Monte *et al. The Journal of Headache and Pain* (2026) 27:96
https://doi.org/10.1186/s10194-026-02344-9

The Journal of Headache and Pain

REVIEW Open Access

Migraine in pediatric population: the role of biochemical markers

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Abstract

Background Diagnosis of migraine remains primarily clinical, and objective biological markers are lacking. Although biomarker research has expanded considerably in adults, evidence in pediatric populations remains fragmented and heterogeneous. This systematic review aimed to synthesize current data on biochemical markers in pediatric migraine and to evaluate their potential diagnostic, monitoring, prognostic, and treatment-response roles.

Methods The review was conducted according to Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines. PubMed, Embase and Scopus were searched up to January 1st, 2026. Studies investigating biochemical markers in individuals younger than 18 years diagnosed with episodic or chronic migraine according to International Classification of Headache Disorders 3rd edition (ICHD-3) criteria were included. Biomarkers assessed in blood, saliva, urine, cerebrospinal fluid, or stool were considered. Due to substantial heterogeneity in study design, sampling timing and laboratory methods, findings were synthesized narratively.

Results Twenty-eight studies met inclusion criteria. Neuropeptides were the most extensively investigated biomarkers. Ictal elevation of calcitonin gene-related peptide (CGRP) was the most reproducible finding, supporting a disease-activity monitoring role, whereas interictal levels showed inconsistent results. Pituitary adenylate cyclase–activating polypeptide 38 (PACAP-38) and vasoactive intestinal peptide (VIP) demonstrated variable associations and may contribute within multimarker panels. Mitochondrial stress markers (growth differentiation factor-15, fibroblast growth factor-21 and hypoxia-inducible factor-1α) were elevated during attacks and in chronic migraine, suggesting links with metabolic stress and disease burden. MicroRNAs emerged as preliminary treatment-responsive candidates. Gut microbiota studies showed consistent β-diversity alterations and shifts in tryptophan metabolism; ratio-based kynurenine metabolites displayed promising diagnostic performance. Among inflammatory markers, tumor necrosis factor (TNF)-axis activation and interleukin (IL)-12p70 were the most reproducible signals.

Conclusions No single biomarker currently supports standalone clinical application. Pathway-based multimarker approaches may improve biological stratification and monitoring in pediatric migraine.

Clinical trial number Not applicable.

Keywords Migraine, Children, Biomarkers, CGRP, Cytokines, Mitochondrial dysfunction, Microbiota

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Introduction

Migraine affects approximately 10% of children and adolescents with substantial impacts on quality of life and functional impairment [1]. Despite its high clinical relevance, diagnosis remains based exclusively on clinical criteria defined by the International Classification of Headache Disorders, 3rd edition (ICHD-3) [2]. This clinic-based approach does not account for the marked clinical variability observed in migraine in pediatric population, suggesting underlying biological heterogeneity and the need for objective biological markers [3]. Biomarker research seeks to identify measurable indicators reflecting disease presence, activity, burden or treatment response [4]. In adults, substantial progress has been made in characterizing molecular pathways involved in trigeminovascular activation, neuroinflammation, and metabolic stress [3]. However, evidence in children and adolescents remains fragmented. Biological heterogeneity and age-related physiological differences may influence biomarker expression, limiting direct extrapolation from adult studies and underscoring the need for age-specific investigation [3]. Several biological pathways have emerged as potential contributors to migraine. Neuropeptides involved in trigeminovascular signaling, such as calcitonin gene-related peptide (CGRP), play a key role in nociceptive transmission and neurovascular signaling [5] and represent the most promising biomarkers [6]. Pituitary adenylate cyclase–activating polypeptide (PACAP)-38, vasoactive intestinal peptide (VIP) and substance P (SP) have also been implicated in migraine pathophysiology, suggesting the involvement of a broader neuropeptide network with potential biological signatures [7–9]. Beyond neuropeptides, growing evidence supports the involvement of neuroinflammatory and systemic inflammatory processes in migraine, including cytokine signaling [10]. In parallel, mitochondrial dysfunction and hypoxia-related metabolic stress may contribute to migraine susceptibility and disease burden, as reflected by circulating growth differentiation factor-15 (GDF-15), fibroblast growth factor-21 (FGF-21), and hypoxia-inducible factor-1α (HIF-1α) [11]. The gut–brain axis has further expanded the biological framework, linking gut microbiota (GM) composition, intestinal permeability, immune activation and tryptophan metabolism to migraine expression [12, 13]. Epigenetic regulators, including microRNAs, have also gained attention as stable and potentially non-invasive indicators of pathway activity [14].

Given the heterogeneity and developmental specificity of migraine in children and adolescents, a comprehensive synthesis of available biomarker evidence is warranted. This systematic review synthesizes the available evidence on biochemical markers in migraine in pediatric age. It evaluates their role in diagnosis, disease monitoring, prognostic stratification, and treatment response, with the aim of supporting clinical translation. Biomarkers measured in blood, saliva, urine, cerebrospinal fluid, and stool will be analyzed for their clinical value.

Methods

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines.

Search strategy

A comprehensive literature search was independently conducted by two authors (G.M. and C.G.) in PubMed, Embase, and Scopus from database inception to January 1, 2026. The search combined controlled vocabulary terms and free-text keywords related to biochemical markers, migraine or headache, and pediatric populations. Only studies conducted in humans and published in English were considered. For the search string we used combinations of the following keywords: (calcitonin gene related peptide OR CGRP OR pituitary adenylate cyclase-activating peptide OR PACAP OR vasoactive intestinal peptide OR VIP OR substance P OR SP OR neuropeptide Y or NPY OR neurokinin A OR NKA OR pentraxin-3 OR PTX-3 OR C-reactive protein OR CRP OR IgG OR immunoglobulin G OR inflammatory burden index OR IBI OR cytokines OR neuropeptide OR adipocytokines OR prostaglandins OR microRNA OR endotelin-1 OR ET-1 OR LPS OR occludin OR 5-HT OR serotonin OR tryptophan metabolites OR kynurenine OR kynurenic acid OR growth differentiation factor-15 OR GDF-15 OR fibroblast growth factor-21 OR FGF-21 OR hypoxia-inducible factor-1α OR HIF-1α OR S100-beta OR immunoglobulin A OR IgA AND (migraine OR headache) AND (pediatric OR children OR child OR adolescent).

Eligibility criteria

We included original studies that investigated potential biochemical markers in individuals younger than 18 years diagnosed with episodic migraine (EM) or chronic migraine (CM) with or without aura according to the ICHD-3 criteria. Eligible study designs comprised randomized clinical trials (RCTs), prospective or retrospective cohort studies, case series, and case reports. We excluded studies conducted exclusively in adults, review articles without original data, conference abstracts not followed by full publication, experimental studies or in vitro studies, animal studies and non–peer-reviewed sources.

Selection and data collection process

All records identified through the database searches were assessed for eligibility in a two-step process. In the first step, titles and abstracts were independently screened by

two authors (G.M. and C.G.) to exclude studies that were clearly irrelevant. Full texts of potentially eligible articles were then retrieved and evaluated in detail according to the predefined inclusion and exclusion criteria. Discrepancies were resolved through discussion until consensus was reached. Data extraction was independently performed by the same two authors (G.M. and C.G.) using a standardized template to ensure consistency. For each included study, we collected information on author and year, study design, sample size, participants' age and sex, diagnostic criteria, methodology and biochemical marker.

