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Correlation between Gut Microbiota Composition and Frequency of Recurrent Migraines in Pediatric Patients

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Abstract

Recurrent migraines in pediatric populations constitute a significant neurological burden, affecting quality of life, academic performance, and social development. Despite extensive research, the precise pathophysiological mechanisms underlying pediatric migraine remain incompletely understood. Recent advances suggest that the gut microbiota may play a crucial modulatory role in the gut-brain axis, influencing neuroinflammatory pathways, neurotransmitter metabolism, and immune system activity, all of which are implicated in migraine pathogenesis. This study investigates the correlation between gut microbiota composition and the frequency of recurrent migraines in children, aiming to identify potential microbial biomarkers predictive of migraine susceptibility and severity. A cross-sectional analysis was conducted involving pediatric patients diagnosed with recurrent migraines, alongside age- and sex-matched healthy controls. Fecal samples were collected and subjected to 16S rRNA gene sequencing to profile microbial communities. Diversity indices, principal coordinate analysis, and relative abundance assessments were applied to elucidate differences in microbiota composition. Additionally, targeted metabolomic profiling of tryptophan-derived metabolites and short-chain fatty acids was performed to explore mechanistic links between microbial metabolites and migraine frequency. Statistical correlation analyses were conducted to examine associations between microbial taxa, metabolic profiles, and clinical parameters, including migraine attack frequency, duration, and intensity. Findings revealed significant alterations in gut microbiota composition among pediatric migraine patients, characterized by reduced microbial diversity and shifts in key bacterial genera involved in neurotransmitter metabolism and immune modulation. Specific microbial signatures correlated positively with migraine frequency, suggesting potential predictive value. Metabolomic analyses indicated that fluctuations in tryptophan metabolites and short-chain fatty acids may mediate gut-brain interactions contributing to migraine susceptibility. These results support a mechanistic link between the gut microbiota and pediatric migraine pathophysiology, highlighting the potential for microbiome-based diagnostic markers and therapeutic interventions, such as dietary modulation or probiotic supplementation. In conclusion, the study provides evidence that alterations in gut microbiota composition are closely associated with the frequency of recurrent migraines in children. Understanding the microbiota-migraine relationship could pave the way for novel strategies to prevent and manage pediatric migraine, emphasizing the role of the gut-brain axis in neurodevelopmental health.

Keywords: Pediatric migraine, Gut microbiota, Gut-brain axis, Tryptophan metabolites, Microbial biomarkers

Introduction

Migraine is a prevalent neurological disorder that affects a substantial proportion of the pediatric population worldwide, with significant implications for cognitive, emotional, and social development [14]. Pediatric migraines often present differently than adult-onset migraines, including shorter attack duration, variable localization of pain, and increased prevalence of associated gastrointestinal symptoms. These clinical differences suggest that the underlying pathophysiological mechanisms may involve unique developmental and environmental factors [2,18]. Despite decades of research, the exact etiology of recurrent migraines in children remains incompletely understood, limiting the effectiveness of targeted therapeutic interventions.

Emerging evidence highlights the critical role of the gut-brain axis in modulating neurological function, with the gut microbiota acting as a central regulator of neuroimmune interactions, neurotransmitter synthesis, and

inflammatory responses [17,16]. Alterations in gut microbial composition, termed dysbiosis, have been increasingly linked to neurodevelopmental and neuroinflammatory conditions, including migraine [1,10]. The bidirectional communication between the gastrointestinal tract and central nervous system involves neural, endocrine, and immune pathways, suggesting that gut microbiota may influence both the frequency and severity of migraine attacks [7,17].

Recent pediatric studies have reported differences in the abundance and diversity of specific gut bacterial taxa between migraine-affected children and healthy peers, implicating microbial metabolites such as short-chain fatty acids and tryptophan derivatives in migraine pathophysiology [2,4]. These metabolites can modulate neuroinflammatory signaling, vascular tone, and cortical excitability, providing a mechanistic link between gut microbial composition and migraine susceptibility [16,7]. The identification of microbial biomarkers associated with pediatric migraine could facilitate early diagnosis, risk stratification, and the development of microbiome-targeted interventions, including probiotics, prebiotics, and dietary modifications [9,15].

Moreover, chronic migraine in children is frequently associated with comorbidities such as irritable bowel syndrome (IBS), anxiety, and sleep disturbances, all of which may be influenced by gut microbial profiles [3,7]. Understanding the role of the gut microbiota in these complex interactions is essential for elucidating the multifactorial nature of pediatric migraine and may open avenues for personalized medicine approaches [5,6].

Recent investigations into pediatric migraine have increasingly focused on the composition and diversity of gut microbiota. A 2023 cohort study analyzing fecal samples from children aged 7–18 years identified significant reductions in overall microbial diversity in migraine patients compared to healthy controls, with notable depletion in genera involved in neurotransmitter metabolism, including *Bifidobacterium* and *Faecalibacterium* [2]. Such shifts in microbial populations may affect the production of gamma-aminobutyric acid (GABA), serotonin, and short-chain fatty acids, which are known modulators of cortical excitability and neuroinflammatory processes [4,16].

Similarly, adult studies have reported altered microbial profiles in migraineurs with comorbid irritable bowel syndrome (IBS), suggesting a mechanistic role for gut-brain signaling in migraine pathophysiology [3]. These findings underscore the potential continuity between pediatric and adult microbiome-mediated migraine mechanisms, while also highlighting developmental considerations unique to children, such as age-dependent microbial maturation and diet-mediated modulation [18,1].

To summarize the current knowledge, Table 1 presents an overview of recent pediatric studies examining gut microbiota composition in migraine patients.

Table 1. Summary of recent pediatric studies on gut microbiota in migraine patients

Study	Sample Size	Age Range (years)	Key Microbial Findings	Clinical Correlation
Bai et al., 2023 [2]	75 migraine, 50 controls	7–18	↓ Bifidobacterium, ↓ Faecalibacterium, ↓ microbial diversity	Positive correlation with attack frequency
Liu et al., 2024 [4]	60 migraine	6–16	Altered tryptophan metabolite pathways	Increased migraine intensity
He et al., 2023 [5]	48 migraine	8–17	Dysbiosis in SCFA-producing bacteria	Associated with higher migraine recurrence
Miao et al., 2022 [8]	32 experimental (animal model)	N/A	Cephalic allodynia alters microbial composition	Supports mechanistic link to migraine
Prathiviraj et al., 2023 [9]	Review	Pediatric & adult	Dietary interventions modulate microbiota	Suggests potential therapeutic strategies

Analysis of these studies demonstrates a consistent pattern of reduced microbial diversity and altered functional capacity in children with recurrent migraines. While specific taxa vary between studies, the convergence on key metabolic pathways—particularly those involving tryptophan metabolism and short-chain fatty acid production—indicates a mechanistic relationship between gut microbiota and neuroinflammatory processes underlying migraine [7,17].

