensive understanding of pediatric IBD. Studies combining metagenomics with metabolomics, transcriptomics, microRNA profiling, epigenomics, and host genomics demonstrate that microbial functional alterations occur in parallel with changes in host molecular pathways rather than in isolation (Lv et al., 2024; Kulecka et al., 2025; Lloyd-Price et al., 2019; Serrano-Gómez et al., 2025). These integrated datasets reinforce the concept that disease-associated dysbiosis reflects disruption of the entire host–microbiome ecosystem.

Functional annotation platforms such as KEGG and MetaCyc have further enhanced interpretation of shotgun metagenomic data by organizing microbial genes into biologically meaningful metabolic pathways. These analytical frameworks consistently demonstrate enrichment of inflammation-associated pathways together with depletion of pathways involved in carbohydrate fermentation and other health-associated microbial functions (Beghini et al., 2021; Caspi et al., 2020; Ning et al., 2023; Franzosa et al., 2019; Heinken et al., 2021; Vich Vila et al., 2023). Although pathway-resolved pediatric datasets remain relatively limited, convergence of metagenomic, metabolomic, and host-derived molecular evidence strongly supports the presence of conserved functional remodelling across childhood-onset disease.

Beyond metabolic pathway alterations, shotgun metagenomics has also improved understanding of strain-level pathogenicity. For example, adherent-invasive Escherichia coli (AIEC) strains have been identified in pediatric CD, suggesting that expansion of Enterobacteriaceae reflects not only ecological imbalance but also increased abundance of bacteria possessing specific virulence characteristics (Conte et al., 2014). Experimental studies further indicate that inflammation-associated increases in epithelial oxygenation selectively favor facultative anaerobes, providing a plausible ecological mechanism for their expansion during disease (Cevallos et al., 2019).

Collectively, these findings demonstrate that shotgun metagenomics has fundamentally transformed the study of pediatric IBD by shifting the focus from identifying which microorganisms are present to understanding which biological functions are altered. Rather than representing isolated taxonomic changes, pediatric dysbiosis is increasingly recognized as coordinated functional remodelling involving microbial metabolism, host signalling, and immune regulation. This functional perspective provides the conceptual foundation for understanding the broader ecological interactions that characterize pediatric IBD, including those involving fungi, viruses, and other members of the intestinal microbiome.

4.Multi-Domain Ecosystem Disruption in Pediatric IBD

Although pediatric microbiome research has historically focused on bacterial community composition, accumulating evidence indicates that pediatric inflammatory bowel disease (IBD) involves disruption of an interconnected microbial ecosystem rather than alterations confined to bacterial communities. This broader ecological perspective recognizes that bacteria, fungi, and viruses interact continuously within the intestinal environment, collectively influencing microbial stability, immune regulation, and mucosal homeostasis. Consequently, dysbiosis in pediatric IBD is increasingly understood as a multi-domain phenomenon involving coordinated perturbations across multiple microbial kingdoms.

Among these non-bacterial components, the intestinal mycobiome has emerged as an important contributor to ecosystem disruption. Fungal dysbiosis has been reported in treatment-naïve pediatric IBD, with alterations in fungal community composition already evident at disease onset (Mukhopadhya et al., 2015). More recently, pediatric studies have shown that bacterial and fungal communities undergo coordinated restructuring during therapeutic interventions such as exclusive enteral nutrition (EEN), indicating that ecological recovery extends beyond bacterial restoration alone (Gerasimidis et al., 2023). Although the underlying mechanisms remain incompletely understood, these observations suggest that intestinal inflammation disrupts ecological equilibrium across microbial kingdoms and that restoration of microbial homeostasis likely requires recovery of both bacterial and fungal communities.

Parallel advances have highlighted the importance of the intestinal virome in pediatric IBD. Early investigations identified alterations in bacteriophage communities associated with pediatric Crohn's disease (Wagner et al., 2013), while more recent work demonstrated distinct gut virome signatures in treatment-naïve Saudi children with ulcerative colitis (El Mouzan et al., 2025b). Because bacteriophages regulate bacterial abundance through predation and facilitate horizontal gene transfer, alterations in viral communities have the potential to reshape both microbial composition and functional capacity. These findings extend the concept of dysbiosis beyond bacterial and fungal communities, recognizing viruses as active participants in host–microbiome interactions rather than passive bystanders.

Beyond community-level alterations, specific bacterial strains may contribute disproportionately to disease through specialized pathogenic traits. Adherent-invasive Escherichia coli (AIEC), for example, has been identified in pediatric Crohn's disease and exhibits enhanced abilities to adhere to, invade, and persist within intestinal epithelial cells and macrophages (Conte et al., 2014). These strain-specific characteristics illustrate that disease progression depends not only on broad shifts in community composition but also on the expansion of microorganisms possessing enhanced virulence potential, adding another layer of complexity to pediatric dysbiosis.

Collectively, these findings support a conceptual shift from viewing pediatric IBD as a disorder of bacterial dysbiosis to recognizing it as disruption of an integrated intestinal ecosystem composed of interacting bacterial, fungal, and viral communities. Understanding how these microbial kingdoms influence one another, together with their interactions with the host immune system, will be essential for developing a comprehensive model of pediatric IBD pathogenesis. Future longitudinal multi-domain studies integrating bacterial, fungal, viral, and host multi-omics data will be critical for defining the ecological mechanisms that govern disease initiation, progression, and therapeutic response.

5.Cross-Study Comparative Synthesis: Convergence, Divergence, and Design-Dependent Signals

Comparison across pediatric microbiome studies reveals several recurring patterns that extend beyond individual datasets. One of the most consistent determinants of study outcomes is the sampling compartment. Mucosal biopsies consistently provide stronger disease discrimination than fecal samples in treatment-naïve Crohn's disease (CD), reflecting microbial communities located at the epithelial interface rather than within the intestinal lumen (Gevers et al., 2014). Likewise, integration of ileal transcriptomics with mucosal microbiome profiling has revealed coordinated host–microbe interactions that are considerably less apparent in stool-based analyses (Haberman et al., 2014). Together, these findings indicate that stool samples primarily capture broad ecological shifts, whereas mucosal sampling more directly reflects biological processes occurring at the host–microbial interface.