Risk of bias, effect measures, and synthesis approach

Given the heterogeneity in biomarker categories, biological matrices, laboratory techniques, sampling timing and study designs, we planned a narrative synthesis. Most included studies were small, single-center and predominantly cross-sectional, which may limit generalizability and increase susceptibility to selection and measurement bias. Risk of bias for observational studies was assessed using the Newcastle–Ottawa Quality Assessment Scale (NOS): selection 0–4 stars, comparability 0–2 stars and exposure/outcome 0–3 stars. The risk of bias was categorized as low, moderate, and high with corresponding NOS scores of 7–9, 4–6, and 0–3, respectively. Stars were conservatively withheld when information was insufficiently reported. For RCTs, risk of bias was assessed using the Cochrane Risk of Bias 2 (RoB 2) tool, considering the five standard domains (randomization process, deviations from intended interventions, missing outcome data, measurement of the outcome and selection of the reported result). The studies were independently evaluated by two researchers (G.M. and C.G.) and disagreements were resolved by consensus. To better characterize the reproducibility of findings across studies, we additionally performed a qualitative assessment of case–control consistency for each biomarker. Consistency was evaluated by examining the direction and reproducibility of case–control differences across studies. Biomarkers were categorized as consistent when the majority of studies reported concordant case–control differences in the same direction, partially consistent when findings were broadly similar but not uniform, inconsistent when results showed conflicting directions or no reproducible pattern, and limited evidence when conclusions were based on a small number of studies or single-study reports.

Primary measures of interest were differences in biomarker concentrations between patients and control groups, across disease phases, migraine subtypes, or disease burden categories. Secondary measures included associations with clinical features such as headache frequency, disability scores, and response to preventive treatment. Owing to marked heterogeneity in biomarkers investigated, biological specimens, laboratory techniques, thresholds and study designs, quantitative pooling and meta-analysis were not considered. Therefore, findings were synthesized narratively, with emphasis on consistency and clinical interpretability rather than statistical aggregation. Finally, an overall level of evidence was qualitatively assigned for each biomarker. This appraisal integrated the number of available studies, methodological quality as assessed by NOS and the consistency of findings across studies. Evidence levels were defined as follows. Moderate evidence: consistent or largely concordant findings across multiple studies of moderate-to-high methodological quality; low evidence: limited number of studies and/or heterogeneous or inconsistent findings across studies; very low evidence: evidence derived from single studies or from studies with important methodological limitations.

Results

The literature search identified 102 articles for eligibility assessment. After exclusion of 74 studies, 28 met inclusion criteria and were included in the qualitative synthesis (Fig. 1). Detailed methodological characteristics, population features, biological specimens, and principal findings of the included studies are summarized in Tables 1, 2, 3, and 4. No eligible studies were identified for inflammatory burden index or IBI, S100-beta, IgG or immunoglobulin G, adipocytokines.

Risk of bias and level of evidence of the included studies

Risk of bias assessed using NOS indicated that most observational studies had moderate methodological quality. Star loss occurred most frequently in the comparability domain, reflecting limited control for confounding (e.g., incomplete matching or adjustment for age/sex, phenotype severity, comorbidities, and treatment exposure). An additional recurring limitation was the heterogeneity in sampling timing (ictal vs. interictal), which may affect the comparability of biomarker measurements across studies. Overall NOS ratings are reported in Supplementary Table S1. The only RCT [15] had high risk of bias, primarily due to open-label design without placebo (deviations from intended interventions), with additional concerns regarding outcome assessment and reporting. Consistency and levels of evidence for each biomarker are summarized in Table 5.

Neuropeptides

Calcitonin gene-related peptide (CGRP)

Eleven studies evaluated circulating CGRP levels in migraine in children and adolescents, including prospective cohorts, cross-sectional analyses and one RCT [9, 11, 15–23]. Details are reported in Table 1. Ictal CGRP

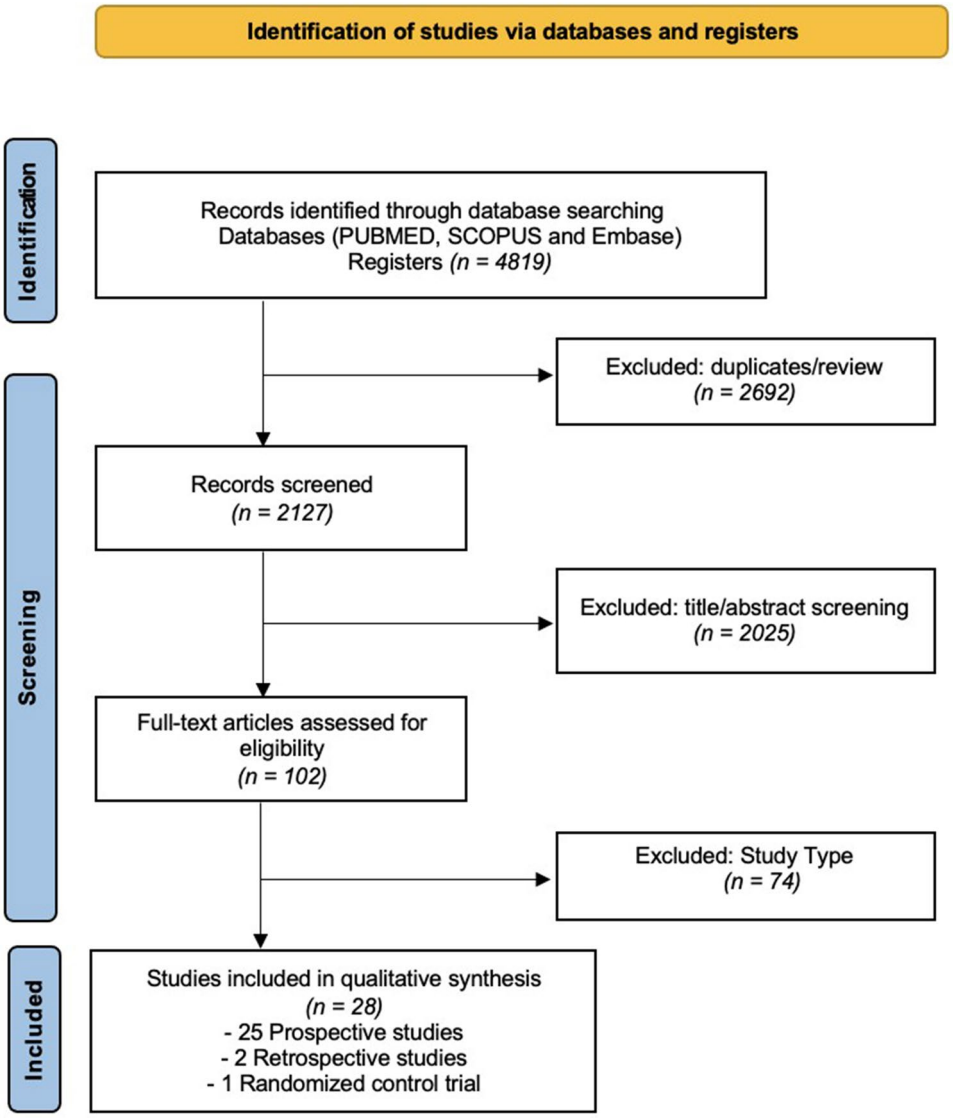

Fig. 1 PRISMA flow diagram of study selection

elevation was the most consistent finding across independent cohorts [11, 16, 17, 19]. In several studies, CGRP levels were significantly higher during attacks compared with interictal phases and healthy controls ($p < 0.05$), particularly when sampling occurred within the first hours of symptom onset. In some cohorts, ictal elevation appeared more pronounced in migraine with aura (MA) ($p < 0.05$) [16, 19], whereas others did not observe clear subtype differences [9, 18]. Interictal findings were heterogeneous. Elevated interictal CGRP levels compared with controls were reported in certain cohorts ($p < 0.05$) [9, 19, 20, 23], in some cases primarily driven by migraine without aura (MO) or CM ($p < 0.05$) [11, 20]. Conversely, other studies, including those comparing migraine with tension-type headache (TTH), did not detect significant interictal differences [18, 21, 22]. Differences in biological matrix (plasma versus serum), assay methodology, and sampling timing were evident across cohorts (Table 1). Associations between CGRP levels and clinical characteristics were inconsistent. Some studies reported correlations with longer attack duration, higher headache frequency, CM or disability scores ($p < 0.05$) [11, 17, 19], whereas others did not find significant relationships [20–22]. Importantly, one RCT demonstrated that effective preventive therapy was associated with a significant reduction in serum CGRP levels over 12 weeks ($p < 0.001$) [15]. Overall, ictal CGRP elevation represents the most reproducible finding across cohorts, whereas interictal levels and clinical correlations show substantial heterogeneity.