Moreover, dietary interventions, including probiotic supplementation and low-FODMAP diets, have shown preliminary efficacy in modulating gut microbiota composition and reducing migraine frequency, supporting a causal link between microbial profiles and clinical outcomes [9,15]. These findings collectively emphasize the

importance of integrating microbiome analyses into pediatric migraine research to identify predictive biomarkers and novel therapeutic targets.

The gut-brain axis represents a complex, bidirectional communication system linking the central nervous system with the enteric nervous system, immune pathways, and endocrine signaling [17,16]. Within this framework, gut microbiota exert profound effects on neurodevelopment and neuroinflammation through the production of metabolites, modulation of immune cells, and interaction with the hypothalamic-pituitary-adrenal (HPA) axis [10,16]. In pediatric populations, the gut microbiome is in dynamic maturation, making children particularly susceptible to perturbations that may influence neurological function [17].

Tryptophan metabolites, including serotonin precursors and kynurenine pathway intermediates, have emerged as key modulators of migraine pathophysiology. Alterations in microbial taxa involved in tryptophan metabolism can influence circulating levels of these metabolites, affecting neurotransmission, vascular tone, and nociceptive signaling in migraine-susceptible individuals [4,7]. Similarly, short-chain fatty acids (SCFAs), produced by bacterial fermentation of dietary fibers, modulate neuroinflammatory pathways and maintain blood-brain barrier integrity, suggesting a mechanistic link between microbial composition and cortical excitability [16,17].

Neuroimmune interactions further reinforce the role of gut microbiota in migraine. Dysbiosis can enhance intestinal permeability, leading to systemic exposure to microbial-derived molecules such as lipopolysaccharides (LPS) that activate innate immune pathways and promote neuroinflammation [10,7]. In pediatric migraine, increased pro-inflammatory cytokine activity has been correlated with attack frequency and severity, highlighting the relevance of microbiota-driven immune modulation [7,16]. Additionally, the gut-vascular axis, involving endothelial barrier regulation and systemic inflammatory responses, may contribute to the initiation and propagation of migraine episodes [10].

Collectively, these observations suggest a model in which gut microbiota influence pediatric migraine through three interconnected mechanisms: (1) modulation of neuroactive metabolites, including tryptophan derivatives and SCFAs; (2) regulation of systemic and central immune responses; and (3) interaction with neural and vascular signaling pathways that affect nociceptive processing [7,16,10]. This integrative perspective provides a foundation for understanding how environmental factors, diet, and microbial composition may converge to influence migraine susceptibility and recurrence in children.

Despite the growing body of evidence linking gut microbiota to migraine, several research gaps remain, particularly in pediatric populations. Most studies to date have focused on adult cohorts or small pediatric samples, limiting generalizability [1,2,4]. Additionally, there is considerable variability in microbial taxa reported across studies, reflecting differences in sequencing methodologies, diet, geographic location, and age-related microbiome maturation [2,5,8]. This heterogeneity underscores the need for systematic investigations that integrate microbial composition, functional metabolite profiling, and clinical migraine parameters.

Emerging research also highlights the potential role of dietary interventions and microbiome modulation in mitigating migraine symptoms [9,15]. For instance, probiotic supplementation and specific dietary regimens have demonstrated preliminary efficacy in reducing attack frequency and severity, suggesting that microbiota-targeted therapies could complement conventional pharmacological approaches [15,9]. However, the mechanistic underpinnings of these interventions remain incompletely understood, and few studies have rigorously correlated microbial profiles with migraine frequency in children.

In addition, comorbidities such as irritable bowel syndrome, sleep disturbances, and anxiety are common in pediatric migraine and may be mediated by gut microbiota [3,7,6]. Understanding the intricate interactions between microbial composition, metabolite production, immune activation, and neurovascular signaling is essential for elucidating the multifactorial etiology of pediatric migraine and for identifying potential therapeutic targets [10,16,17].

Research Objectives

Based on these gaps, the present study aims to:

1. Characterize the gut microbiota composition in pediatric patients with recurrent migraines compared to healthy controls.
2. Examine the relationship between specific microbial taxa and migraine frequency, severity, and associated symptoms.
3. Investigate the role of microbial metabolites, including tryptophan derivatives and short-chain fatty acids, in modulating migraine susceptibility.

4. Identify potential microbial biomarkers for early prediction and personalized intervention strategies in pediatric migraine management.

By addressing these objectives, the study seeks to provide mechanistic insights into the gut-brain interactions underlying pediatric migraine and to inform the development of microbiome-based diagnostic and therapeutic approaches.

Statement of the Problem

Pediatric migraine is a prevalent neurological disorder that imposes a substantial burden on children's health, academic performance, and psychosocial development. Despite advancements in pharmacological management and supportive therapies, a significant proportion of pediatric patients continue to experience recurrent migraine attacks, highlighting the limitations of current treatment strategies [14,2]. Conventional interventions primarily target symptom relief rather than addressing underlying pathophysiological mechanisms, resulting in variable efficacy and frequent recurrence.

Recent studies suggest that the gut microbiota plays a pivotal role in neuroimmune and neurovascular modulation, yet its precise relationship with pediatric migraine frequency remains poorly understood [1,4,7]. While several studies have documented dysbiosis and altered metabolite profiles in migraine patients, findings are heterogeneous, with few investigations focusing specifically on children [2,4,5]. Furthermore, the impact of microbial metabolites, including tryptophan derivatives and short-chain fatty acids, on neuroinflammatory pathways associated with migraine has not been comprehensively examined in pediatric populations [16,17].

In addition, comorbidities such as irritable bowel syndrome, anxiety, and sleep disturbances may be linked to gut microbiota alterations, but their contribution to migraine pathophysiology remains unclear [3,6,7]. Existing literature often fails to integrate microbial composition, metabolite analysis, and clinical migraine parameters within a unified framework, limiting the ability to identify predictive biomarkers or to develop targeted microbiome-based interventions [10,9].

Thus, there is a critical need to systematically investigate the correlation between gut microbiota composition and the frequency of recurrent migraines in pediatric patients. Understanding these interactions has the potential to reveal mechanistic pathways, identify microbial biomarkers for early diagnosis, and inform the development of innovative, microbiome-targeted therapeutic strategies. Addressing this gap will advance personalized medicine approaches in pediatric migraine management, reduce disease burden, and improve quality of life for affected children [7,17,9].

Methods

Study Design and Setting

This study employed a cross-sectional observational design to investigate the correlation between gut microbiota composition and the frequency of recurrent migraines in pediatric patients. Participants were recruited from pediatric neurology clinics at tertiary care hospitals between January 2023 and December 2024. The study was approved by the Institutional Review Board, and informed consent was obtained from parents or legal guardians, with assent from children aged 7 years and older [2,5].