This interpretation is further supported by recent methodological evidence comparing mucosal washes, brushes, biopsies, and luminal samples. Martinez-Medina et al. demonstrated that mucus-associated microbial communities vary according to sampling approach and showed that mucosal washes and brushes recover higher proportions of bacterial DNA with reduced host DNA contamination compared with conventional biopsies while preserving comparable microbial diversity profiles (Martinez-Medina et al., 2025). These findings emphasize that sampling compartment represents a biologically meaningful source of variation rather than merely a technical consideration and likely contributes to some of the heterogeneity reported across pediatric microbiome studies.

Study design similarly influences interpretation of the pediatric microbiome. Most investigations remain cross-sectional and focus on newly diagnosed patients, limiting causal inference and making it difficult to distinguish microbial alterations that precede disease onset from those that develop as secondary consequences of intestinal inflammation (Ni et al., 2017). In contrast, longitudinal therapeutic studies, particularly those evaluating exclusive enteral nutrition (EEN), demonstrate dynamic restructuring of both microbial composition and functional capacity during clinical remission (Quince et al., 2015; Dunn et al., 2016; Diederen et al., 2020; Häcker et al., 2024). These observations indicate that pediatric dysbiosis is not a static phenomenon but retains substantial ecological plasticity. Importantly, treatment-associated restructuring does not necessarily represent restoration of a healthy microbiome; instead, it may reflect the establishment of alternative, partially stabilized ecological states.

Taken together, these observations illustrate the evolution of pediatric IBD microbiome research from descriptive taxonomic profiling toward mechanistic and systems-level understanding. Foundational 16S rRNA studies established reproducible compositional signatures of dysbiosis, whereas shotgun metagenomics and subsequent multi-omics approaches increasingly linked these alterations to microbial metabolism, host–microbe interactions, and developmental biology. Collectively, these converging lines of evidence support the developmental instability framework proposed in this review, which integrates microbial composition, functional remodelling, host genetics, and developmental immune–microbiome interactions into a unified conceptual model of pediatric IBD (Figure 1).

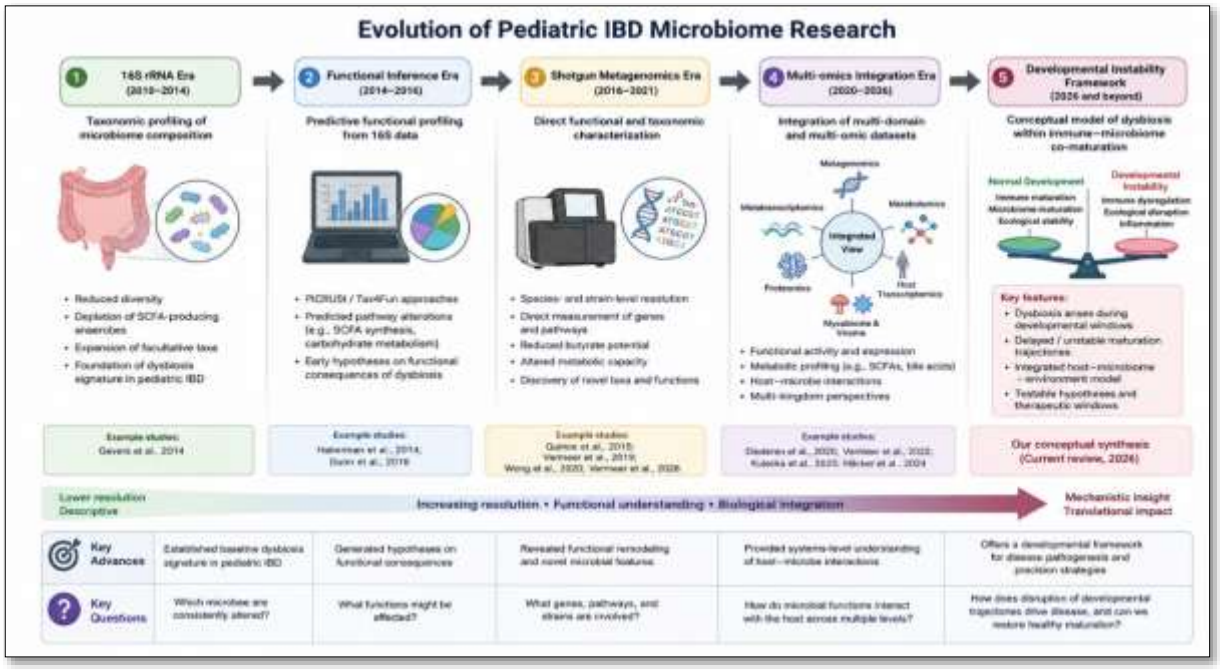

Figure 1. Evolution of pediatric IBD microbiome research from descriptive taxonomic profiling toward a developmental instability framework.

6.A Developmental Instability Framework for Pediatric IBD

When considered collectively, the compositional, functional, and ecological evidence presented throughout this review supports a conceptual model that extends beyond conventional descriptions of microbial dysbiosis. Rather than representing isolated alterations in microbial composition, pediatric inflammatory bowel disease (IBD) appears to reflect disruption of an immune–microbiome ecosystem undergoing active developmental maturation. We therefore propose a

developmental instability framework, in which inflammation emerges within an incompletely stabilized ecological system during childhood rather than from disruption of an already established adult microbial steady state (Figure 2). This framework integrates microbial composition, functional metabolism, host immunity, spatial organization, and genetic susceptibility into a unified biological model capable of explaining many of the reproducible observations reported across pediatric cohorts.