Table 1 Studies investigating CGRP in migraine in the pediatric population

Study	Design	Population	Age (years), Sex	Biomarker	Specimen	Phase	Main findings
Gallai 1995 [16]	Prospective	75 migraine (30 MA, 45 MO) 30 controls	MA: 15.4 ± 2.3 y (12 M); MO: 16.3 ± 2.6 y (20 M); controls: 15.1 ± 2.1 y (15 M)	CGRP	Plasma	Ictal/ Interictal	Ictal CGRP ↑ in MA/MO vs. interictal; no interictal difference vs. controls. Peak <2 h from onset, returns to baseline after pain resolution.
Liu 2022 [19]	Prospective	76 migraine (32 MA, 44 MO) 77 controls	Migraine:10.38 ± 3.73 y (35 M); controls: 9.46 ± 3.91 y (47 M)	CGRP	Plasma	Ictal/ interictal	CGRP ↑ in migraine vs. controls in both phases; stronger ictal increase in MA.
Fures 2024 [20]	Prospective	66 migraine, 59 TTH, 53 controls	Migraine: 13 y (9–14); 30 M; TTH: 12 y (9–16); 20 M; controls: 13 y (6–18); 31 M	CGRP	Serum	Interictal	Interictal CGRP ↑ in migraine. Elevation driven by MO, while MA did not differ from controls. No correlation with headache frequency or severity.
Falsing 2025 [21]	Prospective	29 migraine vs. 16 TTH	Migraine: 12.6 ± 2.9 y; 10 F TTH: 15.2 ± 2.5 y; 26 F	CGRP	Plasma	Interictal	No significant interictal CGRP differences between groups; no clinical associations.
Kilinc 2023 [11]	Cross-sectional	68 MO (EM and CM), 20 controls	Migraine: 14.4 ± 2.2 y; all F controls: 14.5 ± 1.9 y; all F	CGRP	Serum	Ictal/ Interictal	Serum CGRP ↑ in MO, especially during attacks and in chronic migraine.
Puliappadamb 2023 [15]	RCT	54 EM: flunarizine ($n = 24$) vs. flunarizine + ALA ($n = 30$)	Flunarizine: 15.3 ± 3.4 y; 21 F; Flunarizine + ALA: 16.1 ± 2.7 y; 16 F	CGRP	Serum	Baseline and post-treatment (12 weeks)	ALA add-on prophylaxis reduced migraine burden and significantly ↓ serum CGRP over 12 weeks vs. controls.
Fan 2009 [17]	Prospective	66 migraine, 33 non-migraine headache, 22 controls	Migraine: 10.0 ± 0.4 (39 M); non-migraine: 9.2 ± 0.7 (13 M); controls: 10.1 ± 0.8 (13 M/9F)	CGRP	Plasma	Ictal/ Interictal	Ictal CGRP ↑ vs. interictal only in migraine; interictal CGRP did not differ from controls.
Hanci 2021 [18]	Prospective	38 MO vs. 20 healthy controls	MO: 13.0 ± 3.3 y; 10 M controls: 11.3 ± 2.9 y; 7 M	CGRP	Plasma	Ictal/ Interictal	No differences in CGRP between MO and controls; no ictal–interictal variation.
Fan 2019 [23]	Prospective	68 migraine, 30 non-migraine headache, 22 controls	Migraine: 11.7 ± 0.4 y; 28 M Non-migraine: 9.6 ± 0.7 y; 12 M controls: 10.1 ± 0.8 y; 13 M	CGRP	Plasma	Ictal/ Interictal	CGRP ↑ both ictally and interictally vs. non-migraine headache/controls.
Orak 2025 [9]	Prospective	39 MO, 40 healthy controls	MO: 14.12 y; 26 F controls: 14.6 y; 26 F	CGRP	Serum	Ictal/ Interictal	CGRP not different ictal vs. interictal; small interictal ↑ vs. controls.
Aydin 2025 [22]	Cross-sectional	32 migraine, 32 healthy controls	Migraine ictal: 12.31 ± 3.31 y (7 M/9F); Migraine interictal: 11.88 ± 3.22 y (8 M/8F); controls: 12.24 ± 3.42 y (11 M/21F)	CGRP	Plasma	Ictal/ Interictal	Plasma CGRP showed no differences between migraine and controls and no ictal–interictal variation.

ALA: Alpha-Lipoic Acid, CGRP: Calcitonin Gene-Related Peptide, CM: Chronic Migraine, EM: Episodic Migraine, F: Female, M: Male, MA: Migraine with Aura, MO: Migraine without Aura, OR: Odds Ratio, TTH: Tension-Type Headache

Pituitary adenylate cyclase–activating polypeptide 38 (PACAP-38)

Four previously cited studies also evaluated circulating PACAP-38 levels in migraine in children and adolescents [9, 11, 18, 19], with details summarized in Table 2. Both plasma and serum measurements were used, and sampling was performed during ictal and interictal phases. Three independent cohorts reported significantly higher PACAP-38 levels in migraine patients compared with healthy controls [11, 18, 19]. In the largest prospective study [19], plasma PACAP-38 levels were significantly elevated in migraine patients compared with controls during both ictal and interictal phases (both $p < 0.001$), without significant intra-individual phase differences. Similarly, Hanci et al. observed higher PACAP-38 levels in patients compared with controls during both attack and attack-free periods ($p < 0.05$), again without clear ictal–interictal variation [18]. In contrast, Kilinc et al. reported significant ictal–interictal differences ($p < 0.05$), with higher ictal PACAP-38 levels, and demonstrated greater concentrations in CM compared with EM ($p < 0.001$); only the CM subgroup differed significantly from controls ($p < 0.001$) [11]. Conversely, Orak et al. did not identify significant differences between migraine patients and controls, nor between ictal and interictal phases [9]. Associations with clinical parameters were limited; correlation with disease burden was reported only in the cohort by Kilinc et al. ($p < 0.05$) [11]. Overall, PACAP-38 elevation was observed in most cohorts, although phase-dependent variation and replication across studies remain limited.

Additional vasoactive neuropeptides

VIP, SP and neurokinin A (NKA) were evaluated in several cohorts of migraine in children and adolescents, alongside CGRP and PACAP-38 [9, 11, 16, 18]. Study design, population and biological specimen are reported in Table 2. VIP findings were heterogeneous. Elevated VIP levels in migraine patients compared with controls were reported in some cohorts, including higher concentrations during both ictal and interictal phases ($p < 0.05$) [18]. One study observed significantly higher ictal VIP levels compared with interictal levels ($p < 0.05$) and controls ($p < 0.05$) [9], while another reported increased VIP concentration in CM compared with EM ($p < 0.001$), with only the chronic subgroup differing significantly from controls ($p < 0.001$) [11]. However, not all cohorts identified significant differences between migraine patients and healthy controls. In contrast, the two tachykinins, SP and NKA, were largely comparable between migraine patients and controls across studies. Most cohorts did not demonstrate significant ictal–interictal differences or subtype-related variation [11, 16, 18]. One study reported lower interictal SP levels in migraine patients compared with controls ($p < 0.05$) [9], but this finding was not replicated. Evidence regarding neuropeptide Y (NPY) was limited to a study that included 15 children and adolescents with MA and 15 age and sex matched TTH. Interictal plasma NPY levels were lower in MA compared with TTH ($p < 0.05$), not supporting its role in migraine patients [24]. Associations with clinical characteristics were inconsistently reported and generally weak for all four neuropeptides. Overall, VIP demonstrates variable but statistically supported elevation in selected cohorts, whereas current evidence does not support a consistent discriminative role for SP, NKA or NPY in migraine in children and adolescents.