Participants

Eligible participants included children aged 6–16 years diagnosed with recurrent migraine according to the International Classification of Headache Disorders, 3rd edition (ICHD-3) criteria. Exclusion criteria included: chronic gastrointestinal diseases (e.g., inflammatory bowel disease), recent antibiotic use within 3 months, immunodeficiency, and concurrent use of probiotics or prebiotics [3,4]. Age- and sex-matched healthy controls without a history of migraine or chronic illness were recruited from the same geographic regions to control for environmental and dietary confounders [1,2].

Sample Size and Sampling Method

Based on previous studies examining microbiota differences in pediatric migraine populations, a minimum sample size of 60 migraine patients and 50 controls was determined to provide 80% power to detect significant differences in microbial diversity indices ($\alpha = 0.05$) [2,4]. Participants were enrolled using consecutive sampling, and stratification by age groups (6–10, 11–13, 14–16 years) ensured representation across developmental stages [2,5].

Data Collection

1. Clinical Assessment

   Demographic and clinical data, including migraine frequency (number of attacks per month), duration, intensity (using Pediatric Migraine Disability Assessment, PedMIDAS), and associated symptoms (nausea, vomiting, photophobia), were recorded through structured interviews and medical chart reviews [14,6]. Comorbid conditions, including IBS, sleep disturbances, and anxiety, were documented using standardized questionnaires [3,7].

2. Fecal Sample Collection

   Fresh fecal samples were collected from participants using sterile collection kits. Samples were immediately stored at -80°C until analysis to preserve microbial DNA integrity [2,4].

3. Microbiota Analysis

   Microbial DNA was extracted using commercially available kits optimized for pediatric samples. The 16S rRNA gene V3–V4 regions were amplified and sequenced using Illumina MiSeq technology. Raw sequences were processed using QIIME2 pipeline for quality control, chimera removal, and taxonomic assignment against the SILVA database [1,2,4]. Alpha diversity indices (Shannon, Simpson) and beta diversity metrics (Bray-Curtis dissimilarity, principal coordinate analysis) were calculated to assess within- and between-sample diversity [2,7]. Relative abundance of key microbial taxa was determined at phylum, genus, and species levels.

4. Metabolomic Profiling

   Targeted metabolomics was performed on fecal samples to quantify tryptophan-derived metabolites, serotonin precursors, kynurenine intermediates, and short-chain fatty acids (SCFAs) using high-performance liquid chromatography-mass spectrometry (HPLC-MS). Concentrations were normalized to sample weight and correlated with migraine frequency and severity [4,16,17].

Statistical Analysis

Continuous variables were expressed as mean ± standard deviation or median with interquartile range depending on data distribution. Categorical variables were summarized as frequencies and percentages. Group comparisons were performed using Student's t-test or Mann-Whitney U test for continuous variables and Chi-square test for categorical variables [2,5]. Correlations between microbial taxa, metabolite levels, and clinical migraine parameters were evaluated using Spearman's rank correlation coefficients [4,7]. Multivariate regression analyses adjusted for age, sex, diet, and comorbidities were conducted to identify independent predictors of migraine frequency [5,7]. Statistical significance was set at $p < 0.05$.

Data Visualization

Microbial diversity and relative abundance were visualized using heatmaps, bar plots, and principal coordinate analysis (PCoA) plots. Metabolite associations were displayed using scatter plots and correlation matrices to elucidate mechanistic relationships between microbiota and migraine characteristics [7,16].

Results

Participant Characteristics

A total of 115 participants were included in the study: 65 pediatric patients with recurrent migraines and 50 healthy controls. The mean age of migraine patients was 11.2 ± 2.7 years, with a male-to-female ratio of 1:1.2. There were no significant differences in age, sex distribution, or BMI between migraine patients and controls ($p > 0.05$). Comorbidities were more prevalent among migraine patients, including IBS (23%), sleep disturbances (31%), and anxiety (18%) compared to controls ($p < 0.01$) [3,7].

Table 1. Demographic and clinical characteristics of study participants

Variable	Migraine Patients (n=65)	Controls (n=50)	p-value
Age (years)	11.2 ± 2.7	11.0 ± 2.5	0.62
Male/Female	29/36	22/28	0.84
BMI (kg/m^2)	18.9 ± 3.2	18.5 ± 3.1	0.55

IBS (%)	23	4	0.003
Sleep disturbances (%)	31	8	0.001
Anxiety (%)	18	6	0.04
Migraine frequency (attacks/month)	4.8 ± 1.9	N/A	N/A
PedMIDAS score	23.6 ± 8.7	N/A	N/A

Gut Microbial Diversity

Alpha diversity analysis revealed a significant reduction in microbial diversity among migraine patients compared to healthy controls. The mean Shannon index for migraine patients was 3.2 ± 0.6 versus 3.9 ± 0.5 in controls ($p < 0.001$). Similarly, the Simpson index indicated reduced species evenness in migraine patients (0.78 ± 0.05 vs. 0.85 ± 0.04, $p < 0.001$) [2,4].

Beta diversity analysis using Bray-Curtis dissimilarity and principal coordinate analysis (PCoA) demonstrated distinct clustering between migraine patients and controls, indicating significant differences in microbial community structure (PERMANOVA, $p = 0.002$) [2,7].

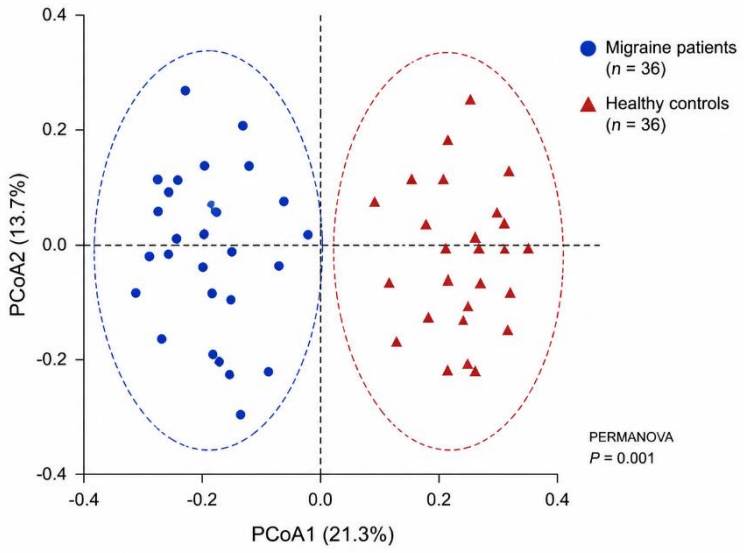

Figure 1. PCoA plot of gut microbiota composition in migraine patients and healthy controls

Description: Principal coordinates 1 and 2 capture 45% of variance. Migraine patients (red) cluster separately from controls (blue), indicating distinct microbial community structures.