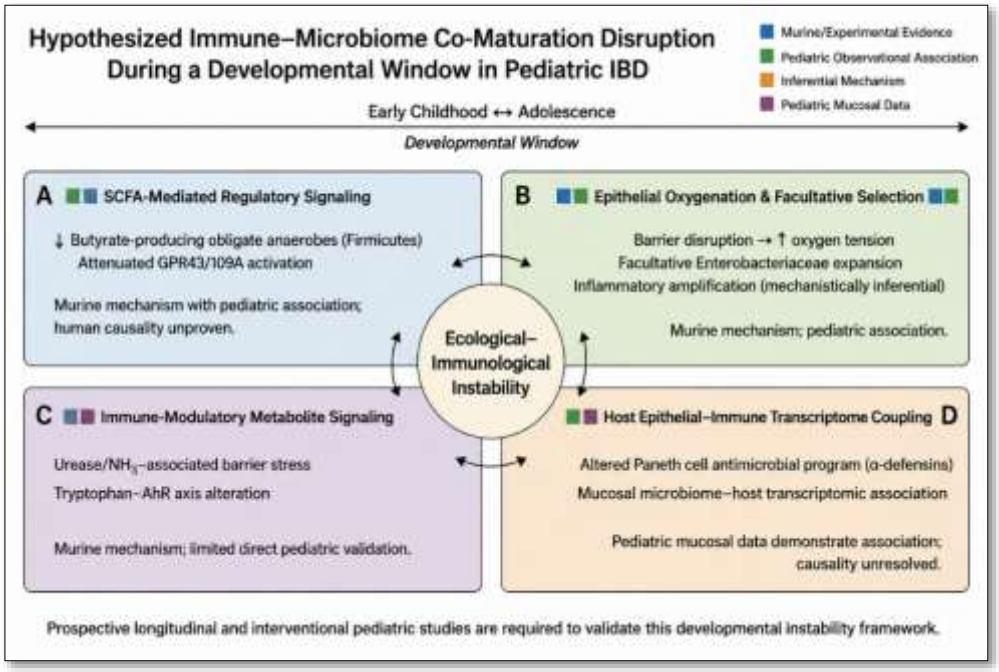

Figure 2. Proposed developmental instability framework illustrating disruption of immune–microbiome co-maturation in pediatric inflammatory bowel disease.

Unlike adults, children develop IBD during a period in which both the intestinal microbiome and the mucosal immune system continue to undergo coordinated maturation. Early-life microbial communities progressively diversify while simultaneously educating epithelial and immune regulatory networks responsible for maintaining intestinal homeostasis (Belkaid & Hand, 2014; Gensollen et al., 2016). Perturbations during this developmental window may therefore exert biological effects that extend beyond transient microbial imbalance.

Rather than simply reducing microbial diversity, dysbiosis may interrupt the normal establishment of immune tolerance, epithelial barrier function, and host–microbiome equilibrium, thereby increasing susceptibility to persistent intestinal inflammation. In this context, the biological consequences of microbial disruption may be greater during childhood than in adulthood because regulatory immune networks and ecological resilience have not yet fully stabilized.

Among the most consistent functional alterations identified in pediatric Crohn's disease is depletion of obligate anaerobic bacteria responsible for producing short-chain fatty acids (SCFAs), particularly butyrate. Experimental studies have demonstrated that SCFAs promote epithelial integrity, enhance regulatory T-cell differentiation, and contribute to mucosal immune homeostasis (Furusawa et al., 2013; Smith et al., 2013). Pediatric cohorts consistently demonstrate depletion of major butyrate-producing genera, including Faecalibacterium, Roseburia, and Blautia (Gevers et al., 2014; Kowalska-Duplaga et al., 2019), while shotgun metagenomic studies reveal reduced representation of butyrate-associated metabolic pathways during active disease (Quince et al., 2015).

Within a developmental context, these findings suggest that impaired microbial metabolism may influence immune calibration during a period when regulatory networks are still being established. Loss of butyrate-producing capacity may therefore have broader and more persistent consequences than a comparable perturbation occurring after immune and microbial maturation has been achieved.

Functional disruption extends beyond SCFA depletion to encompass multiple microbial metabolic pathways that regulate intestinal homeostasis. Altered bile acid transformation may impair FXR- and TGR5-mediated signalling involved in epithelial repair and inflammatory regulation, while disturbances in microbial tryptophan metabolism reduce production of aryl hydrocarbon receptor (AhR) ligands that normally support IL-22 secretion and mucosal barrier maintenance (Zelante et al., 2013; Lamas et al., 2016).

Alterations in nitrogen metabolism and other microbial metabolic networks further illustrate that pediatric dysbiosis represents coordinated functional remodelling rather than isolated taxonomic change. Microbial metabolites may therefore constitute a central mechanistic interface through which ecological disruption influences epithelial function and immune regulation during childhood.

Inflammation itself may further amplify developmental instability through reciprocal ecological feedback. Experimental models have shown that inflammatory increases in epithelial oxygen availability selectively favor facultative anaerobes, particularly members of the Enterobacteriaceae, thereby reinforcing microbial imbalance (Cevallos et al., 2019).

Consistent with this mechanism, treatment-naïve pediatric Crohn's disease cohorts reproducibly demonstrate expansion of Enterobacteriaceae together with depletion of obligate anaerobes (Gevers et al., 2014; Kowalska-Duplaga et al., 2019). The identification of adherent-invasive Escherichia coli (AIEC) strains in pediatric CD further suggests that inflammation not only alters community composition but also promotes expansion of bacterial populations possessing enhanced pathogenic potential (Conte et al., 2014). Oxygen-driven ecological restructuring and inflammatory activation may therefore form a self-reinforcing cycle in which microbial imbalance intensifies mucosal inflammation, and inflammation in turn creates ecological conditions that further destabilize the microbial community.

These ecological interactions are also influenced by the spatial organization of the intestinal microbiome. Mucosal profiling studies consistently demonstrate stronger host–microbe coupling than stool-based analyses, emphasizing that microbial communities residing at the epithelial surface more directly reflect disease-associated biological processes. Integration of ileal transcriptomics with mucosal microbiome profiling revealed coordinated epithelial antimicrobial dysfunction and microbial imbalance in pediatric Crohn's disease (Haberman et al., 2014).