Mitochondrial and hypoxia-related biomarkers

Serum levels of HIF-1α, GDF-15, and FGF-21 were evaluated in the previously reported prospective cohort by Kilinc et al. [11]. In that cohort, all three markers were significantly elevated in migraine patients compared with healthy controls (all $p < 0.001$). Ictal levels of HIF-1α and FGF-21 were higher than interictal levels (both $p < 0.05$), and CM was associated with greater biomarker elevation compared with EM ($p < 0.001$). In subgroup analyses, only the CM group consistently differed from controls across all three markers (all $p < 0.001$). Correlations between mitochondrial markers and circulating CGRP and PACAP-38 levels were also reported within the same study [11]. These findings derive from a single-center cohort and have not yet been independently replicated. Overall, mitochondrial and hypoxia-related markers were associated with disease activity and greater burden, particularly in CM, although evidence is currently limited to a single study.

MicroRNAs

Circulating and salivary microRNAs were evaluated in a prospective pilot study including 24 children and adolescents with MO and 12 age- and sex-matched healthy controls [14]. Serum and salivary levels of hsa-miR-34a-5p and hsa-miR-375 were significantly higher in untreated migraine patients compared with controls ($p < 0.05$). Expression profiles were comparable between serum and saliva, with parallel upregulation in both biological specimens. Following pharmacological treatment (magnesium supplementation combined with nonsteroidal anti-inflammatory drugs or acetaminophen), both microRNAs demonstrated approximately 50% reduction in treated patients compared with untreated patients ($p < 0.05$), again consistently across serum and saliva. No additional independent studies have evaluated these microRNAs in migraine in children and adolescents. Overall, elevated hsa-miR-34a-5p and hsa-miR-375 levels and their modulation after treatment were reported in a single pilot cohort, without external replication.

Table 2 Studies investigating PACAP-38, VIP, SP, NKA and NPY in migraine in the pediatric population

Study	Design	Population	Age (years), Sex	Biomarker	Specimen	Phase	Main findings
Liu 2022 [19]	Prospective	76 migraine (32 MA, 44 MO) 77 controls	Migraine: 10.38 ± 3.73 y (35 M) controls: 9.46 ± 3.91 y (47 M)	PACAP-38	Plasma	Ictal/ Interictal	PACAP-38 higher in pediatric migraine vs. controls during ictal and interictal phases; no ictal–interictal differences
Hanci 2021 [18]	Prospective	38 MO 20 healthy controls	MO: 13.0 ± 3.3 y (10 M) controls: 11.3 ± 2.9 y (7 M)	PACAP-38	Plasma	Ictal/ Interictal	PACAP-38 higher in pediatric migraine vs. controls during attack and attack-free periods; no ictal–interictal differences
Kilinc 2023 [11]	Cross-sectional	68 MO (EM and CM) 20 controls	Migraine: 14.4 ± 2.2 y (all F) controls: 14.5 ± 1.9 y (all F)	PACAP-38	Serum	Ictal/ Interictal	PACAP-38: ictal higher than interictal; CM > EM; CM > controls; limited correlation with disease burden
Orak 2025 [9]	Prospective	39 MO 40 healthy controls	MO: 14.12 y; (26 F) controls: 14.6 y; (26 F)	PACAP-38	Serum	Ictal/ Interictal	No significant differences in PACAP-38 between migraine patients and controls; no ictal–interictal variation
Hanci 2021 [18]	Prospective	38 MO 20 healthy controls	MO: 13.0 ± 3.3 y (10 M) controls: 11.3 ± 2.9 y (7 M)	VIP	Plasma	Ictal/ Interictal	VIP ↑ in pediatric migraine vs. controls; ictal and interictal levels elevated
Orak 2025 [9]	Prospective	39 MO, 40 healthy controls	MO: 14.12 y (26 F) controls: 14.6 y (26 F)	VIP	Serum	Ictal/ Interictal	VIP ↑ ictally vs. interictally; ↑ in migraine vs. controls
Kilinc 2023 [11]	Cross-sectional	68 MO (EM and CM) 20 controls	Migraine: 14.4 ± 2.2 y (all F) controls: 14.5 ± 1.9 y (all F)	VIP	Serum	Ictal/ Interictal	VIP ↑ in CM vs. EM; CM > controls; ictal > interictal
Gallai 1995 [16]	Prospective	75 migraine (30 MA, 45 MO) 30 controls	MA: 15.4 ± 2.3 y (12 M); MO: 16.3 ± 2.6 y (20 M); controls: 15.1 ± 2.1 y (15 M)	SP; NKA	Plasma	Ictal/ Interictal	SP NS vs. controls and across phases; NKA ↑ ictal vs. interictal
Hanci 2021 [18]	Prospective	38 MO 20 healthy controls	MO: 13.0 ± 3.3 y (10 M) controls: 11.3 ± 2.9 y (7 M)	SP	Plasma	Ictal/ Interictal	SP levels NS vs. controls and across ictal–interictal phases
Kilinc 2023 [11]	Cross-sectional	68 MO (EM and CM) 20 controls	Migraine: 14.4 ± 2.2 y (all F) controls: 14.5 ± 1.9 y (all F)	SP	Serum	Ictal/ Interictal	SP levels NS vs. controls, across phases and between CM and EM
Orak 2025 [9]	Prospective	39 MO 40 healthy controls	MO: 14.12 y (26 F) controls: 14.6 y (26 F)	SP	Serum	Ictal/ Interictal	No ictal change; interictal SP lower than controls
Gallai 1994 [24]	Prospective	15 MA, 20 MO 15 TTH 20 healthy controls	MA: 14.2 ± 4.1 y (8 F); MO: 16.1 ± 3.2 y (12 F); TTH: 14.3 ± 2.2 y (8 F); controls: 15.7 ± 2.8 y (10 F)	NPY	Plasma	Ictal/ Interictal	Interictal NPY ↓ vs. controls; ictal ↑ vs. interictal but NS vs. controls

CM: Chronic migraine, EM: Episodic Migraine, F: Female, M: Male, MA: Migraine with Aura, MO: Migraine without aura, NKA: Neurokinin A, NPY: Neuropeptide Y, NS: Not Significant, PACAP-38: Pituitary Adenylate Cyclase-activating Polypeptide-38, SP: Substance P, TTH: Tension-Type Headache, VIP: Vasoactive Intestinal Peptide, y: years

Serotonin

Peripheral serotonin (5-HT) levels were evaluated in three independent cohorts of children and adolescents with migraine [25–27], with detailed results summarized in Table 3. In a cohort including adolescents with EM and CM, Pakalnis et al. did not observe significant differences in whole-blood 5-HT levels between migraine patients and healthy controls ($p > 0.05$), nor correlations with headache frequency or Pediatric Migraine Disability Assessment (PedMIDAS) scores. A non-significant trend toward higher 5-HT levels was noted in CM compared with EM [25]. In contrast, Liu et al. reported significantly higher plasma 5-HT levels in migraine patients compared with controls ($p < 0.05$). Subgroup analyses showed lower 5-HT levels in MA compared with MO and controls ($p < 0.05$). Ictal 5-HT concentrations were higher than interictal levels and higher than levels in healthy controls ($p < 0.05$) [26]. Wang et al. evaluated interictal serum 5-HT in pediatric patients with migraine and patent foramen ovale (PFO) and found significantly higher levels compared with both PFO patients without migraine and healthy controls ($p < 0.001$). However, no significant associations were detected between 5-HT levels and attack frequency, duration, pain intensity, or associated symptoms [27]. Overall, peripheral 5-HT findings were heterogeneous across cohorts, with significant elevation observed in selected phenotypes but inconsistent associations with disease burden.