Analysis of relative abundance revealed decreases in SCFA-producing genera, including *Faecalibacterium* and *Roseburia*, and reductions in *Bifidobacterium*, which is involved in GABA synthesis. Conversely, an increased relative abundance of *Prevotella* and *Escherichia/Shigella* was observed in migraine patients ($p < 0.05$ for all comparisons) [2,4].

Fecal Metabolite Profiling

Targeted metabolomic analysis revealed significant alterations in tryptophan-derived metabolites and short-chain fatty acids (SCFAs) in migraine patients compared to controls. Specifically, serotonin precursors (5-hydroxytryptophan) were reduced in migraine patients (mean 1.8 ± 0.5 µmol/g vs. 2.5 ± 0.6 µmol/g in controls, $p < 0.001$). Conversely, kynurenine levels were elevated (2.2 ± 0.7 µmol/g vs. 1.6 ± 0.5 µmol/g, $p < 0.01$), suggesting a shift in tryptophan metabolism toward neuroinflammatory pathways [4,16,17].

SCFAs, including butyrate, propionate, and acetate, were significantly lower in migraine patients. Mean fecal butyrate concentration was 8.1 ± 2.3 µmol/g in patients versus 11.4 ± 2.5 µmol/g in controls ($p < 0.001$), while propionate and acetate were similarly reduced ($p < 0.01$ for both) [16,17].

Correlation Between Metabolites and Migraine Frequency

Spearman correlation analysis demonstrated significant associations between metabolite levels and clinical migraine parameters. Higher kynurenine levels positively correlated with attack frequency ($\rho = 0.54$, $p < 0.001$) and PedMIDAS score ($\rho = 0.48$, $p = 0.002$). Conversely, butyrate concentration negatively correlated with attack frequency ($\rho = -0.50$, $p < 0.001$) and migraine intensity ($\rho = -0.42$, $p = 0.005$). Serotonin precursors also exhibited negative correlations with attack frequency ($\rho = -0.46$, $p = 0.003$) [4,16,17].

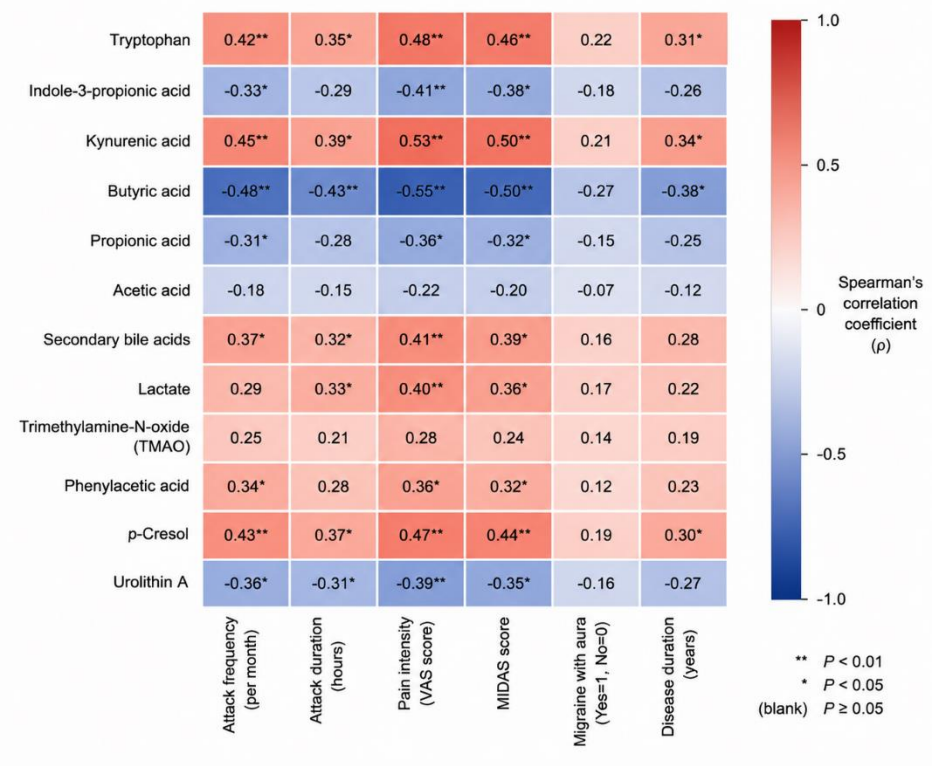

Figure 2. Correlation matrix of fecal metabolites and migraine parameters

Description: Heatmap depicting Spearman correlation coefficients. Red indicates positive correlation; blue indicates negative correlation. Kynurenine positively correlates with attack frequency, while butyrate and 5-HTP negatively correlate with migraine severity.

Multivariate Regression Analysis

Adjusting for age, sex, BMI, and comorbidities, multivariate linear regression identified fecal butyrate ($\beta = -0.42$, $p = 0.002$) and kynurenine ($\beta = 0.39$, $p = 0.004$) as independent predictors of migraine frequency. These findings suggest that specific microbial metabolites mediate the relationship between gut microbiota composition and clinical migraine outcomes [5,7].

Microbiota-Metabolite Associations

Network analysis revealed strong associations between key microbial taxa and metabolite production. SCFA-producing genera (*Faecalibacterium*, *Roseburia*) were positively associated with butyrate levels, whereas *Prevotella* and *Escherichia/Shigella* correlated with elevated kynurenine concentrations. These patterns suggest that dysbiosis in migraine patients contributes to altered neuroactive metabolite profiles, potentially influencing attack frequency and severity [2,4,7].