Subsequent work demonstrated age-dependent immune intensification despite broadly similar dysbiotic microbial profiles (Haberman et al., 2019). Together with recent methodological comparisons of mucosal sampling strategies (Martinez-Medina et al., 2025), these findings indicate that the biological consequences of dysbiosis depend not only on microbial composition but also on developmental timing and spatial proximity to the intestinal epithelium (Conrad et al., 2024). Pediatric IBD may therefore represent a failure of immune–microbiome co-stabilization occurring during active developmental change rather than a simple alteration in luminal microbial composition.

This developmental instability does not occur independently of host genetics. Several major IBD susceptibility genes influence microbial ecology through distinct but interconnected biological mechanisms. NOD2, the first and most consistently replicated Crohn's disease susceptibility gene, regulates bacterial sensing and Paneth cell antimicrobial peptide production, with loss-of-function variants associated with reduced microbial diversity and altered mucosal community composition (Uniken Venema et al., 2017).

ATG16L1 links autophagy to bacterial handling and epithelial antimicrobial function, complementing the epithelial abnormalities observed in pediatric ileal disease (Liu et al., 2022). IL23R influences Th17- and IL-22-mediated barrier responses, whereas CARD9 directly affects microbial conversion of tryptophan into AhR ligands, providing one of the clearest examples of a genotype–microbiome–metabolite–immune axis in IBD (Lamas et al., 2016). FUT2 secretor status further modifies the mucosal glycan environment available to commensal bacteria, thereby influencing the composition of mucosa-associated microbial communities (Rausch et al., 2011).

These observations demonstrate that host genetics shape not only immune responsiveness but also the ecological landscape within which microbial communities develop and function. The developmental instability framework therefore positions genotype as an active determinant of ecological resilience rather than as a background susceptibility factor operating independently of the microbiome.

The influence of these susceptibility loci varies substantially across populations, providing a strong biological rationale for population-specific pediatric microbiome studies. Saudi Arabian investigations have reported that several NOD2 and IL23R variants identified in European genome-wide association studies show limited association with pediatric IBD in Saudi cohorts (Alharbi et al., 2022). Additional computational and structural analyses suggest that the biological consequences of these variants may differ according to population-specific genetic architecture (Nasser & Shinawi, 2023). Similar observations have been reported in neighboring Middle Eastern populations (Siddique et al., 2021), indicating that both host genetic susceptibility and microbiome composition are influenced by ancestry. Genotype–microbiome interactions characterized predominantly in European and North American cohorts therefore cannot be assumed to apply universally.

This limitation is particularly relevant for Saudi Arabia, where patterns of genetic diversity, consanguinity, diet, and environmental exposure differ from those of the populations underpinning many foundational pediatric microbiome studies (El-Sayed et al., 2024). Population-specific investigations integrating shotgun metagenomics with host genomic profiling are therefore essential for determining whether developmental instability follows shared biological pathways across populations or is modified by ancestry-specific genetic and ecological contexts.

Overall, the evidence reviewed here supports a shift from viewing pediatric IBD as a disorder of isolated microbial dysbiosis toward understanding it as a disease of immune–microbiome developmental instability. Within this framework, microbial composition, metabolic function, host immunity, spatial microbial organization, and genetic susceptibility interact continuously throughout childhood to determine ecological resilience or vulnerability.

Rather than emerging from disruption of a mature microbial ecosystem, pediatric IBD may arise because this ecosystem fails to achieve stable co-maturation with the developing immune system. The framework integrates findings from taxonomic, metagenomic, metabolomic, and host molecular studies while also generating testable hypotheses regarding disease mechanisms, age-dependent therapeutic responsiveness, and optimal windows for microbiome-targeted intervention.

Future longitudinal, compartment-resolved, age-stratified, and genotype-informed multi-omics studies will be essential to determine whether restoration of normal immune–microbiome developmental trajectories can modify disease progression and ultimately enable precision microbiome-based therapeutic strategies for pediatric IBD.

7.Limitations and Evidence Gaps

While the developmental instability framework provides a biologically plausible interpretation of the current evidence, several important limitations continue to constrain mechanistic inference and cross-study comparability. First, most pediatric IBD cohorts remain modest in size compared with large adult multicentre studies, limiting statistical power for

analyses stratified by age, disease location, clinical phenotype, and treatment history. Second, substantial heterogeneity in sampling strategies—including stool versus mucosal specimens, anatomical site, and timing relative to diagnosis or therapy—as well as differences in laboratory protocols and bioinformatic pipelines reduce reproducibility and complicate comparisons across studies. Third, cross-sectional case–control designs continue to dominate the literature, limiting causal inference and making it difficult to distinguish microbial alterations that precede disease onset from those that arise secondary to intestinal inflammation.

Additional knowledge gaps remain within specific pediatric disease subgroups. Pediatric ulcerative colitis (UC) and very early-onset IBD (VEO-IBD) are considerably underrepresented, particularly in shotgun metagenomic investigations, resulting in weaker functional resolution and less consistent taxonomic characterization than is available for Crohn's disease (CD). Likewise, multi-kingdom profiling of the intestinal mycobiome and virome remains relatively limited and is frequently underpowered, restricting interpretation of cross-domain ecological interactions and their contribution to disease pathogenesis.

Another important limitation is the limited population diversity represented in current pediatric microbiome research. Most microbiome and host-genetic cohorts, including the majority of the foundational studies summarized in Table 1, have been conducted in European and North American populations. However, genetic association studies from Saudi Arabia and neighboring Arab populations suggest that several IBD susceptibility variants identified in European cohorts do not consistently replicate in these populations (Alharbi et al., 2022; Siddique et al., 2021). Consequently, the extent to which currently recognized taxonomic signatures, functional pathways, and host–microbiome interactions can be generalized across genetically distinct populations remains largely unknown.

Finally, many functional conclusions continue to rely on inference from microbial gene and pathway abundance rather than direct biological validation through paired metabolomics, host transcriptomics, proteomics, or experimental models. As a result, several mechanistic pathways proposed to contribute to pediatric IBD remain insufficiently validated within pediatric tissue contexts.