Gut-brain axis

Gut microbiota

GM composition was investigated in three independent pediatric cohorts using fecal 16 S rRNA sequencing [12, 26, 28], with results summarized in Table 3. Across studies, β-diversity (between-sample microbial community dissimilarity) consistently demonstrated significant separation between migraine and control groups ($p < 0.05$) [12, 26, 28], indicating distinct microbial community structures in migraine. In contrast, α-diversity (within-sample diversity and species richness) findings were heterogeneous: Papetti et al. reported increased α-diversity indices in migraine patients (all $p < 0.05$), Liu et al. observed reduced α-diversity ($p < 0.05$), whereas Fan et al. did not detect significant α-diversity differences between groups. Taxonomic analyses revealed differential abundance of multiple genera across cohorts, although the specific taxa varied between studies. Some studies reported enrichment in migraine of genera within the *Bacteroides/Parabacteroides* lineage whereas controls were relatively enriched in taxa including *Bifidobacterium* and other short-chain fatty acid–producing bacteria [12, 28]. Liu et al. identified a multigenera signature discriminating migraine from controls and reported significant differences in taxa implicated in microbial tryptophan metabolism, including *Bacteroides* and *Sutterella* ($p < 0.05$) [26]. Functional inference analysis demonstrated enrichment of metabolic pathways related to tryptophan and phenylalanine metabolism in migraine ($p < 0.05$) [12]. Fan et al. additionally conducted a randomized pilot intervention showing that Bifidobacterium longum supplementation was associated with significant reductions in pain scores and weekly headache days after 12 weeks ($p < 0.05$) [28]. Overall, while α-diversity findings were inconsistent, altered β-diversity and differential abundance of taxa, particularly those linked to tryptophan metabolism, were reproducible across independent cohorts.

Gut barrier integrity and microbial translocation markers

Markers of gut barrier integrity and microbial translocation included circulating lipopolysaccharides (LPS), immunoglobulin A (IgA), and the tight junction protein occludin. They were evaluated in pediatric cohorts of migraine in children and adolescents [12, 26], with results summarized in Table 3. Migraine patients showed significantly higher levels of permeability-related biomarkers compared with controls. Increased serum LPS concentrations ($p < 0.05$) and elevated occludin levels ($p < 0.05$) were reported, suggesting alterations in epithelial tight junction integrity [12]. Elevated IgA levels were also observed in migraine patients compared with controls ($p < 0.05$), indicating enhanced mucosal immune activation in the same cohort [12]. In contrast, in a case-control study of 73 children and adolescents with migraine and 147 age and sex matched controls serum IgA levels were normal in all participants [29]. In parallel, indirect markers of altered intestinal permeability were described in a separate cohort, including significantly increased urinary indican levels in migraine patients ($p < 0.05$) [12]. However, associations between these biomarkers and clinical measures such as headache frequency or disease duration were not consistently demonstrated. Overall, available data indicate increased markers of gut permeability and microbial translocation in migraine cohorts, although findings derive from a limited number of studies and require independent replication.

Tryptophan metabolism

Alterations in tryptophan metabolism were investigated in independent pediatric cohorts of migraine in children and adolescents [12, 26, 30], with detailed results summarized in Table 3. Across systemic metabolomic studies, migraine patients demonstrated significant shifts within the kynurenine pathway compared with controls, although the direction of specific metabolite changes varied between cohorts. Elevated kynurenine pathway activity was reflected by increased kynurenine (KYN) levels and higher KYN/tryptophan (TRP) ratios in one

Table 3 Studies investigating the gut–brain and tryptophan pathways in migraine in the pediatric population

Study	Design	Population	Age (years), Sex	Biomarker	Specimen	Phase	Main findings
Pakalnis 2009 [25]	Prospective	15 EM, 15 CM, 18 controls	EM: 13.9 y (9 F/6 M); CM: 15.2 y (10 F/5 M); controls: 15.9 y (8 F/10 M)	Serotonin (5-HT)	Whole blood	Interictal	5-HT levels did not differ between EM, CM, and controls; no correlation with headache frequency.
Liu 2024 [26]	Cross-sectional	51 migraine, 120 controls	Migraine: 10.75 ± 2.20 y (31 M/20F); controls: 9.91 ± 5.33 y (68 M/52F)	Serotonin (5-HT)	Plasma	Ictal/ Interictal	Plasma 5-HT higher in migraine than controls; lower in MA; ictal levels ↑ interictal.
Wang 2025 [27]	Prospective	78 PFO + MA, 71 PFO-MA, 70 controls	PFO + MA: 13.15 ± 9.67 y (38 M/40F); PFO-MA: 15.23 ± 9.49 y (33 M/38F); controls: 14.19 ± 9.28 y (32 M/38F)	Serotonin (5-HT)	Serum	Interictal	Interictal serum 5-HT higher in PFO + MA than PFO-only and controls; no correlation with attack frequency, duration, intensity.
Fan 2025 [28]	Prospective	126 migraine, 50 controls	Migraine: 11.9 ± 0.3 y (61 M/65F)	Gut microbiome	Fecal samples	Interictal	Altered β-diversity vs. controls; no α-diversity difference; B. longum reduced pain scores and weekly headache days.
Liu 2024 [26]	Cross-sectional	51 migraine, 120 controls	Migraine: 10.75 ± 2.20 y (31 M/20F); controls: 9.91 ± 5.33 y (68 M/52F)	Gut microbiome	Fecal samples	Interictal	Altered β-diversity vs. controls; reduced α-diversity; multigenera signature including taxa involved in tryptophan metabolism.
Papetti 2024 [12]	Prospective	98 migraine, 98 controls	Migraine: 12.5 ± 2.87 y (64 F/34 M)	Gut microbiome	Fecal samples	Interictal	Altered β-diversity vs. controls; increased α-diversity; differential abundance of Bacteroides/ Parabacteroides lineage.
Sahin 2025 [30]	Case-control	45 migraine, 48 controls	Migraine: 13.8 ± 2.6 y (30 F); controls: 13.6 ± 3.0 y (25 F)	Tryptophan metabolites	Serum	Interictal	Increased KYN levels and KYN/TRP ratio vs. controls; kynurenine pathway activation; metabolite ratios correlated with headache burden.
Liu 2024 [26]	Cross-sectional	51 migraine, 120 controls	Migraine: 10.75 ± 2.20 y (31 M/20F); controls: 9.91 ± 5.33 y (68 M/52F)	Gut permeability markers; Tryptophan metabolites	Serum	Ictal and interictal	QUIN and ↓ KYNA; pathway ratios indicated shift toward neurotoxic kynurenine branch.
Papetti 2024 [12]	Prospective	98 migraine, 98 controls	Migraine: 12.5 ± 2.87 y (64 F/34 M)	Gut permeability markers; Tryptophan metabolism	Serum and urine	Interictal	↑ LPS and ↑ occludin; ↑ urinary indican; enrichment of tryptophan metabolic pathways.

CM: Chronic migraine, EM: Episodic Migraine, F: Female, KYN: Kynurenine, KYNA: Kynurenic acid, LPS: Lipopolysaccharide, M: Male, MA: Migraine with Aura, PFO: Patent Foramen Ovale, QUIN: Quinolinic acid, TRP: Tryptophan, 5-HT: Serotonin (5-hydroxytryptamine), y: years

interictal cohort ($p < 0.05$) [30], whereas another study identified significantly increased quinolinic acid (QUIN) levels ($p < 0.001$) and reduced kynurenic acid (KYNA) concentrations ($p < 0.001$) [26]. In the latter, pathway ratios consistently indicated imbalance toward the neurotoxic branch (lower KYNA/KYN and KYNA/QUIN; higher QUIN/KYN; all $p < 0.001$), with similar patterns observed in both MA and MO and across ictal and interictal phases. While absolute TRP levels did not differ consistently across cohorts, metabolite ratios appeared more sensitive in discriminating migraine from controls. Associations with clinical burden were modest: KYNA/3-hydroxykynurenine (KYNA/3-HK) ratio correlated negatively with headache frequency and PedMIDAS, whereas 3-HK correlated positively with disability in one cohort [30]. Complementary microbiota-based functional inference demonstrated enrichment of tryptophan metabolic pathways in migraine ($p < 0.05$), together with elevated urinary indican levels ($p < 0.05$), suggesting increased intestinal tryptophan degradation [12]. Overall, pediatric migraine cohorts exhibit consistent evidence of KYN pathway imbalance and altered TRP-related metabolism, supported by both systemic metabolite shifts and gut-derived functional alterations, despite variability in individual metabolite patterns.