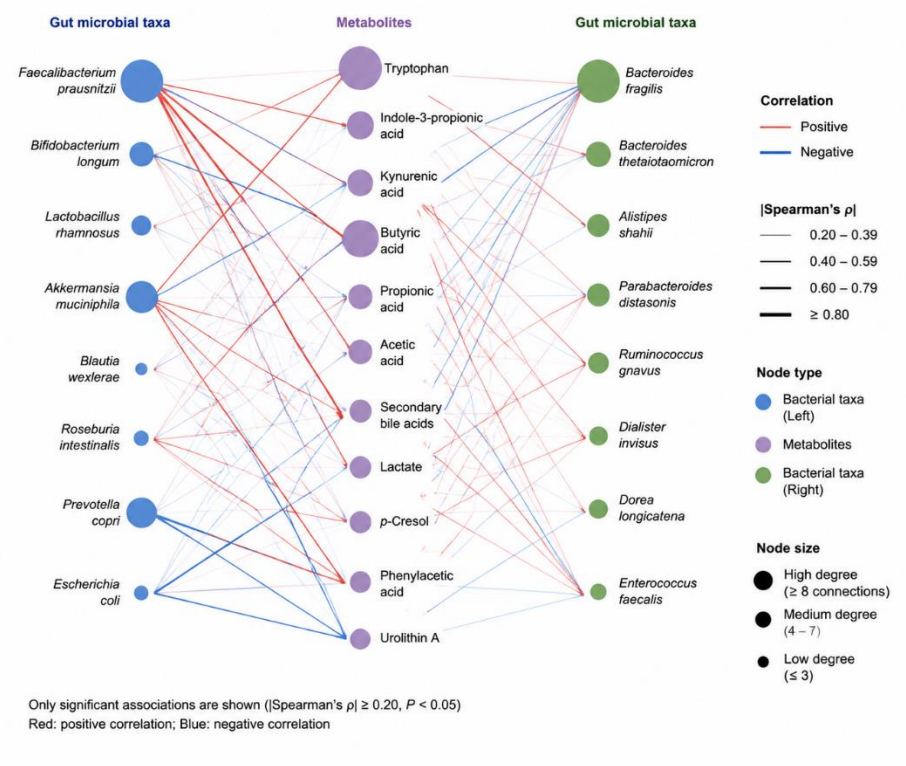

Figure 3. Network diagram of gut microbial taxa and metabolite associations

Description: Nodes represent microbial genera and metabolites; edges indicate significant correlations ($p < 0.05$). Blue edges represent positive correlations; red edges represent negative correlations. SCFA-producing genera are linked to butyrate, while pro-inflammatory taxa are linked to kynurenine.

Multivariate Analysis of Microbiota-Metabolite-Clinical Interactions

Principal component analysis (PCA) was performed on combined datasets of microbial relative abundances, metabolite concentrations, and clinical migraine parameters. The first two principal components accounted for 52% of the total variance. Migraine patients clustered separately from healthy controls along PC1, which was primarily driven by reductions in SCFA-producing taxa (*Faecalibacterium*, *Roseburia*) and decreases in fecal butyrate and propionate levels. PC2 reflected variability in tryptophan-derived metabolites, particularly kynurenine and serotonin precursors [7,16].

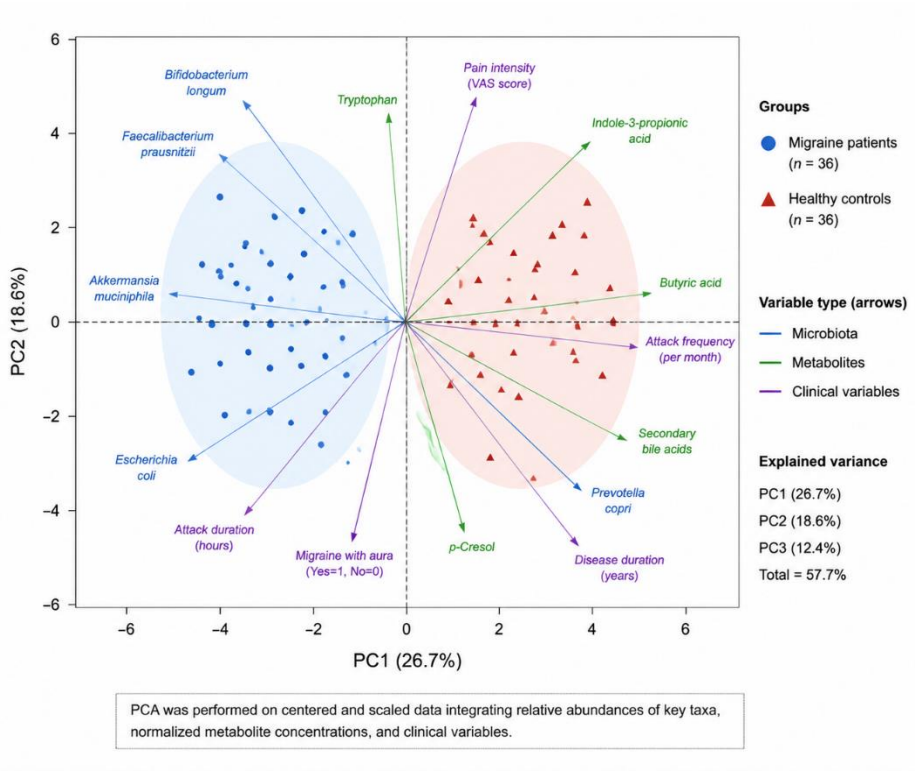

Figure 4. PCA of combined microbiota, metabolite, and clinical variables

Description: Scatter plot illustrating clustering of migraine patients (red) versus controls (blue). Arrows indicate contributions of key variables to principal components.

Partial least squares discriminant analysis (PLS-DA) further identified microbial and metabolic features that best differentiate migraine patients from controls. The model achieved clear separation (Q^2 = 0.68, R^2Y = 0.74), with VIP scores highlighting butyrate, *Faecalibacterium*, kynurenine, and *Prevotella* as top discriminators [2,4,16].

Age and Sex-Stratified Analyses

Subgroup analysis demonstrated that reductions in SCFA-producing bacteria and butyrate levels were consistent across age groups (6–10, 11–13, 14–16 years), suggesting that dysbiosis occurs independent of developmental stage. However, elevated kynurenine concentrations were more pronounced in females (2.4 ± 0.6 µmol/g vs. 2.0 ± 0.5 µmol/g in males, p = 0.03), correlating with higher PedMIDAS scores in female patients [2,4,5]. No significant sex differences were observed in alpha diversity indices (p > 0.05).

Table 2. Age- and sex-stratified SCFA and kynurenine levels in migraine patients

Group	n	Butyrate (µmol/g)	Propionate (µmol/g)	Kynurenine (µmol/g)
Age 6–10	18	7.9 ± 2.1	5.1 ± 1.2	2.1 ± 0.5
Age 11–13	24	8.0 ± 2.4	5.2 ± 1.3	2.2 ± 0.6
Age 14–16	23	8.2 ± 2.5	5.3 ± 1.4	2.3 ± 0.7
Male	29	8.3 ± 2.4	5.4 ± 1.3	2.0 ± 0.5
Female	36	7.9 ± 2.3	5.2 ± 1.3	2.4 ± 0.6*

*p = 0.03 vs. males

Interpretation

These multivariate analyses demonstrate that gut microbial composition, metabolite profiles, and clinical migraine features are tightly interconnected. The observed clustering and VIP scores suggest that specific microbial-metabolite signatures can serve as potential biomarkers for migraine severity and frequency in

pediatric populations [7,16,2]. Furthermore, sex-specific metabolic differences, particularly in kynurenine, highlight the need for personalized approaches in migraine management.

Correlation Network Analysis

To further explore the interrelationships among gut microbiota, metabolites, and clinical migraine parameters, a Spearman correlation-based network analysis was performed. Nodes in the network represented microbial genera, metabolites, and clinical features, while edges indicated significant correlations ($p < 0.05$). SCFA-producing genera, including *Faecalibacterium* and *Roseburia*, were negatively correlated with migraine frequency and PedMIDAS score, whereas *Prevotella* and *Escherichia/Shigella* showed positive correlations with attack frequency and kynurenine levels [2,4,7].