Addressing these limitations will require well-powered longitudinal studies that integrate compartment-resolved sampling, host genomics, shotgun metagenomics, complementary multi-omics, and experimental validation. Such approaches will be essential for distinguishing causal mechanisms from secondary inflammatory effects, refining the proposed developmental instability framework, and advancing precision microbiome-based therapeutic strategies for pediatric IBD.

8.CONCLUSION AND FUTURE DIRECTIONS

Evidence from treatment-naïve 16S rRNA studies and emerging pediatric shotgun metagenomic investigations supports the existence of a reproducible dysbiosis signature in pediatric Crohn's disease (CD), characterized by reduced microbial diversity, depletion of obligate anaerobic short-chain fatty acid (SCFA)-producing communities, and expansion of facultative taxa. Viewed collectively across independent cohorts, sampling compartments, and analytical platforms, these findings suggest that pediatric inflammatory bowel disease (IBD) is not simply a disorder of altered microbial composition but rather reflects ecological instability arising during immune–microbiome co-maturation.

The developmental instability framework proposed in this review provides a conceptual model that integrates microbial composition, functional remodelling, metabolic perturbation, host immunity, and genetic susceptibility within the unique biological context of childhood. Although current evidence remains insufficient to establish causality, the convergence of taxonomic, functional, experimental, and host-derived molecular findings supports the hypothesis that disruption of developmental ecological trajectories contributes to pediatric IBD pathogenesis.

Future progress will require well-powered longitudinal, age-stratified, compartment-resolved multi-omics studies supported by standardized analytical pipelines capable of tracking microbiome development throughout childhood. Priorities include strengthening mechanistic validation through paired metabolomics and host tissue profiling, expanding shotgun metagenomic investigations in pediatric ulcerative colitis (UC) and very early-onset IBD (VEO-IBD), and incorporating multi-kingdom approaches that simultaneously characterize bacterial, fungal, and viral communities. In addition, emerging long-read sequencing technologies may improve strain-level resolution, genome reconstruction, and characterization of mobile genetic elements, providing new opportunities to refine our understanding of host–microbiome interactions. Such strain-resolved analyses may help determine whether disease-associated microbial signatures primarily reflect broad taxonomic shifts or expansion of specific pathogenic lineages, including adherent-invasive Escherichia coli (AIEC) and other functionally important microorganisms.

Beyond technological advances, the developmental instability framework also generates several testable biological hypotheses. First, children who subsequently develop IBD may exhibit delayed, divergent, or unstable microbiome maturation trajectories before clinical disease onset. Second, age-dependent differences in immune–microbiome interactions may influence disease phenotype, progression, and therapeutic responsiveness, indicating that pediatric IBD should not be regarded as a biologically uniform condition across developmental stages. Third, interventions capable of restoring normal developmental ecological trajectories may prove more effective than strategies focused solely on suppressing inflammation. Testing these hypotheses will require prospective pediatric cohorts integrating longitudinal microbiome profiling, host immune phenotyping, environmental exposure assessment, and repeated sampling across critical developmental windows.

Continued integration of metagenomics, metabolomics, transcriptomics, epigenomics, and host genomic data will further accelerate the development of precision microbiome medicine for pediatric IBD. Future therapeutic strategies may move beyond broad anti-inflammatory approaches toward individualized interventions guided by microbial functional capacity, ecological maturity, and host–microbiome interaction profiles. Such approaches may ultimately identify critical therapeutic windows during immune maturation when restoration of microbial ecosystem stability is most achievable.

Ultimately, one of the most important unanswered questions in pediatric IBD research is whether restoration of normal immune–microbiome developmental trajectories can alter disease course. Addressing this challenge will require moving beyond descriptive characterization of dysbiosis toward a mechanistic understanding of how microbial ecosystem development, immune maturation, host genetics, and environmental exposures interact to shape disease susceptibility and progression throughout childhood. If successful, this transition will not only refine our understanding of pediatric IBD biology but also establish the foundation for developmentally informed, precision microbiome-based therapeutic strategies.