Cytokines and inflammatory markers

Cytokines

Circulating cytokines were evaluated in independent pediatric cohorts of migraine in children and adolescents [31–33], with detailed results summarized in Table 4. Across studies, activation of the tumor necrosis factor (TNF) axis emerged as the most consistent inflammatory signal. Bockowski et al. reported significantly elevated soluble TNF receptor 1 (sTNFR1) levels in migraine patients compared with controls ($p < 0.001$), with higher concentrations observed in both MA and MO [31]. TNF-α showed a non-significant increasing trend in the same cohort [31]. Similarly, Hirfanoglu et al. identified significantly higher baseline levels of TNF-α in migraine patients compared with controls ($p < 0.05$), together with increased interleukin (IL)-1β and IL-6 ($p < 0.05$) [32]. Following four months of prophylactic treatment, TNF-α, IL-1β, and IL-6 levels decreased significantly across treatment groups (all $p < 0.05$) [32]. In contrast, anti-inflammatory cytokines did not demonstrate consistent differences. Interictal IL-4, IL-10, and IL-13 levels were not significantly different between migraine patients and controls [34]. More recently, a broader cytokine panel analysis comparing migraine patients with two disease-control groups (encephalitis with headache and pneumonia without headache) identified IL-12p70 as the most distinctive cytokine [33]. IL-12p70 levels were significantly higher in migraine patients compared with both control groups ($p < 0.001$), and IL-12p70 and IL-17 A were independently associated with migraine (odds ratio (OR) 1.267 and 1.066, respectively). In that cohort, IL-6, IL-5, and IL-8 were lower in migraine patients, while IL-10, IFN-γ, and IL-1β did not differ significantly. Overall, TNF-axis activation represents the most reproducible inflammatory signal across cohorts, whereas other cytokines demonstrate heterogeneous and panel-dependent findings.

Systemic inflammatory and endothelial biomarkers

Acute-phase proteins and endothelial vasoactive mediators including C-reactive protein (CRP), pentraxin-3 (PTX-3), endothelin-1 (ET-1) and prostaglandin F2α (PGF2α) were evaluated in pediatric cohorts of migraine in children and adolescents. Findings for CRP were heterogeneous across studies. Wang et al. evaluated 219 pediatric subjects, including 78 children with PFO and migraine, 71 PFO patients without migraine, and 70 healthy controls. Serum CRP levels were significantly higher in migraine patients compared with both non-migraine PFO subjects and healthy controls ($p < 0.05$) [27]. However, independent cohorts did not detect significant interictal differences between migraine patients and controls ($p > 0.05$) and associations with migraine subtype or clinical characteristics were not consistently observed [35, 36]. In one study, modest CRP elevation did not remain significant after multivariable adjustment [37]. Markers of endothelial activation showed more consistent signals in selected phenotypes. Serum ET-1 levels were significantly higher in pediatric migraine patients compared with both non-migraine PFO subjects and healthy controls with all $p < 0.001$ [27]. Similarly, PTX-3 concentrations were significantly elevated in PFO-associated migraine compared with both comparison groups with all $p < 0.001$ [27]. In an independent cohort, interictal serum PTX-3 levels were significantly higher in children with migraine compared with controls ($p < 0.001$) [36]. PTX-3 levels correlated positively with PedMIDAS scores [36]. Prostaglandin F2α demonstrated phase-dependent variation rather than consistent group differences [38]. In a pediatric cohort of 64 children with migraine, ictal and interictal urinary PGF2α concentrations did not differ between migraine patients and controls overall. However, within the migraine group, ictal levels were significantly higher than interictal levels ($p < 0.05$), and no differences were observed between migraine subtypes [38]. Overall, CRP demonstrates inconsistent elevation across cohorts, whereas PTX-3 and ET-1 show more reproducible associations in selected phenotypes, and PGF2α appears primarily attack dependent rather than discriminatory.

Table 4 Studies investigating cytokines in migraine in the pediatric population

Study	Design	Population	Age (years), Sex	Biomarker	Specimen	Phase	Main findings
Bockowski 2009 [31]	Cross-sectional	21 migraine (9 MA, 12 MO), 24 TTH	Migraine: 14.0 ± 2.3 y (10 F); TTH: 12.1 ± 3.5 y (18 F)	IL-1α, TNF-α, sTNFR1	Plasma	Interictal	↑ sTNFR1 in migraine vs. TTH; IL-1α detectable mainly in migraine and higher in MA. TNF-α showed only a trend.
Bockowski 2010 [34]	Cross-sectional	35 migraine (14 MA, 21 MO), 33 TTH	Migraine: 14,04 ± 2,29 y; 20 M TTH: 12,11 ± 3,46 y; 11 M	IL-4, IL-10, IL-13	Plasma	Interictal	Anti-inflammatory cytokines detectable in few patients; no differences vs. TTH; no clinical correlations.
Hirfanoglu 2009 [32]	Prospective	77 migraine (9 MA, 68 MO), 19 controls	Migraine: 12.98 ± 3.11 y; 33 M; controls: 12.26 ± 2.8 y	TNF-α, IL-1β, IL-6	Serum	Interictal	↑ TNF-α, IL-1β, IL-6 at baseline vs. controls; all ↓ after prophylaxis, paralleling clinical improvement.
Yang 2024 [33]	Retrospective	44 migraine, 27 encephalitis + HA, 44 pneumonia	Migraine: 9,80 ± 2,29 y; 25 M/19F	IL-12p70, IL-17 A, IL-10, IFN-γ, IL-1β, IL-6, IL-5, IL-8	Serum	Interictal	↑ IL-12p70 and IL-17 A in migraine vs. both controls; IL-12p70 (OR 1.27) and IL-17 A (OR 1.07) independently associated with migraine.

CGRP: Calcitonin Gene-Related Peptide, CM: Chronic Migraine, F: Female, IL: interleukin, M: Male, MA: Migraine with Aura, MO: Migraine without Aura, NDPH: New Daily Persistent Headache, OR: Odds Ratio, sTNFR1: soluble Tumor Necrosis Factor Receptor 1, TTH: Tension-Type Headache, TNF-α: Tumor Necrosis Factor alpha