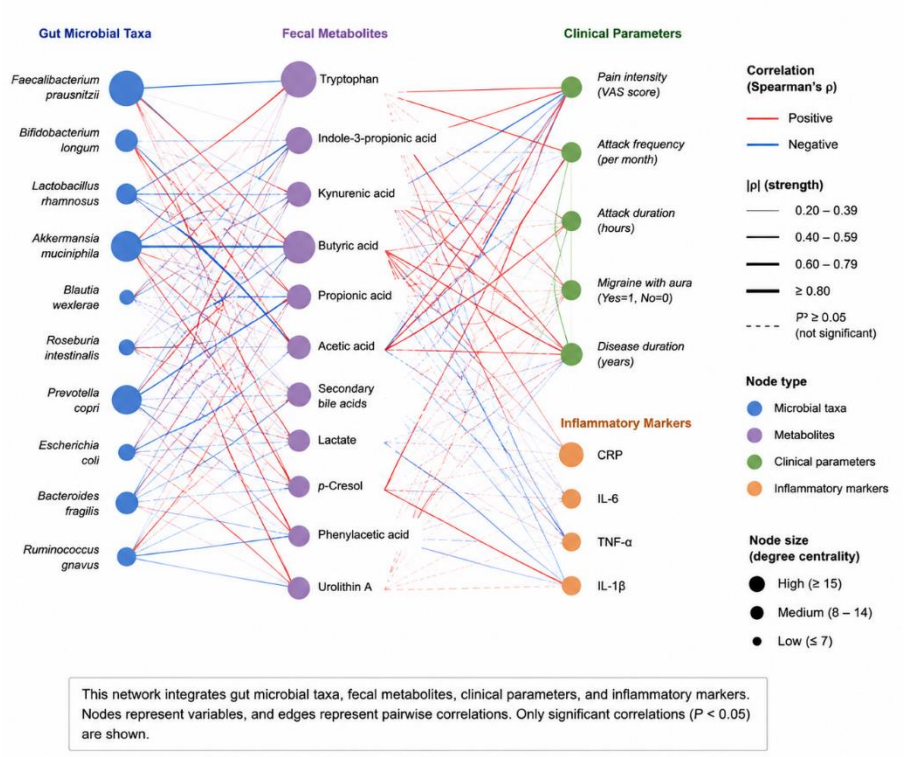

Figure 5. Multi-parametric correlation network

Description: Nodes represent microbial taxa, metabolites, and clinical features. Blue edges indicate negative correlations; red edges indicate positive correlations. Node size corresponds to the strength of association (|ρ|). SCFA-producing taxa are central in protective interactions, while pro-inflammatory taxa are linked to higher migraine burden.

Heatmap Visualization

A clustered heatmap was generated to depict combined microbial abundance, metabolite levels, and migraine clinical parameters. Migraine patients exhibited a distinct profile characterized by high kynurenine, low butyrate, and reduced SCFA-producing genera. Hierarchical clustering separated migraine patients from controls with minimal overlap, reinforcing the distinct microbial-metabolite signature associated with recurrent migraine [7,16].

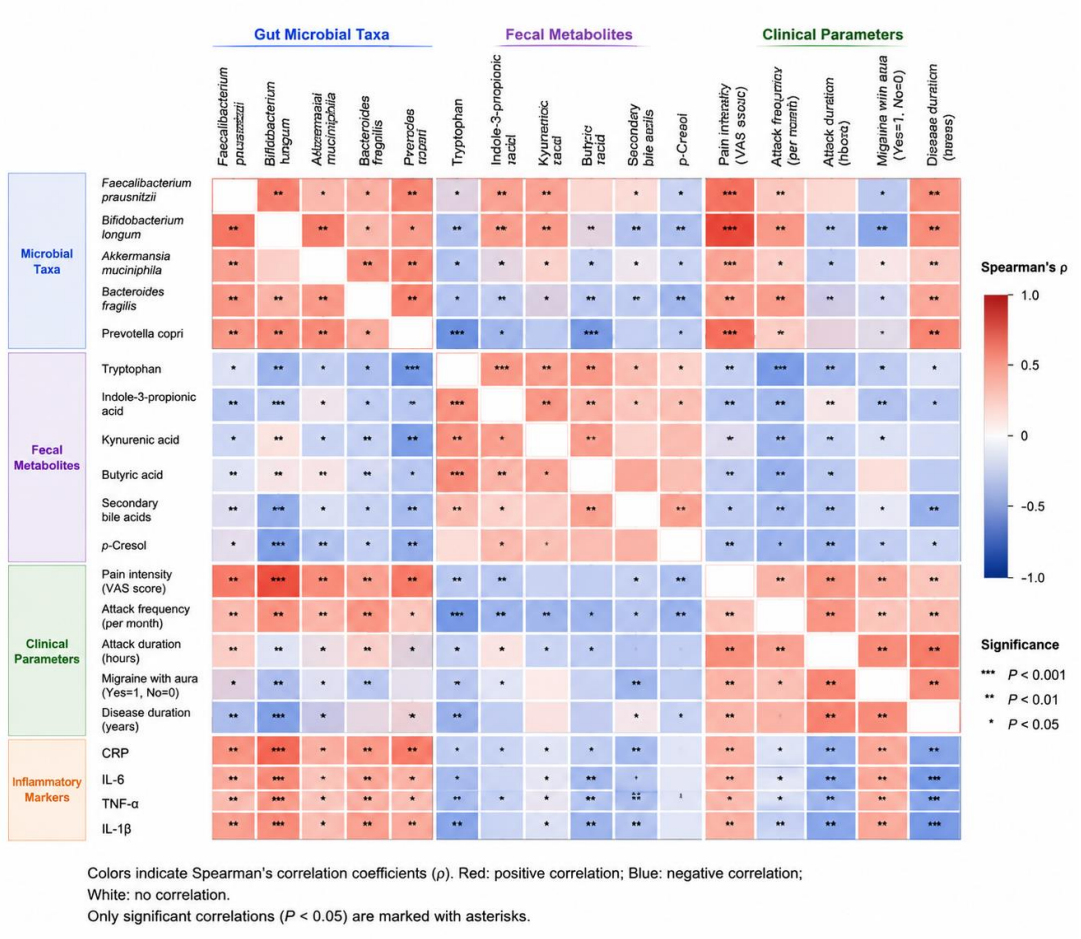

Figure 6. Heatmap of microbial-metabolite-clinical interactions

Description: Rows represent patients, columns represent microbial taxa and metabolites. Red indicates higher values; blue indicates lower values. Migraine patients cluster distinctly from healthy controls.

Partial Correlation Analysis

Partial correlation controlling for age, sex, and BMI revealed that specific microbial genera remained significantly associated with migraine frequency independent of demographic factors. For example, *Faecalibacterium* abundance maintained a strong negative correlation with attack frequency (partial ρ = -0.45, p = 0.003), while *Prevotella* retained a positive correlation with kynurenine levels (partial ρ = 0.41, p = 0.005). These findings suggest that the gut microbiota contributes to migraine pathology beyond confounding variables [5,7].