REFERENCES

1. Alharbi, R. S., Shaik, N. A., Almahdi, H., ElSokary, H. A., Jamalalail, B. A., Mosli, M. H., Alsufyani, H. A., Al-Aama, J. Y., Elango, R., Saadah, O. I., & Banaganapalli, B. (2022). Genetic association study of NOD2 and IL23R amino acid substitution polymorphisms in Saudi inflammatory bowel disease patients. Journal of King Saud University – Science, 34(1), 101726.
2. Bäckhed, F., Roswall, J., Peng, Y., Feng, Q., Jia, H., Kovatcheva-Datchary, P., Arora, T., Hallen, A., ... Wang, J. (2015). Dynamics and stabilization of the human gut microbiome during the first year of life. Cell Host & Microbe, 17(5), 690–703.
3. Beghini, F., McIver, L. J., Blanco-Míguez, A., Dubois, L., Asnicar, F., Maharjan, S., Mailyan, A., Manghi, P., Scholz, M., Thomas, A. M., Valles-Colomer, M., Weingart, G., Zhang, Y., Zolfo, M., Huttenhower, C., Franzosa, E. A., & Segata, N. (2021). Integrating taxonomic, functional, and strain-level profiling of diverse microbial communities with bioBakery 3. eLife, 10, e65088.
4. Belkaid, Y., & Hand, T. W. (2014). Role of the microbiota in immunity and inflammation. Cell, 157(1), 121–141.
5. Caspi, R., Billington, R., Keseler, I. M., Kothari, A., Krummenacker, M., Midford, P. E., Ong, W. K., Paley, S., Subhraveti, P., & Karp, P. D. (2020). The MetaCyc database of metabolic pathways and enzymes – a 2019 update. Nucleic Acids Research, 48(D1), D445–D453.
6. Cevallos, S. A., Lee, J.-Y., Velazquez, E. M., Foegeding, N. J., Shelton, C. D., Tiffany, C. R., ... Winter, S. E. (2019). Increased epithelial oxygenation links colitis to an expansion of tumorigenic Enterobacteriaceae. mBio, 10(5), e02244-19.
7. Conrad, M. A., Bittinger, K., Ren, Y., Kachelries, K., Vales, J., Li, H., Wu, G. D., Bushman, F. D., Devoto, M., Baldassano, R. N., & Kelsen, J. R. (2024). The intestinal microbiome of inflammatory bowel disease across the pediatric age range. Gut Microbes, 16(1), 2317932.
8. Conte, M. P., Schippa, S., Zamboni, I., Penta, M., Chiarini, F., Seganti, L., ... Longhi, C. (2014). Adherent-invasive Escherichia coli (AIEC) in pediatric Crohn's disease patients: Phenotypic and genetic pathogenic features. BMC Research Notes, 7, 748.
9. de Meij, T. G. J., de Groot, E. F. J., Peeters, C. F. W., Boot, F., & Budding, A. E. (2018). Microbiota composition of treatment-naïve pediatric inflammatory bowel disease patients. Gut Microbes, 9(6), 509–521.
10. Diederen, K., Li, J. V., Donachie, G. E., de Waart, D. R., Hakvoort, T. B. M., Kindermann, A., Wagner, J., de Groot, P. F., van Bodegraven, A. A., de Meij, T. G. J., & van Goudoever, J. B. (2020). Exclusive enteral nutrition mediates gut microbial and metabolic changes associated with remission in children with Crohn's disease. Scientific Reports, 10, 18879.
11. Dunn, K. A., Moore-Connors, J., MacIntyre, B., Stadnyk, A. W., Thomas, N. A., Noble, A., Mahdi, G., Rashid, M., Otley, A. R., Bielawski, J. P., & Van Limbergen, J. (2016). Early changes in microbial community structure are associated with sustained remission after nutritional treatment of pediatric Crohn's disease. Inflammatory Bowel Diseases, 22(12), 2853–2862.
12. El Mouzan, M., Al Mofarreh, M., Alsaleem, B., Al Sarkhy, A., Alanazi, A., Khormi, M., Almasoud, A., & Assiri, A. (2025a). Bacterial dysbiosis in newly diagnosed treatment-naïve pediatric ulcerative colitis in Saudi Arabia. Saudi Journal of Gastroenterology, 31(1), 14–21.
13. El Mouzan, M., Savidge, T. C., Al Sarkhy, A., Badu, S., Alsaleem, B., Al Mofarreh, M., Almasood, A., & Assiri, A. (2025b). Gut virome profile in new-onset treatment-naïve Saudi children with ulcerative colitis. Saudi Journal of Gastroenterology, 31(4), 212–218.
14. El-Sayed, A., Kapila, D., Taha, R. S. I., El-Sayed, S., Mahen, M. R. A., Taha, R., & Alrubaiy, L. (2024). The role of the gut microbiome in inflammatory bowel disease: The Middle East perspective. Journal of Personalized Medicine, 14(6), 652.
15. Franzosa, E. A., Sirota-Madi, A., Avila-Pacheco, J., Fornelos, N., Haiser, H. J., Reinker, S., Vatanen, T., Hall, A. B., Mallick, H., McIver, L. J., Sauk, J. S., Wilson, R. G., Stevens, B. W., Scott, J. M., Pierce, K., Deik, A. A., Bullock, K., Imhann, F., Porter, J. A., ... Huttenhower, C. (2019). Gut microbiome structure and metabolic activity in inflammatory bowel disease. Nature Microbiology, 4(2), 293–305.
16. Furusawa, Y., Obata, Y., Fukuda, S., Endo, T. A., Nakato, G., Takahashi, D., ... Ohno, H. (2013). Commensal microbe-derived butyrate induces differentiation of colonic regulatory T cells. Nature, 504(7480), 446–450.
17. Gensollen, T., Iyer, S. S., Kasper, D. L., & Blumberg, R. S. (2016). How colonization by microbiota in early life shapes the immune system. Science, 352(6285), 539–544.
18. Gerasimidis, K., Nichols, B., Papadopoulou, R., Ijaz, U., Rajendran, R., Quince, C., Russell, R. K., Hansen, R., & Ramage, G. (2023). The faecal bacterial and fungal microbiome of newly diagnosed treatment-naïve children with Crohn's disease and the modifying effects of exclusive enteral nutrition. Journal of Crohn's and Colitis, 17(Suppl. 1), i122–i124.
19. Gevers, D., Kugathasan, S., Denson, L. A., Vázquez-Baeza, Y., Van Treuren, W., Ren, B., ... Xavier, R. J. (2014). The treatment-naive microbiome in new-onset Crohn's disease. Cell Host & Microbe, 15(3), 382–392.