Discussion

The available biomarker data in migraine in children and adolescents largely belong to the principal biological domains described in adults, although quantitative and methodological differences remain. The convergence across age groups suggests biological continuity of core pathophysiological mechanisms, whereas differences likely reflects developmental modulation rather than distinct processes. Together, these findings indicate that trigeminovascular activation, metabolic stress, immune signaling and gut–microbial interactions are already biologically detectable early in life, but their magnitude and clinical expression appear shaped by developmental stage and disease duration (Fig. 2). Neuropeptide findings show the strongest cross-age concordance. In adults, CGRP represents the most validated molecular mediator of migraine, supported by consistent ictal elevation and by the therapeutic efficacy of anti-CGRP monoclonal antibodies and receptor antagonists [39, 40]. Pediatric cohorts similarly demonstrate reproducible ictal CGRP elevation [11, 16, 19], indicating that trigeminovascular activation is already measurable early in life. However, interictal CGRP levels appear more heterogeneous in children than in adults, where baseline elevation is more consistently reported in CM [41]. This discrepancy may reflect developmental neurovascular regulation, hormonal influences or shorter disease duration [42, 43]. The observed reduction of CGRP following preventive therapy [15] is highly concordant with adult data and supports a treatment-responsive role across age groups. PACAP-38 shows elevation in several pediatric cohorts [11, 19], in line with adult experimental data demonstrating PACAP-induced migraine-like attacks [44]. However, phase-dependent variation appears less consistent in children than in adult migraine attacks models [45]. As regards other neuropeptides, VIP shows variable elevation, while SP, NKA and NPY lack reproducible discriminative value [9, 18], similar to the limited clinical biomarker utility observed in adults [4]. Markers of mitochondrial dysfunction and hypoxia-related stress have been increasingly implicated in adult migraine, particularly in chronic forms [46]. Elevated HIF-1α, GDF-15, and FGF-21 levels in pediatric cohorts with migraine, especially in CM mirror adult data, suggesting metabolic stress involvement [11]. However, as in adults, these markers lack disease specificity and likely reflect systemic metabolic burden rather than migraine-specific processes. MicroRNA research is more developed in adult migraine, where several circulating signatures have been proposed [47]. In children, only a single pilot cohort has reported elevated and treatment-responsive hsa-miR-34a-5p and hsa-miR-375 levels [14]. The concordance between serum and salivary expression suggests potential feasibility for noninvasive monitoring, but pediatric

Table 5 Research and potential clinical relevance of biochemical markers in migraine in the pediatric population

Biomarker(s)	Case–control discrimination	Monitoring	Prognostic / Burden	Treatment-response	Studies	Case-control consistency	Overall evidence
CGRP	Moderate case–control discrimination; inconsistent interictal separation	Ictal elevation; variable interictal findings	Higher in chronic vs. episodic migraine (some cohorts)	Decreases after prophylaxis	[9, 11, 15–23]	Partially consistent	Moderate
PACAP-38	Possible case–control discrimination (selected cohorts)	Limited ictal–interictal differentiation	Possible burden association	Not established	[9, 11, 18, 19]	Limited evidence	Low
VIP	Limited standalone discrimination	Possible ictal increase	Weak evidence	Not established	[9, 11, 18]	Limited evidence	Low
SP	No consistent discrimination	Transient ictal changes (isolated studies)	Not established	Not established	[9, 11, 16, 18]	Inconsistent	Low
NKA NPY	No consistent discrimination	Transient ictal changes (isolated studies)	Not established	Not established	[16, 24]	Limited evidence	Very low
HIF-1α GDF-15 FGF-21	No validated discrimination	Elevated during attacks	Higher in chronic migraine	Not studied longitudinally	[11]	Limited evidence	Very low
miR-34a-5p miR-375	No validated discrimination	Reflect disease activity; saliva mirrors serum	Not established	Decrease after treatment	[14]	Limited evidence	Very low
5-HT	Phenotype-dependent discrimination; negative in unselected cohorts	Not consistently phase-dependent	Not established	Not established	[25–27]	Inconsistent	Low
Gut microbiota	Promising discrimination in selected cohorts	Not established	Not established	Early probiotic signal	[12, 25, 26]	Partially consistent	Moderate
Tryptophan pathway (incl. KYNA/QUIN ratio)	Promising discrimination mainly for ratio metrics; single metabolites variable	Correlates with frequency/disability (some studies)	Potential burden association	Not longitudinally tested	[26, 30]	Limited evidence	Low
CRP PTX-3	Limited and phenotype-dependent discrimination	Weak	Weak	Not established	[27, 36, 37]	Inconsistent	Low
Pro-inflammatory cytokines (TNF-α, sTNFR1, IL-1β, IL-6, IL-12p70, IL-17 A)	Limited standalone discrimination; potential utility in panels	Baseline elevation in some cohorts; reflects immune activation	Weak evidence	Decrease after prophylaxis (TNF-axis)	[31–33]	Partially consistent	Low
Anti-inflammatory cytokines (IL-4, IL-13, IL-10)	No demonstrated discrimination	Not established	Not established	Not established	[34]	Limited evidence	Very low

CGRP: Calcitonin Gene-Related Peptide, CRP: C reactive protein, FGF-21: Fibroblast Growth Factor-21, GDF-15: Growth Differentiation Factor-15, HIF-1α: Hypoxia-inducible Factor-1α, IL: Interleukin, KYNA: Kynurenic acid, NKA: neurokinin A, NPY: Neuropeptide Y, PACAP: Pituitary Adenylate Cyclase–Activating Polypeptide, PTX-3: Pentraxin-3, QUIN: Quinolinic acid, SP: Substance P, sTNFR1: soluble Tumor Necrosis Factor Receptor 1, TNF-α: Tumor Necrosis Factor alpha, TTH: Tension-Type Headache, VIP: Vasoactive Intestinal Peptide, 5-HT: serotonin

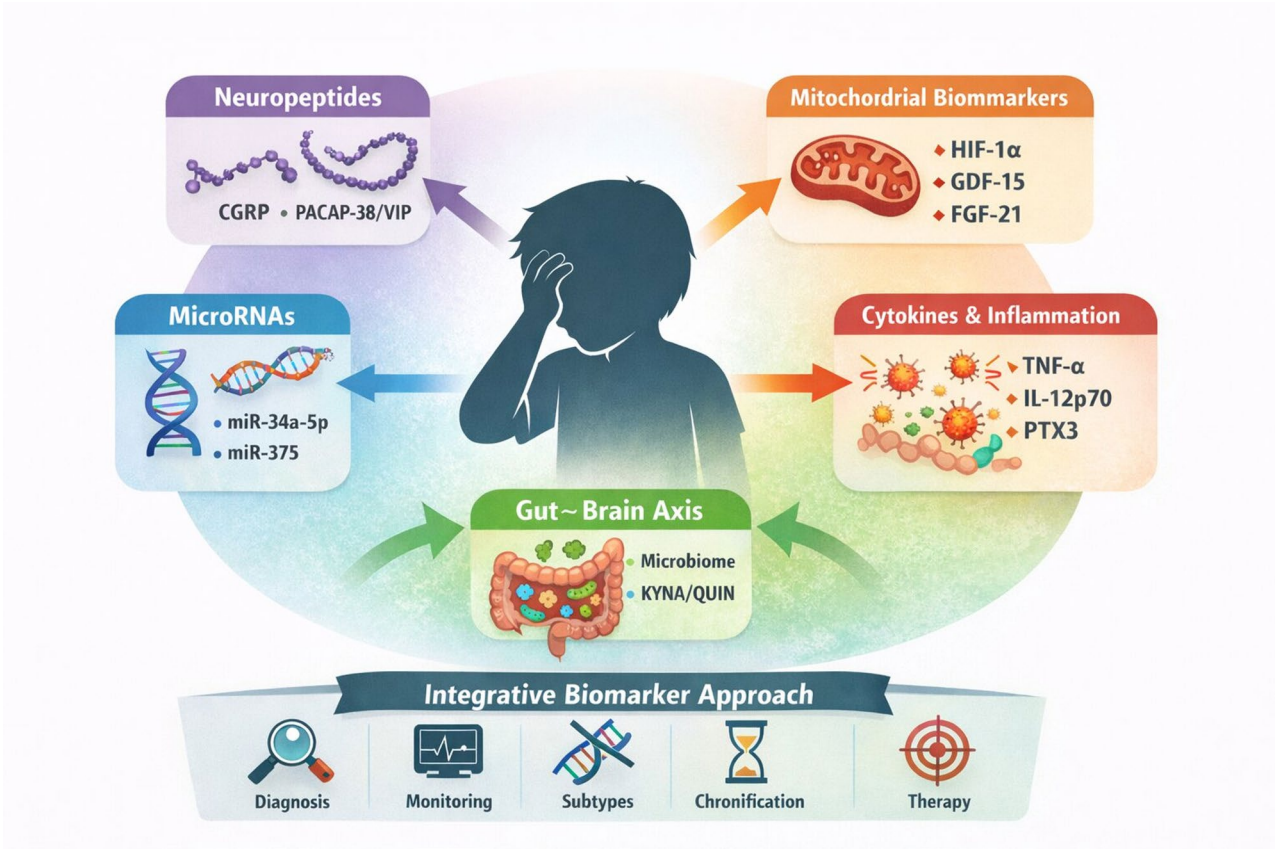

Fig. 2 Conceptual framework of biochemical markers in migraine in the pediatric population. Overview of the principal biomarker categories investigated, including neuropeptides (CGRP, PACAP-38, VIP), mitochondrial biomarkers (HIF-1α, GDF-15, FGF-21), inflammatory mediators (TNF-α, IL-12p70, PTX3), microRNAs (miR-34a-5p, miR-375), and gut–brain axis components (microbiome composition and kynurenine pathway metabolites). The figure illustrates the potential convergence of these pathways within an integrative biomarker approach, encompassing research applications in diagnosis, disease monitoring, subtype characterization, chronification and therapeutic response