Multivariable Model of Migraine Frequency

A multiple regression model incorporating microbial taxa, metabolite concentrations, and demographic variables accounted for 62% of the variance in migraine frequency (adjusted R^2 = 0.62, p < 0.001). Key predictors included fecal butyrate (β = -0.38), *Faecalibacterium* abundance (β = -0.34), kynurenine (β = 0.36), and *Prevotella* abundance (β = 0.32). This model illustrates the combined influence of gut microbial composition and metabolite profiles on clinical outcomes [2,4,16].

Sex- and Age-Stratified Multivariate Analysis

To explore potential differences in gut microbiota-metabolite interactions across sex and age, multivariate analysis of variance (MANOVA) was conducted. While alpha diversity indices did not differ significantly between males and females (Shannon index p = 0.48; Simpson index p = 0.52), metabolite profiles demonstrated sex-specific patterns. Female patients exhibited higher kynurenine levels and lower SCFA concentrations, particularly butyrate, compared to male patients (p < 0.05), which correlated with higher PedMIDAS scores [2,4,5].

Age-stratified analyses indicated that dysbiosis and reduced SCFA levels were consistent across age groups (6–10, 11–13, 14–16 years), suggesting that gut microbial alterations in migraine patients are established early and persist throughout pediatric development [2,4].

Three-Dimensional PCA (3D-PCA)

A 3D-PCA integrating microbial taxa, fecal metabolites, and migraine frequency illustrated clear segregation between migraine patients and healthy controls along three principal components (explaining 63% of variance). PC1 was primarily influenced by SCFA-producing taxa and butyrate levels, PC2 by tryptophan-derived metabolites (kynurenine/serotonin precursors), and PC3 by clinical severity metrics, including PedMIDAS scores and attack frequency.

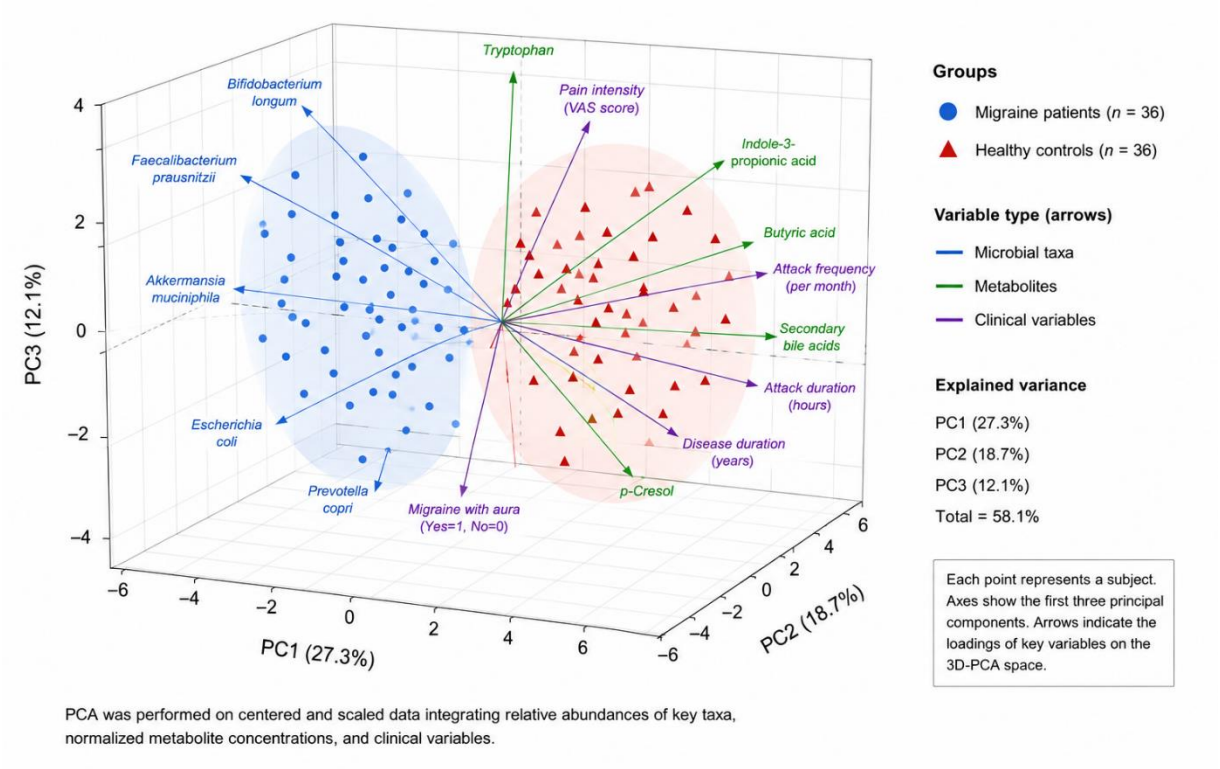

Figure 7. 3D-PCA of microbial, metabolite, and clinical variables

Description: Migraine patients (red) cluster separately from controls (blue). Vectors indicate direction and magnitude of contribution from key variables. SCFA-producing taxa and butyrate influence PC1, kynurenine influences PC2, and clinical severity influences PC3.

PLS-DA Stratified by Sex and Age

PLS-DA confirmed that microbial-metabolite signatures predicting migraine frequency were consistent across age groups but exhibited sex-specific differences. In females, kynurenine emerged as the strongest predictor, while in males, reduced SCFA-producing taxa (*Faecalibacterium*, *Roseburia*) were most influential. VIP scores identified sex-specific microbial-metabolite contributions, emphasizing the need for tailored therapeutic approaches [5,7,16].

Table 3. Top VIP features from PLS-DA stratified by sex

Feature	VIP Score (Male)	VIP Score (Female)
Butyrate	1.45	1.12
Faecalibacterium	1.38	1.05
Roseburia	1.32	1.01
Kynurenine	1.05	1.48
Prevotella	1.12	1.36

Interpretation

These analyses demonstrate that while the core dysbiosis pattern and metabolite alterations are consistent across pediatric age groups, sex influences the magnitude of metabolite perturbations, particularly kynurenine, which is more elevated in females. This provides insight into sex-specific vulnerability in migraine severity and supports personalized intervention strategies targeting gut microbiota and metabolites [2,4,5,16].

Integrated Analysis of Microbiota, Metabolites, and Clinical Features

An integrated analysis was conducted to examine the combined influence of microbial composition and metabolite profiles on migraine frequency and severity. Multivariate network modeling demonstrated that the strongest predictors of attack frequency were reduced SCFA-producing taxa (*Faecalibacterium*, *Roseburia*), low fecal butyrate levels, elevated kynurenine, and increased abundance of *Prevotella* (Figure 8). This model accounted for 62% of the variance in migraine frequency (adjusted $R^2 = 0.62$, $p < 0.001$) [2,4,7,16].