20. Haberman, Y., Tickle, T. L., Dexheimer, P. J., Kim, M.-O., Tang, D., Karns, R., Baldassano, R. N., Noe, J. D., Rosh, J., Markowitz, J., Heyman, M. B., Griffiths, A. M., Crandall, W. V., Mack, D. R., Baker, S. S., Huttenhower, C., Keljo, D. J., Hyams, J. S., Kugathasan, S., Walters, T. D., Aronow, B., Xavier, R. J., Gevers, D., & Denson, L. A. (2014). Pediatric Crohn disease patients exhibit specific ileal transcriptome and microbiome signature. Journal of Clinical Investigation, 124(8), 3617–3633.
21. Haberman, Y., Schirmer, M., Dexheimer, P. J., Karns, R., Braun, T., Kim, M.-O., Walters, T. D., Baldassano, R. N., Noe, J. D., Rosh, J., Markowitz, J., Crandall, W. V., Mack, D. R., Griffiths, A. M., Heyman, M. B., Baker, S. S., Kellermayer, R., Moulton, D. E., Patel, A. S., Gulati, A. S., Steiner, S. J., LeLeiko, N., Otley, A., Oliva-Hemker, M., Ziring, D., Kirschner, B. S., Keljo, D. J., Guthery, S. L., Cohen, S. A., Snapper, S., Evans, J., Dubinsky, M., Aronow, B., Hyams, J. S., Kugathasan, S., Huttenhower, C., Xavier, R. J., & Denson, L. A. (2019). Age-of-diagnosis dependent ileal immune intensification and reduced alpha-defensin in older versus younger pediatric Crohn disease patients despite already established dysbiosis. Mucosal Immunology, 12(2), 491–502.
22. Häcker, D., Siebert, K., Smith, B. J., Köhler, N., Riva, A., Mahapatra, A., ... Haller, D. (2024). Exclusive enteral nutrition initiates individual protective microbiome changes to induce remission in pediatric Crohn's disease. Cell Host & Microbe, 32(11), 2019–2034.e8.
23. Heinken, A., Hertel, J., & Thiele, I. (2021). Metabolic modelling reveals broad changes in gut microbial metabolism in IBD patients with dysbiosis. NPJ Systems Biology and Applications, 7(1), 19.
24. Jagt, J. Z., Struys, E. A., Ayada, I., Bakkali, A., Jansen, E. E. W., Claesen, J., van Limbergen, J. E., Benninga, M. A., de Boer, N. K. H., & de Meij, T. G. J. (2022). Fecal amino acid analysis in newly diagnosed pediatric inflammatory bowel disease: A multicenter case-control study. Inflammatory Bowel Diseases, 28(5), 755–763.
25. Korpela, K., Salonen, A., Virta, L. J., Kekkonen, R. A., de Vos, W. M., & Kalliomäki, M. (2016). Intestinal microbiome is related to lifetime antibiotic use in Finnish pre-school children. Nature Communications, 7, 10410.
26. Kowalska-Duplaga, K., Gosiewski, T., Kapusta, P., Sroka-Oleksiak, A., Wędrychowicz, A., Pieczarkowski, S., ... Fyderek, K. (2019). Differences in the intestinal microbiome of healthy children and patients with newly diagnosed Crohn's disease. Scientific Reports, 9, 18880.
27. Kuenzig, M. E., Lee, S. M., El-Matary, W., Kaplan, G. G., & Benchimol, E. I. (2022). Increasing incidence of pediatric inflammatory bowel disease in Canada. Gastroenterology, 162(4), 1140–1151.
28. Kulecka, M., O'Sullivan, J., Fitzgerald, R., Velikonja, A., Huseyin, C. E., Laserna-Mendieta, E. J., Ruiz-Limón, P., Eckenberger, J., Vidal-Marín, M., Truppel, B. A., Singh, R., Naik, S., Croft, N. M., Temko, A., Zomer, A., MacSharry, J., Melgar, S., Deb, P., Sanderson, I. R., & Claesson, M. J. (2025). Combining mucosal microbiome and host multi-omics data shows prognostic potential in paediatric ulcerative colitis. Nature Communications, 16(1), 7157.
29. Lamas, B., Richard, M. L., Leducq, V., Pham, H. P., Michel, M. L., Da Costa, G., ... Sokol, H. (2016). CARD9 impacts colitis by altering gut microbiota metabolism of tryptophan into aryl hydrocarbon receptor ligands. Nature Medicine, 22(6), 598–605.
30. Levine, A., Griffiths, A., Markowitz, J., Wilson, D. C., Turner, D., Russell, R. K., ... Ruemmele, F. M. (2011). Pediatric modification of the Montreal classification for inflammatory bowel disease: The Paris classification. Inflammatory Bowel Diseases, 17(6), 1314–1321.
31. Liu, H., Gao, P., Jia, B., Lu, N., Zhu, B., & Zhang, F. (2022). IBD-associated Atg16L1 T300A polymorphism regulates commensal microbiota of the intestine. Frontiers in Immunology, 12, 772189.
32. Lloyd-Price, J., Arze, C., Ananthakrishnan, A. N., Schirmer, M., Avila-Pacheco, J., Poon, T. W., ... Xavier, R. J. (2019). Multi-omics of the gut microbial ecosystem in inflammatory bowel disease. Nature, 569(7758), 655–662.
33. Lv, Y., Zhen, C., Liu, A., Hu, Y., Yang, G., Xu, C., Lou, Y., Cheng, Q., Luo, Y., Yu, J., Fang, Y., Zhao, H., Peng, K., Yu, Y., Lou, J., Chen, J., & Ni, Y. (2024). Profiles and interactions of gut microbiome and intestinal microRNAs in pediatric Crohn's disease. mSystems, 9(9), e00783-24.
34. Martinez-Medina, J. N., Ghazisaeedi, F., Kramer, C., Ziegler, J. F., McParland, V., Mönch, P. W., Siegmund, B., Jarquín-Díaz, V. H., Fulde, M., & Forslund-Startceva, S. K. (2025). Mucosal washes are useful for sampling intestinal mucus-associated microbiota despite low biomass. Gut Microbes, 17(1), 2464296.
35. Mottawea, W., Chiang, C.-K., Mühlbauer, M., Starr, A. E., Butcher, J., Abujamel, T., Deeke, S. A., Brandel, A., Zhou, H., Shokralla, S., Hajibabaei, M., Singleton, R., Benchimol, E. I., Jobin, C., Mack, D. R., Figeys, D., & Stintzi, A. (2016). Altered intestinal microbiota–host mitochondria crosstalk in new onset Crohn's disease. Nature Communications, 7, 13419.
36. Moutusy, S. I., & Ohsako, S. (2024). Gut microbiome-related anti-inflammatory effects of aryl hydrocarbon receptor activation on inflammatory bowel disease. International Journal of Molecular Sciences, 25(6), 3372.
37. Mukhopadhya, I., Hansen, R., El-Omar, E. M., & Hold, G. L. (2015). The fungal microbiota of de-novo paediatric inflammatory bowel disease. Microbes and Infection, 17(4), 304–310.
38. Nasser, K. K., & Shinawi, T. (2023). Genotype-protein phenotype characterization of NOD2 and IL23R missense variants associated with inflammatory bowel disease: A paradigm from molecular modelling, dynamics, and docking simulations. Frontiers in Medicine, 9, 1090120.
39. Ng, S. C., Shi, H. Y., Hamidi, N., Underwood, F. E., Tang, W., Benchimol, E. I., ... Kaplan, G. G. (2017). Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century. The Lancet, 390(10114), 2769–2778.
40. Ning, L., Zhou, Y.-L., Sun, H., Zhang, Y., Shen, C., Wang, Z., Xuan, B., Zhao, Y., Ma, Y., Yan, Y., Tong, T., Huang, X., Hu, M., Zhu, X., Ding, J., Zhang, Y., Cui, Z., Fang, J.-Y., Chen, H., & Hong, J. (2023). Microbiome and metabolome features in inflammatory bowel disease via multi-omics integration analyses across cohorts. Nature Communications, 14, 7135.