data remain preliminary. Peripheral serotonin findings in children are heterogeneous [25–27], paralleling inconsistencies in adult literature, where peripheral 5-HT levels do not reliably reflect central serotonergic activity [48]. The association of elevated 5-HT with specific phenotypes such as PFO-associated migraine [27] suggests that peripheral serotonin may act as a context-dependent susceptibility marker rather than a universal biomarker, consistent with adult observations. One of the most compelling parallels with adult research concerns the gut–brain axis. In adult migraine, alterations in microbiota composition and tryptophan metabolism have been increasingly reported [49, 50]. Pediatric cohorts similarly demonstrate consistent β-diversity differences despite heterogeneous α-diversity findings [12, 26, 28], suggesting a reproducible migraine-associated microbial profile. Altered gut permeability markers including LPS, occludin, IgA and urinary indican [12] further support the involvement of intestinal barrier function, although these markers lack specificity and replication in both adult and pediatric settings. Alterations in tryptophan metabolism may represent an integrative bridge between microbial and central mechanisms. In adults, shifts within the kynurenine pathway, particularly imbalances in KYNA and QUIN, have been associated with migraine susceptibility [50]. Pediatric data show similar pathway imbalance patterns, especially when considering metabolite ratios rather than absolute concentrations [26, 30]. Microbiota-derived enrichment of tryptophan metabolic pathways and elevated urinary indican levels [12] reinforce the biological role of gut-mediated modulation. Small probiotic intervention studies demonstrating clinical improvement [28] are consistent with exploratory adult interventional data, although evidence remains preliminary in both age groups. Inflammatory profiling in adults frequently demonstrates low-grade immune activation rather than overt systemic inflammation [51]. Pediatric data similarly indicate modest activation of the TNF axis [31, 32], while broader cytokine panels yield heterogeneous findings [33]. Acute-phase markers such as CRP show inconsistent elevation, mirroring adult inconsistencies [27, 35, 36]. Pentraxin-3 and endothelin-1 demonstrate stronger associations in selected phenotypes [27, 36], comparable to adult data suggesting endothelial

dysfunction in migraine [52, 53]. Prostaglandin F2α appears attack-dependent [38], similar to observations in adult acute-phase studies [54]. Overall, migraine biomarker patterns in pediatric age align with the principal biological domains described in adult migraine, including trigeminovascular activation, metabolic stress, gut–microbial modulation, and low-grade immune activation. However, the consistency and strength of these signals remain variable, and no single marker currently achieves sufficient specificity or reproducibility for clinical implementation. The emerging picture suggests that migraine in children and adolescents is a biologically measurable condition, but its molecular signatures appear developmentally modulated and context-dependent. These findings support a mechanistic continuity across the lifespan while highlighting the complexity of translating adult-derived biomarker frameworks directly into pediatric practice. An overall level of evidence for each biomarker category was qualitatively assigned and is reported in Table 5.

The evidence base is dominated by small, single-center observational studies, limiting causal inference and increasing susceptibility to residual confounding and selection bias. Future research should prioritize multicenter and longitudinal designs capturing developmental transitions, harmonized sampling protocols, and multimarker integration strategies. Rather than relying on single-molecule candidates, combinatorial biomarker panels reflecting interconnected neurovascular, metabolic, and microbial pathways may offer greater potential for clinical translation.

Conclusion

Migraine in children and adolescents is associated with measurable alterations across trigeminovascular, metabolic, microbial and inflammatory pathways. CGRP shows the most consistent evidence, particularly as a disease-activity and treatment-responsive biomarker. Other candidates, including PACAP-38, mitochondrial stress markers, and kynurenine pathway ratios, may reflect disease burden or specific phenotypes but lack diagnostic specificity and remain insufficiently validated. The certainty of findings is limited by the predominance of observational studies with moderate risk of bias, mainly related to comparability and residual confounding, and heterogeneity in sampling phase and assays. At present, no single biomarker supports routine clinical use. Future advances will require standardized sampling frameworks, assay harmonization and prospective multicenter validation, with emphasis on integrative multimarker approaches that capture the complex biology of pediatric migraine.

Abbreviations

ALA	Alpha-Lipoic Acid
CGRP	Calcitonin gene-related peptide
CM	Chronic migraine
EM	Episodic migraine
FGF-21	Fibroblast growth factor-21
GDF-15	Differentiation factor-15
GM	Gut microbiota
HIF-1α	Hypoxia-inducible factor-1α
HK	3-hydroxykynurenine
ICHD-3	International Classification of Headache Disorders, 3rd edition
IgA	Immunoglobulin A
IL	Interleukin
KYN	Kynurenine
KYNA	Kynurenic acid
LPS	Lipopolysaccharides
MA	Migraine with aura
MO	Migraine without aura
NPY	Neuropeptide Y
OR	Odds ratio
PACAP	Pituitary adenylate cyclase–activating polypeptide
PedMIDAS	Pediatric Migraine Disability Assessment
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-analyses
QUIN	Quinolinic acid
RCT	Randomized clinical trials
SP	Substance P
sTNFR1	Soluble TNF receptor 1
TNF	Tumor necrosis factor
TRP	Tryptophan
TTH	Tension-type headache
VIP	Vasoactive intestinal peptide
5-HT	Serotonin

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s10194-026-02344-9.

Supplementary Material 1: Table S1. Risk of bias assessment using the Newcastle–Ottawa Scale (NOS) for observational studies. Scoring: Selection (0–4), Comparability (0–2), Exposure (0–3); total 0–9 stars. Overall: Low (7–9), Moderate (4–6), High (0–3).

Author contributions

GM: drafting of the manuscript; acquisition and interpretation of data. CG: acquisition and interpretation of data, review writing. FU, GS: acquisition of data. MV: review writing and supervision. LP: conceptualization, review writing and supervision.

Funding

This work was supported by the Italian Ministry of Health (Current Research funds) and by the project MNESYS (PE0000006 – A Multiscale integrated approach to the study of the nervous system in health and disease (DN. 1553 11.10.2022).

Data availability

The datasets generated during the current study are available upon reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

GM, CG, FU, GS have nothing to declare. MV: Chief editor of Pain Research and Management; editorial board of Journal Head Pain, Clinical Neurophysiology, European Journal of Pain, Neurological Sciences. LP: member of the Junior Editorial Board of The Journal of Headache and Pain, Section Board Member for Children in the area of Pediatric Neurology & Neurodevelopmental Disorders, Editorial Board Member of Confinia Cephalalgica, and Associate Editor for the Pediatric Neurology section of Frontiers in Neurology. She has received financial support from TEVA, Eli Lilly, Lundbeck, Pfizer, AbbVie, and Epitech for the organization of scientific meetings and support from attending meetings from MERCK and Biogen.

Received: 16 February 2026 / Accepted: 20 March 2026
Published online: 04 April 2026

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