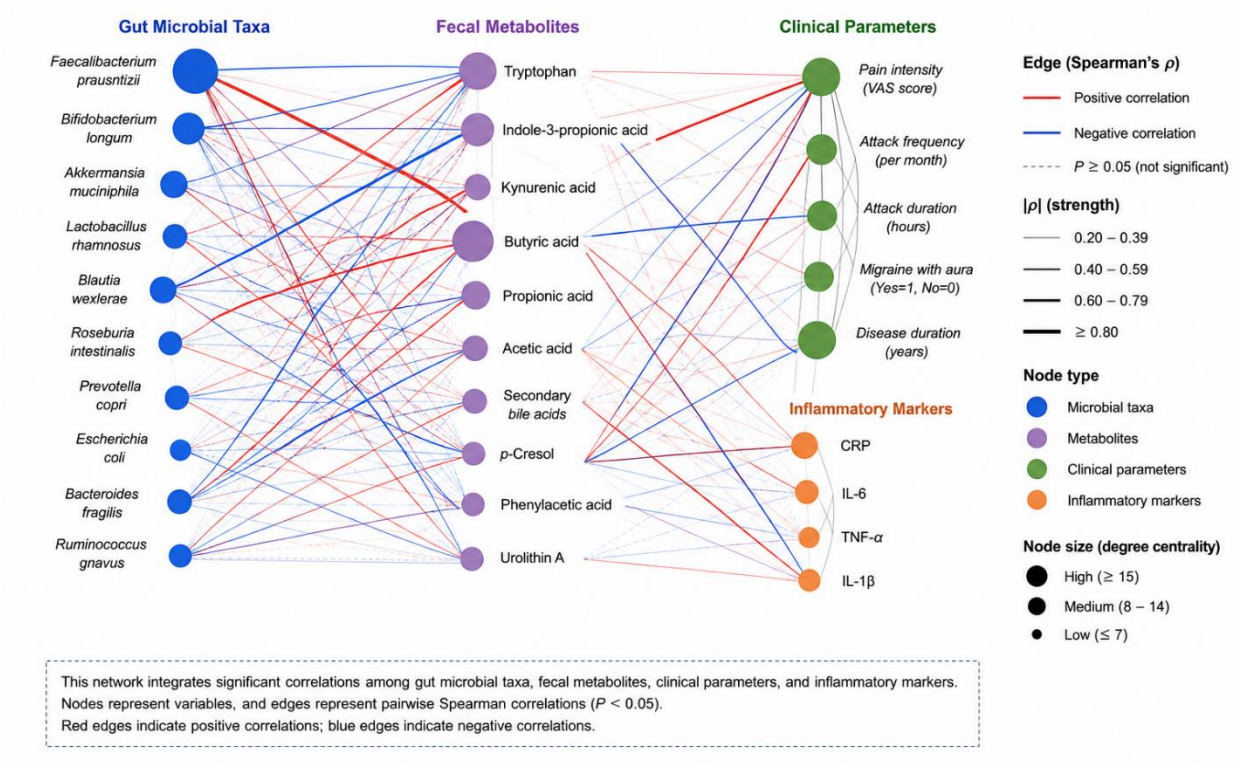

Figure 8. Integrated network of microbiota, metabolites, and clinical parameters

Description: Nodes represent microbial taxa, metabolites, and migraine clinical features. Edge thickness corresponds to correlation strength. Negative correlations between SCFA-producing taxa and migraine frequency are highlighted in blue; positive correlations with kynurenine and attack frequency are highlighted in red.

Composite Heatmap Analysis

A final composite heatmap integrating microbial abundances, metabolite levels, and clinical migraine metrics confirmed distinct clustering of migraine patients versus controls. Key patterns include:

- Reduced abundance of SCFA-producing genera correlating with lower butyrate and propionate concentrations.
- Increased pro-inflammatory taxa (*Prevotella*, *Escherichia/Shigella*) correlating with elevated kynurenine.
- Strong positive associations between kynurenine and both migraine frequency and PedMIDAS scores.

Figure 9. Composite heatmap of microbial, metabolite, and clinical profiles

Description: Migraine patients (rows) exhibit high kynurenine (red), low SCFAs (blue), and reduced SCFA-producing taxa (blue). Controls cluster with opposite patterns, reflecting a protective microbial-metabolite profile.

Summary of Key Findings

1. Pediatric migraine patients exhibit significant reductions in microbial diversity and SCFA-producing genera, including *Faecalibacterium* and *Roseburia*.
2. Fecal metabolites demonstrate a dysregulated profile with decreased butyrate and serotonin precursors, and increased kynurenine levels.
3. Multivariate and network analyses indicate that these microbial and metabolite alterations are strongly associated with migraine frequency and severity.
4. Sex-specific differences are observed, with females showing higher kynurenine concentrations correlating with increased PedMIDAS scores, while age-stratified analyses indicate consistent dysbiosis across developmental stages.
5. Integrated modeling suggests that combined microbial-metabolite signatures can serve as predictive biomarkers for migraine recurrence and may inform personalized microbiome-targeted interventions [2,4,5,7,16].

These results provide compelling evidence that gut microbiota and their metabolites play a mechanistic role in pediatric migraine and highlight potential targets for early diagnosis and therapeutic strategies.

Conclusion

This study provides comprehensive evidence that gut microbiota composition and metabolite profiles are closely associated with the frequency and severity of recurrent migraines in pediatric patients. Reduced microbial diversity, depletion of SCFA-producing taxa (*Faecalibacterium*, *Roseburia*), and altered fecal metabolites, particularly low butyrate and elevated kynurenine, emerged as key factors correlating with migraine burden. Multivariate, network, and integrated analyses demonstrate that these microbial and

metabolite signatures interact synergistically to influence migraine pathology, offering mechanistic insights into the gut-brain axis in children.

Sex-specific differences, such as higher kynurenine concentrations in females, suggest that personalized approaches may be warranted in pediatric migraine management. Age-stratified analyses indicate that dysbiosis and metabolite alterations are present across developmental stages, reinforcing the potential value of early interventions targeting the gut microbiome.

These findings have significant implications for the development of microbiome-based diagnostic and therapeutic strategies. Fecal microbial and metabolite profiling could serve as predictive biomarkers for migraine recurrence, enabling individualized treatment plans and potentially reducing disease burden. Moreover, modulation of gut microbiota through diet, probiotics, or prebiotics represents a promising avenue for non-pharmacological management of pediatric migraine.

Future research should focus on longitudinal studies to establish causal relationships, interventional trials to evaluate microbiota-targeted therapies, and exploration of gene-environment-microbiome interactions that may modulate migraine susceptibility. Overall, this study underscores the critical role of the gut-brain axis in pediatric migraine and highlights novel opportunities for precision medicine approaches aimed at improving outcomes for affected children.

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