41. Peterson, D., Weidenmaier, C., Timberlake, S., & Gura Sadovsky, R. (2025). Depletion of key gut bacteria predicts disrupted bile acid metabolism in inflammatory bowel disease. Microbiology Spectrum, 13(2), e0199924.
42. Quince, C., Ijaz, U. Z., Loman, N., Eren, A. M., Saulnier, D., Russell, J., ... Segata, N. (2015). Extensive modulation of the fecal metagenome in children with Crohn's disease during exclusive enteral nutrition. The American Journal of Gastroenterology, 110(12), 1718–1729.
43. Quince, C., Walker, A. W., Simpson, J. T., Loman, N. J., & Segata, N. (2017). Shotgun metagenomics, from sampling to analysis. Nature Biotechnology, 35(9), 833–844.
44. Rausch, P., Rehman, A., Künzel, S., Häsler, R., Ott, S. J., Schreiber, S., Rosenstiel, P., Franke, A., & Baines, J. F. (2011). Colonic mucosa-associated microbiota is influenced by an interaction of Crohn disease and FUT2 (Secretor) genotype. Proceedings of the National Academy of Sciences, 108(47), 19030–19035.
45. Ruemmele, F. M., Veres, G., Kolho, K. L., Griffiths, A., Levine, A., Escher, J. C., ... Turner, D. (2014). Consensus guidelines of ECCO/ESPGHAN on the medical management of pediatric Crohn's disease. Journal of Crohn's and Colitis, 8(10), 1179–1207.
46. Serrano-Gómez, G., Yañez, F., Soler, Z., Pons-Tarín, M., Mayorga, L., Herrera-deGuise, C., Borruel, N., Rodriguez-Sinovas, A., Consegal, M., Manjón, I., Vega-Abellaneda, S., & Manichanh, C. (2025). Microbiome multi-omics analysis reveals novel biomarkers and mechanisms linked with CD etiopathology. Biomarker Research, 13, 85.
47. Shaw, K. A., Bertha, M., Hofmekler, T., Chopra, P., Vatanen, T., Srivatsa, A., ... Kugathasan, S. (2016). Dysbiosis, inflammation, and response to treatment. Genome Medicine, 8, 75.
48. Siddique, I., Mustafa, A. S., Khan, I., Ziyab, A. H., Altarrah, M., Sulaiman, R., Kadungothayil, N., & Shaheed, F. (2021). Detection of mutations in NOD2/CARD15 gene in Arab patients with Crohn's disease. Saudi Journal of Gastroenterology, 27(4), 240–248.
49. Smith, P. M., Howitt, M. R., Panikov, N., Michaud, M., Gallini, C. A., Bohlooly-Y, M., ... Garrett, W. S. (2013). The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. Science, 341(6145), 569–573.
50. Sokol, H., Pigneur, B., Watterlot, L., Lakhdari, O., Bermudez-Humaran, L. G., Gratadoux, J.-J., Blugeon, S., Bridonneau, C., Furet, J.-P., Corthier, G., Grangette, C., Vasquez, N., Pochart, P., Trugnan, G., Thomas, G., Blottiere, H. M., Doré, J., Marteau, P., Seksik, P., & Langella, P. (2008). Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. Proceedings of the National Academy of Sciences, 105(43), 16731–16736.
51. Uniken Venema, W. T., Voskuil, M. D., Dijkstra, G., Weersma, R. K., & Festen, E. A. (2017). The genetic background of inflammatory bowel disease: From correlation to causality. Journal of Pathology, 241(2), 146–158.
52. Vermeer, E., Prins, F. M., Hidding, I. J., Jagt, J. Z., de Jonge, R., Benninga, M. A., ... de Meij, T. G. J. (2026). Metagenomic sequencing reveals distinct gut microbiome profiles. Inflammatory Bowel Diseases, 32(2), 207–219.
53. Vich Vila, A., Hu, S., Andreu-Sánchez, S., Collij, V., Jansen, B. H., Augustijn, H. E., Bolte, L. A., Ruigrok, R. A. A. A., Abu-Ali, G., Giallourakis, C., Schneider, J., Parkinson, J., Al-Garawi, A., Zhernakova, A., Gacesa, R., Fu, J., & Weersma, R. K. (2023). Faecal metabolome and its determinants in inflammatory bowel disease. Gut, 72(8), 1472–1485.
54. Wagner, J., Maksimovic, J., Farries, G., Sim, W. H., Bishop, R. F., Cameron, D. J. S., & Catto-Smith, A. G. (2013). Bacteriophages in gut samples from pediatric Crohn's disease patients. Inflammatory Bowel Diseases, 19(8), 1598–1608.
55. Yatsunenko, T., Rey, F. E., Manary, M. J., Trehan, I., Dominguez-Bello, M. G., Contreras, M., ... Gordon, J. I. (2012). Human gut microbiome viewed across age and geography. Nature, 486(7402), 222–227.
56. Zelante, T., Iannitti, R. G., Cunha, C., De Luca, A., Giovannini, G., Pieraccini, G., ... Romani, L. (2013). Tryptophan catabolites from microbiota engage aryl hydrocarbon receptor. Nature, 494(7438), 361–365.
57. Zheng, M., Han, R., Yuan, Y., Xing, Y., Zhang, W., Sun, Z., Liu, Y., Li, J., & Mao, T. (2023). The role of Akkermansia muciniphila in inflammatory bowel disease: Current knowledge and perspectives. Frontiers in Immunology, 13, 1089600.